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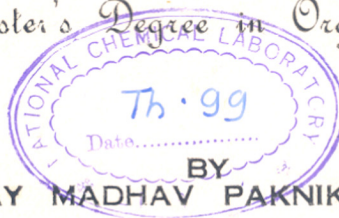
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Chemical Investigation on Terpenes
and
COMPUTERISED
Related Compounds

A
Thesis submitted to
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for
The Master's Degree in Organic Chemistry



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A C K N O W L E D G M E N T

I wish to express my deep sense of gratitude to Dr. K.K. Chakravarti for his valuable guidance throughout the course of this investigation. Also, I take this opportunity to thank Dr. S.K. Paknikar whose constant inspiration and keen interest made this work possible.

The immense and valuable services from the micro-analytical, spectral, gas-liquid chromatographic and glass-blowing sections of this laboratory are gratefully acknowledged.

Finally, I am grateful to the Director, National Chemical Laboratory, Poona, for allowing me to submit this work in the form of a thesis.

(U.M. Paknikar)

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Introduction

INTRODUCTION

The study of natural products isolated from the vegetable sources constitutes a very fascinating aspect of organic chemistry. The plant kingdom yields a very large number of compounds including some pleasantly smelling oils and solids, e.g. camphor, which by virtue of their sweet fragrance, attracted the attention of mankind from the earliest times.

The term 'essential oil' according to present day terminology, includes, besides the steam distilled oils of volatile nature, the solvent extracts of roots, barks, flowers, leaves etc. Essential oils are found to be rich sources of the well-known group of natural products viz. terpenoids. Besides essential oils, terpenoids have been found to occur in several other sources such as rosin, which is a complex mixture of resin acids having molecular formula $C_{19}H_{29}.COOH$. These substances find amazingly wide and varied applications in many industries for the scenting and flavouring all kinds of consumer finished products. Many of these products are used in medicinal preparations. Indian 'Materia Medica' describes innumerable drug preparations, their botanical origin, composition and medicinal properties.

The importance of the study of natural products in India can hardly be over emphasised especially in view of the fact that India possesses a wealth of most abundant and

diversified flora. This is but natural as this sub-continent is fortunate in having a variety of climatic and soil conditions, suitable for healthy growth of plants, herbs, grasses etc.

Historically, terpenoid chemistry has seen three broad phases. First, a phase, in which structures of simple bicyclic monoterpenoids such as camphor, α -pinene, Δ^2 -carene, camphene, followed by few sesquiterpenes were established mainly through the efforts of Wallach, Semmler and several others. The structure elucidation of these substances posed serious problems because the molecular rearrangements taking place during a chemical reaction were not realised. These substances then attracted the attention of eminent organic chemists because of the diversified carbon skeletons and the ease with which they undergo molecular rearrangements. The second phase was dominated by the pioneering researches of Ruzicka, Sir John Simonsen, D.H.R. Barton, Prelog and others, during which the presently accepted ideas of 'biogenetic isoprene rule' and conformational analysis were developed. These can be regarded as outstanding achievements of this century. During this phase, structures a very large number of mono, sesqui-di and triterpenes were established.

Lastly, the most recent phase or the third phase, in which more refined ideas of stereochemistry and reaction mechanism, stereospecific synthetic reactions have been developed. During this phase, highly effective separation

techniques such as analytical and preparative GLC, thin and preparative thick layer chromatography and instrumental methods of analysis such as infrared, nuclear magnetic resonance, optical rotatory dispersion, mass spectroscopy, X-ray analysis were developed. Although mass spectroscopy and X-ray analysis have been known for many years, their applications to structural problems in natural product chemistry are of much recent origin and hence their inclusion in the recent phase is justified. Due to the availability of these techniques and a better understanding of reaction mechanisms, biogenetic concepts and stereochemical aspects, an enormous amount of research has been carried out in the field of natural product chemistry. And the research work is being continued with more vigour.

We have also developed keen interest in the study of terpenoids and related compounds. As a part of this programme, the candidate has carried out some research work at the National Chemical Laboratory, Poona and the results are incorporated in the present thesis.

S U M M A R Y

The catalytic hydrogenation products of cyperene have been studied. The two saturated dihydroderivatives ($C_{15}H_{26}$) have been isolated in pure form for the first time. Contrary to the earlier reports, only the minor product was found to be identical with isopatchoulane. Structure (including stereochemistry) has been assigned to the major product of hydrogenation. During this study, a new sesquiterpene, which always accompanies cyperene has been isolated in pure form. A tentative structure has been assigned to this new hydrocarbon. Rotundene, has been isolated from the hydrocarbon fraction. The results are incorporated in Chapter I.

A systematic chemical investigation of the sesquiterpene hydrocarbon fraction of Singapore patchouli oil (Bogostemon gablin Benth) has been carried out. A careful combination of column and thin layer chromatography over alumina, silica gel and silica gel impregnated with silver-nitrate, has led to the isolation of three pure sesquiterpene hydrocarbons, β -patchoulene, α -gurjunene and seychellene. Petroleum-ether eluate also contains caryophyllene contaminated with traces of β -elemene.

The steam distilled oil of gurjun balsam contains mainly two sesquiterpene hydrocarbons. These have been identified as caryophyllene and humulene. The results are included in Chapter II.

In order to support the assigned structure to the condensation product of citral, β -naphthylamine and pyruvic acid, we undertook a further study of condensation of citral with other amines in presence of pyruvic acid. The Chapter III contains the results of this investigation.

The details of an attempted synthesis of hydrocarbons having bicyclo (3,2,0) heptane skeleton have been described.

GENERAL REMARKS

All m.ps and b.ps are uncorrected. Pet.ether refers to the fraction b.p. 60-80°. All solvent extracts were finally washed with brige, before drying over anhydrous Na_2SO_4 . Optical rotations were measured in CHCl_3 unless otherwise stated.

IR spectra were measured as liquid films (neat) or nujol mulls (solids) on a Perkin-Elmer Infracord, model 137B. NMR spectra were recorded as 10 to 20% solution in CCl_4 (unless otherwise stated) with TMS as the internal standard on a Varian A-60 spectrometer and Varian T-60, model; signals recorded on 'G' units. Mass spectra were taken on Mass spectrometer 21-110B, model GEC. Analytical GLC was run on Griffin and George apparatus, MK-IIA or Aerograph, model A-350B.

Micro-analyses were carried out in the micro-analytical section of NCL.

Neutral alumina used for chromatography was prepared according to Evans and Shoppe and graded according to Brockman.

Silica gel (100-150 mesh) used for chromatography was activated by heating at 120° for 4-5 hrs. Silver nitrate impregnated silica-gel was prepared according to the literature procedure with different percentages of silver nitrate.

The numbers given to the structures and charts and also the numbers given to the figures in each chapter of the thesis refer to that particular chapter only.

Chapter-I

Hydrocarbon Fraction of *Cyperus* *Scariosus* Oil.

Essential Oil of Cyperus Scariosus R.Br.

Cyperus scariosus is one of the most important species of the genus cyperus (Fam: Cyperaceae). C.scariosus R.Br. [Sanskrit-Nagarmusta; Hindi and Bengali- Nagarmotha; Marathi - Lawala] is a delicate, slender sedge with small compound umbel, short leaves, scanty involucre and can be easily distinguished from other members of the genus. The plant produces deep brown or black tubers. The sedge is found in damp places in Bengal, Uttar Pradesh, eastern and southern parts of India.

The tubers which find extensive use in indigenous medicine¹ are rich in aromatic odour. The essential oil² (0.08%) obtained from these tubers is dark amber coloured viscous liquid resembling the odour of camphor. Rhizomes are used for certain dye preparations to impart perfume to the fabric. Perfumers use it as a fixative for heavy odour blends. It is a substitute for patchouli oil in soap preparation and also is used in agarbatti preparations.

Chemical Composition

In 1954, Naves³ isolated α -cyperene and an α,β -unsaturated ketone (2,4,D.N.P.H., m.p. 228-90) and reported the presence of tricyclic (40%) and bicyclic (33%) sesquiterpenes in the essential oil of C.scariosus. Recently, the essential oil of C.scariosus (Indian origin) has been examined systematically^{4a,b}. The hydrocarbon fraction which constitutes about 40% of the total oil,

contains cyperene (I) as a overwhelming constituent. The hydrocarbon fraction also contains another sesquiterpene hydrocarbon, rotundene⁵ of unestablished structure*.

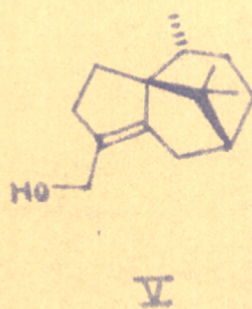
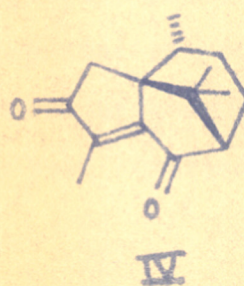
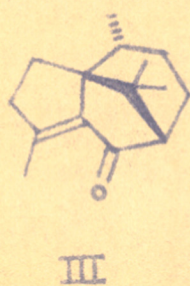
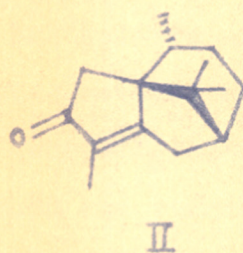
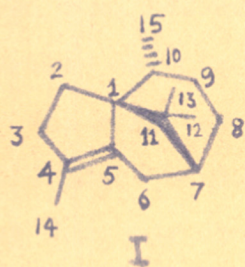
The structure of cyperene including stereochemical details as indicated in (I) has been established by Czech⁶ and Japanese workers⁷. The assigned structure is fully supported by an unambiguous synthesis⁸. Several oxygenated derivatives of cyperene, such as cyperotundone (II), patchoulone (III), scariodione (IV) cyperenol (V) and other related allylic alcohols have been isolated and characterised from Cyperus scariosus. Since compounds (II - V) have been directly related to cyperene (I) it would be appropriate to discuss briefly the structure of cyperene.

Structure of Cyperene

Cyperene, $C_{15}H_{24}$ was first isolated by Naves et al.³ and later by Nigam from *C. scariosus*^{4b}. Trivedi et al.⁶ isolated this hydrocarbon from the oil of *C. rotundus* of Chinese origin. Its Raman spectrum showed a band at 1695 cm^{-1} characteristic of a tetrasubstituted olefinic linkage. It gave a saturated dihydroderivative having molecular formula $C_{15}H_{26}$ and hence must be tricyclic. The IR spectrum of the saturated hydrocarbon (dihydrocyperene) was found to be identical with isopatchoulane (VI) prepared from patchouli alcohol (VII) via α - or γ -patchoulene (VIII, IX).

Structure (I) was, therefore, assigned to cyperene.

*The structure elucidation is in progress. On the basis of the spectral and chemical data collected so far, rotundene belongs to a new tricyclic sesquiterpene skeleton (Dr.S.K. Paknikar, Personal communication).



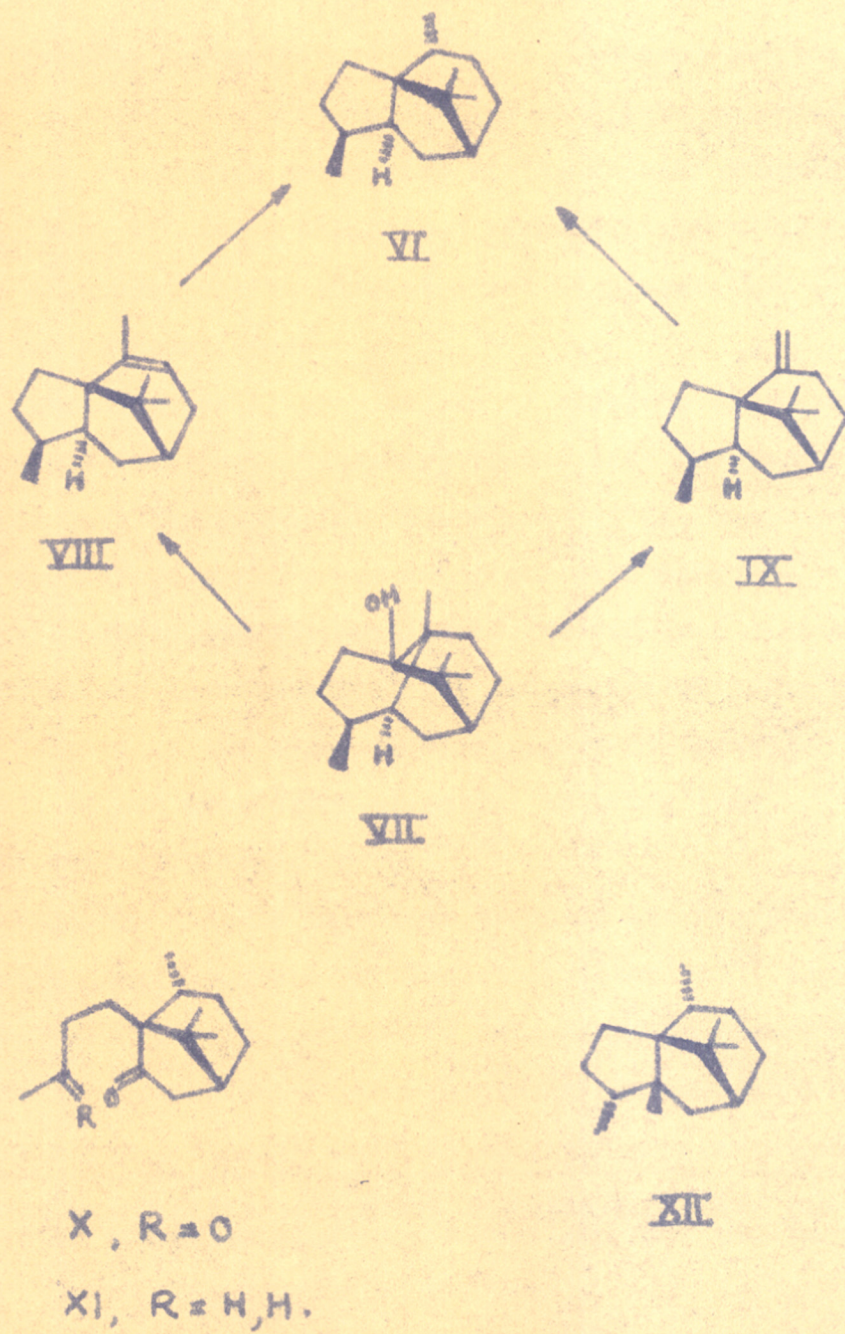
Further chemical proof was obtained by ozonolysis studies. Cyperene, on ozonolysis gave the solid diketone (X). The presence of a methyl ketone and a five membered ring ketone was confirmed from spectral studies and further degradative work. The NMR spectrum of cyperene showed the presence of three saturated methyls ($2, >C-CH_3$; $1-CH-CH_3$) and one vinyl methyl ($-C=C-CH_3$) group. It did not show any absorption above 2.5 μ and therefore the olefinic linkage must be fully substituted.

Stereochemistry of Cyperene

The stereostructure (I) was assigned mainly on the ORD studies on monoketone (XI), a degradation product of cyperene and NMR studies⁷ on the parent hydrocarbon (cyperene). These conclusions have been verified by a direct synthesis⁸.

Present Study

The hydrogenation of cyperene (I) is reported to proceed smoothly in acetic acid with Adam's catalyst to afford isopatchoulane⁶ (VI). Similarly α - and γ -patchoulenes (VIII & IX) are reported to yield isopatchoulane (VI) on catalytic hydrogenation⁹. Moreover all the three isopatchoulanes from these three different hydrocarbons show exactly the same infrared spectrum. On the contrary, the completely hydrogenated cyperene, although had superimposable infrared spectrum was reported to be a mixture of two components (in the ratio 7:3) on a



GLC column¹⁰. In view of lack of spectral data on pure saturated hydrocarbons, it was of interest to resolve the mixture into individual components and record the spectral data.

Previous investigators^{6,4b} have also reported that cyperene on ozonolysis gives a mixture of diketo compounds. This mixture on further processing yields a solid diketone, $C_{15}H_{24}O_2$, m.p. 77° and a colourless viscous liquid diketone. Both the diketones can be characterised by preparation of crystalline semicarbazones.

Stereochemically pure sample of cyperene (I) should give only one diketone. Secondly, it has been observed previously that the retention time of one of the components of dihydrocyperene mixture was identical with dihydro-rotundene*. The above observations suggested that cyperene used in previous investigations may not be analytically pure.

In spite of the apparent homogeneity of cyperene (TLC/GLC), it was considered essential to study the reactions more carefully.

Cyperene used in this investigation was obtained from Cyperus scariosus oil as previously described¹¹ (see experimental). Its IR spectrum is reproduced in Figure 1. Catalytic hydrogenation of cyperene in acetic acid over Adam's catalyst proceeded smoothly to yield the corresponding

*Rotundene has a different carbon skeleton and hence the identity of retention time on a GLC column may just be a coincidence.

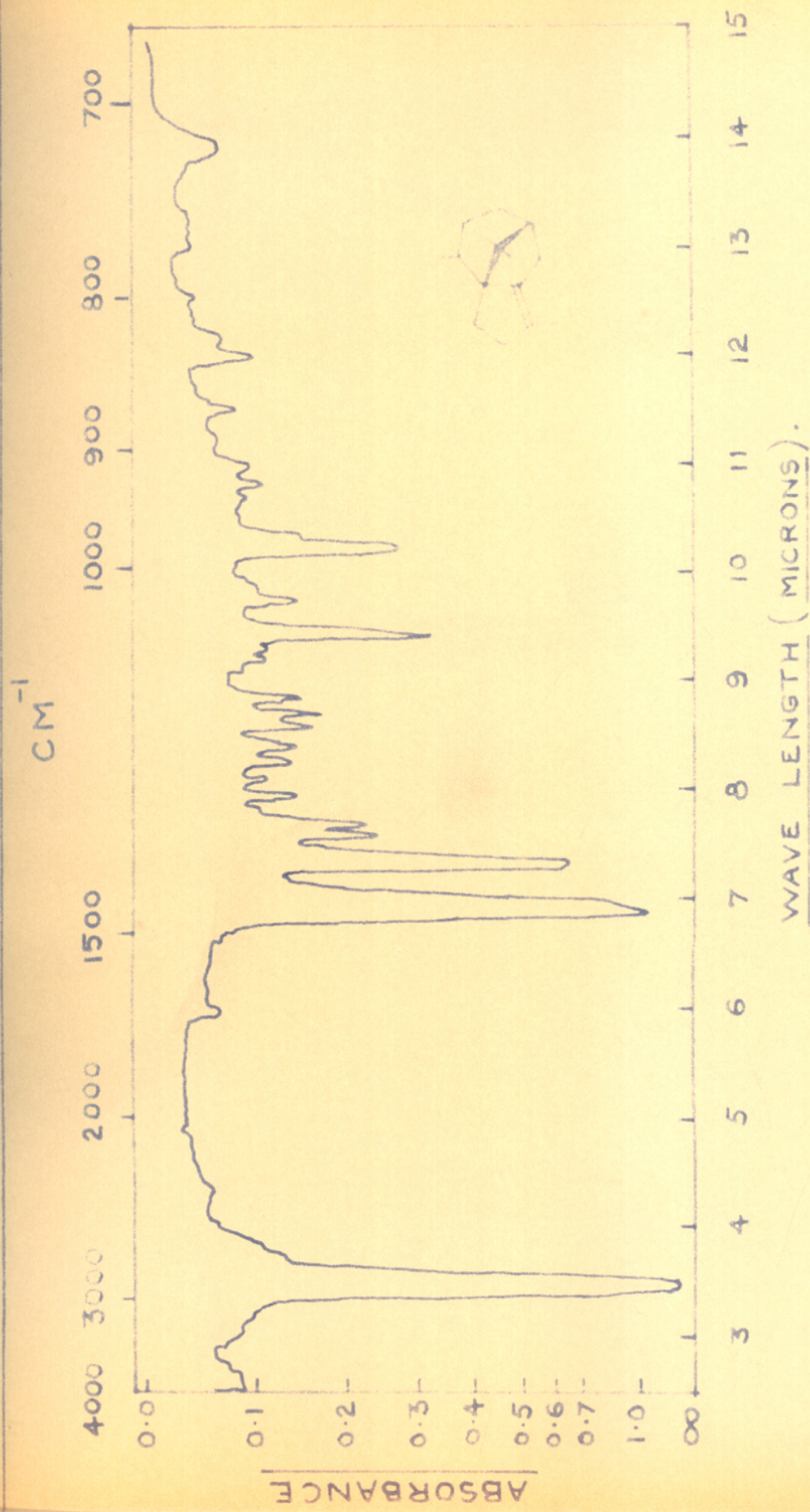


FIG. 1 IR SPECTRUM OF CYFERENE (LIQUID CELL).

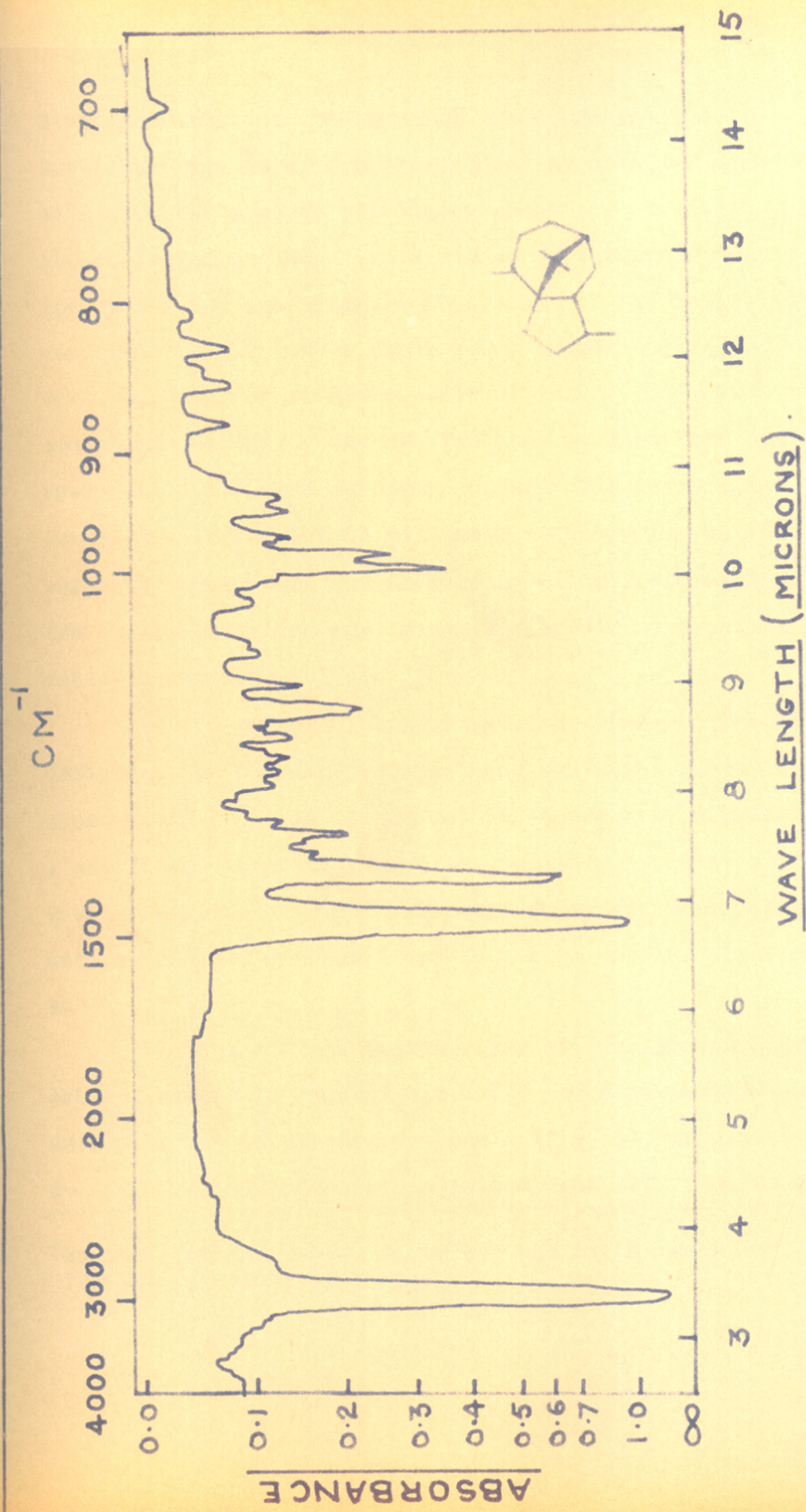


FIG. 2 IR SPECTRUM OF DIHYDRO CYPERENE (Liq. Cell).

dihydroderivative. The specific rotation and the IR spectrum (Fig. 2) of dihydrocyperene so obtained agreed well with the product previously prepared. However, the GLC analysis indicated the presence of three components (two major and one minor). Separation of the individual components could not be achieved by column/thin layer chromatography or by combination of both. Individual components (major) were then isolated in pure form by preparative GLC on a carbowax column*. The third minor constituent could not be collected. The purity of the separated components was checked by GLC on two columns. The two components which were separated by GLC were present in the ratio, approximately 1:3.

In the following discussion, the minor component is designated as dihydrocyperene-I and the major one as dihydrocyperene-II. While the IR and NMR spectra of dihydrocyperene I and II are strikingly similar, the mass spectra showed some characteristic differences. Thus, the mass spectral data on dihydrocyperene I and II can be used to distinguish them.

The IR, NMR and mass spectra of dihydrocyperenes I and II are reproduced in Figures 3,4,5,6,7 and 8 respectively. The mass spectrum of dihydrocyperene-I (Fig. 5) was proved to be identical with that of isopatchoulane (VI, Fig. 9) prepared

*We are thankful to Dr. G. Ohloff and Dr. F. Gautschi for the GLC analysis.

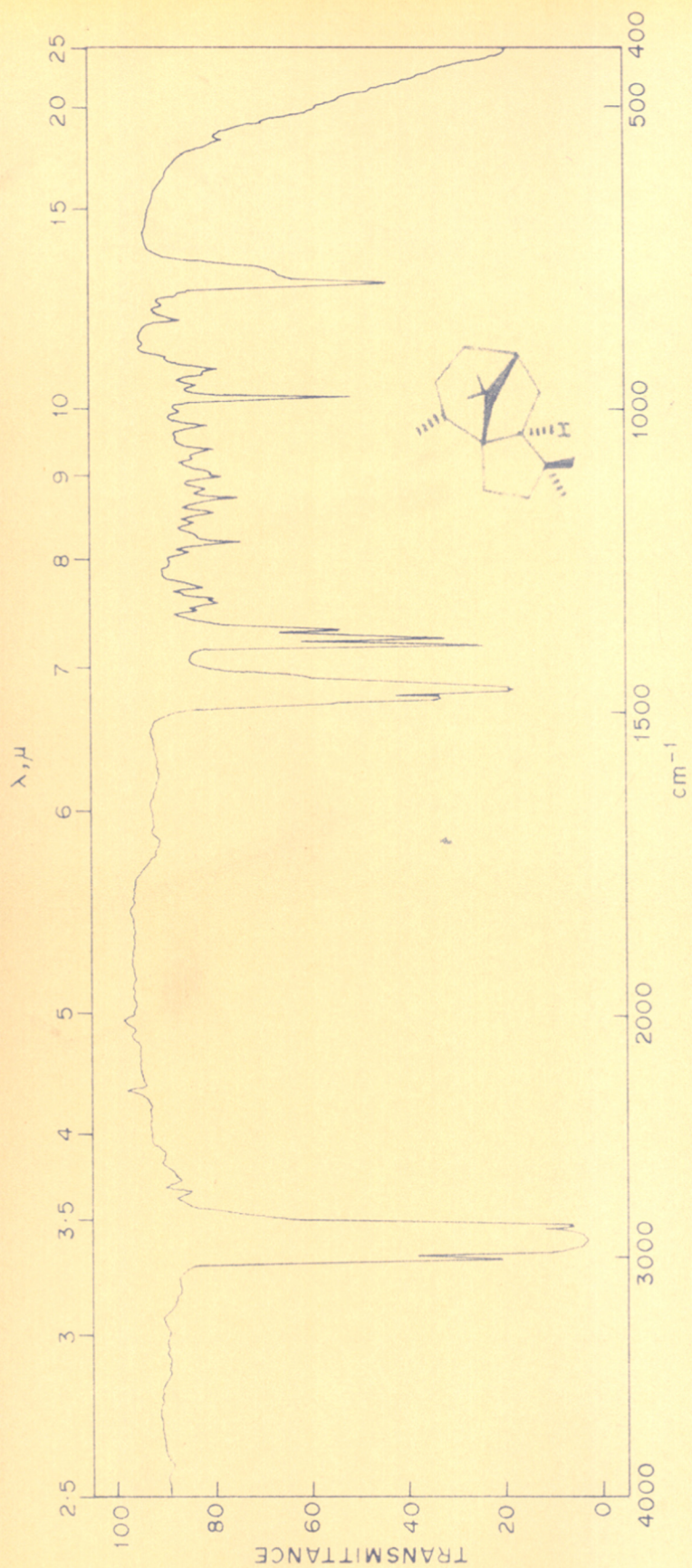
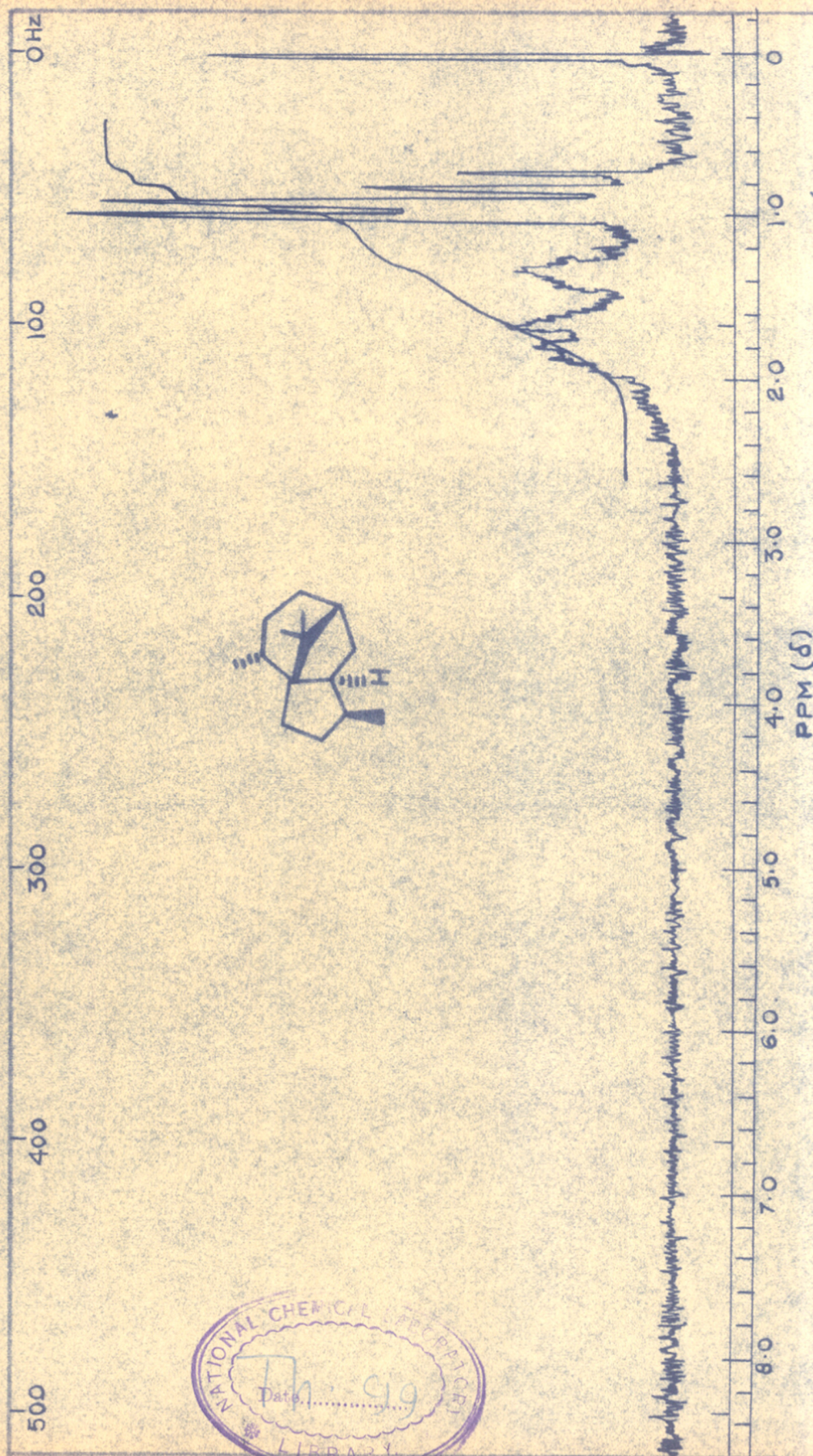


FIG. 3 IR SPECTRUM OF DIHYDROCYPERENE - I (liq. film)

FIG. 4 NMR SPECTRUM OF DIHYDROCYPERENE I (IN CCl_4).

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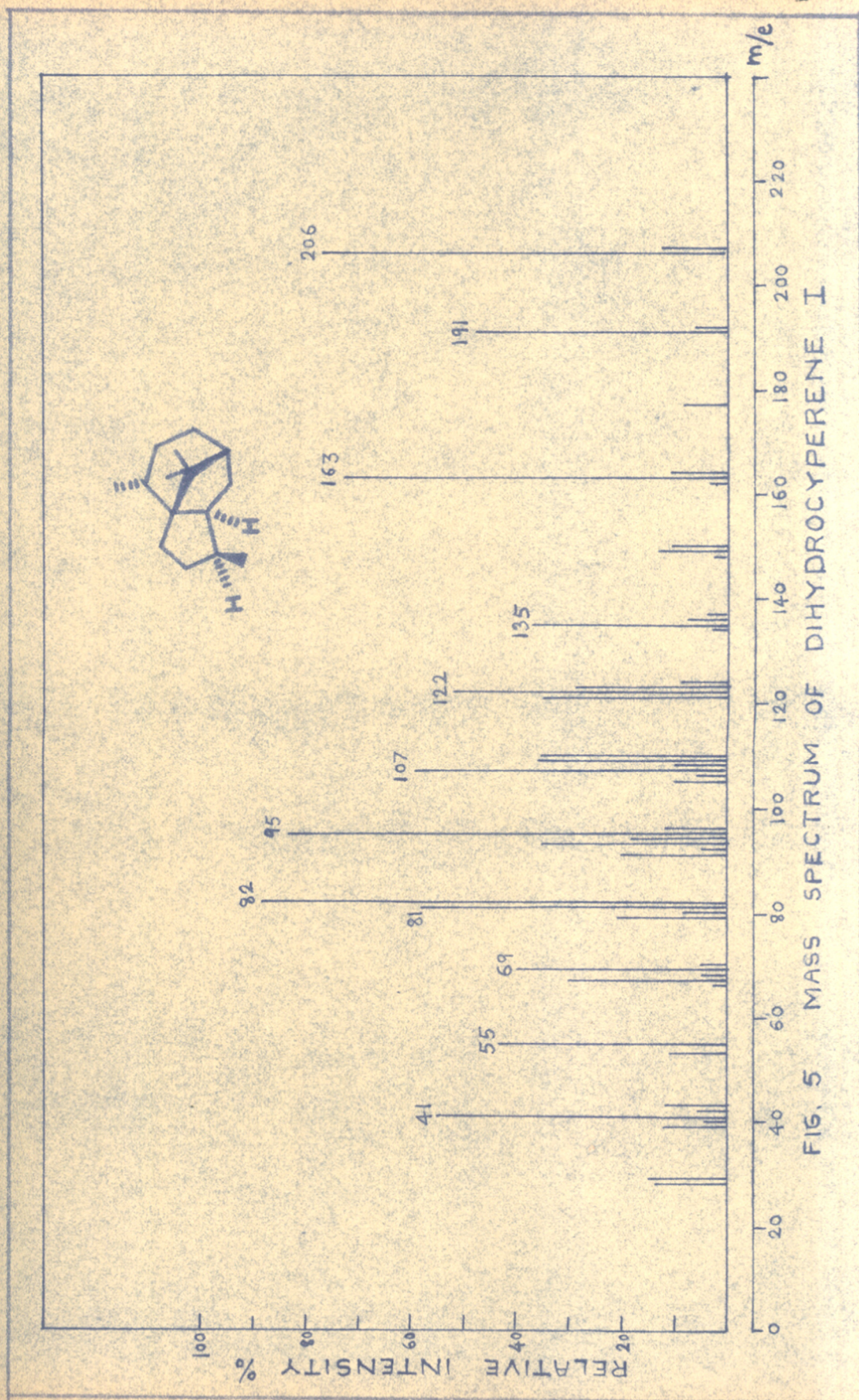


FIG. 5 MASS SPECTRUM OF DIHYDROCYPERENE I

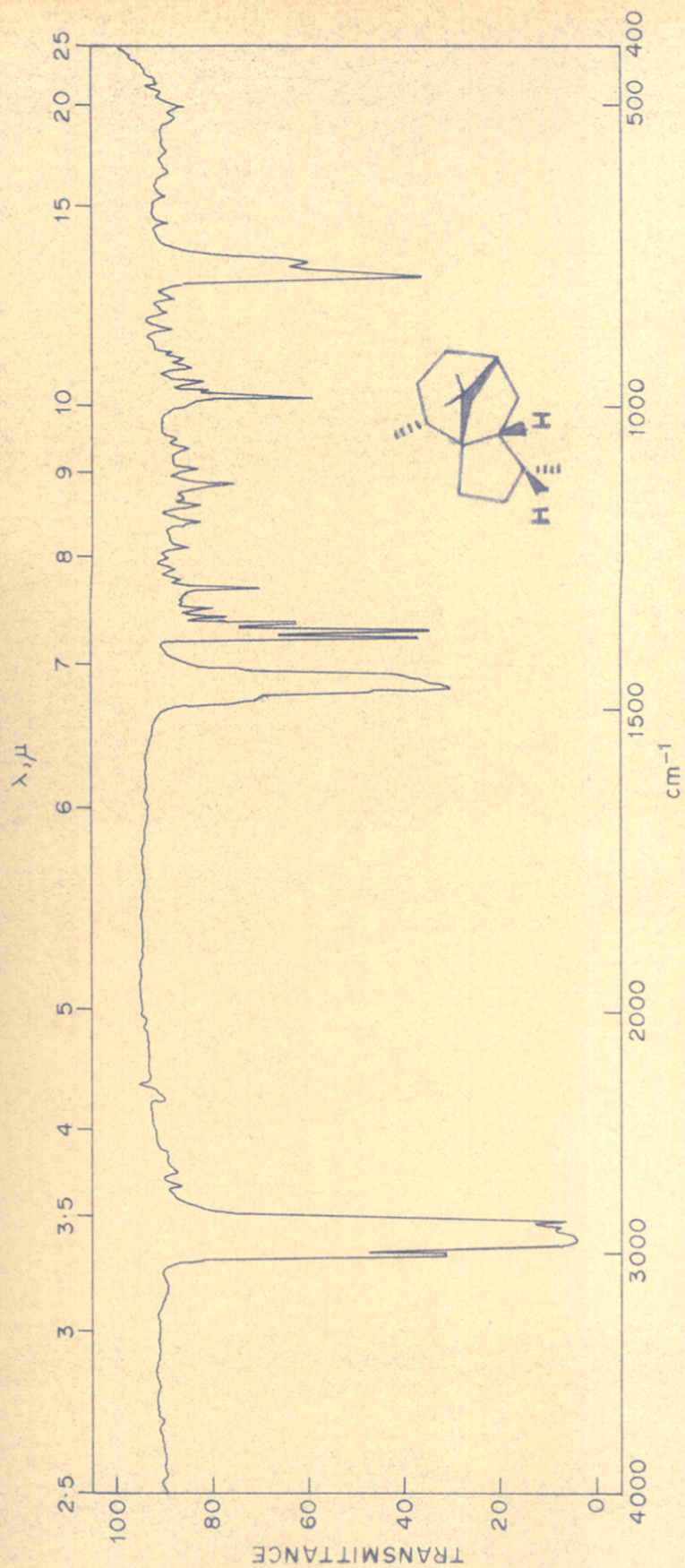


FIG.6 IR SPECTRUM OF DIHYDROCYPERENE - II (liq. film)

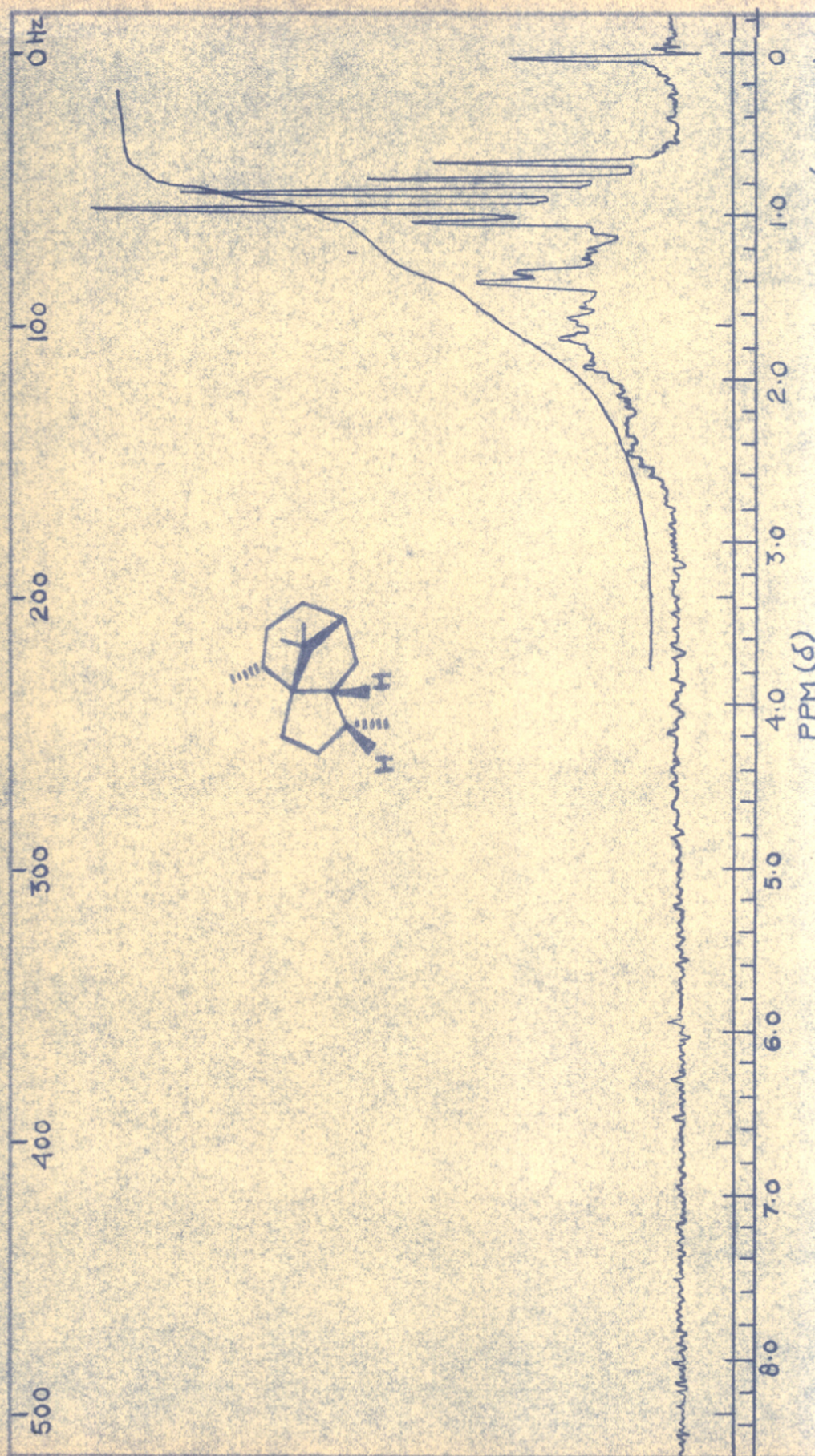
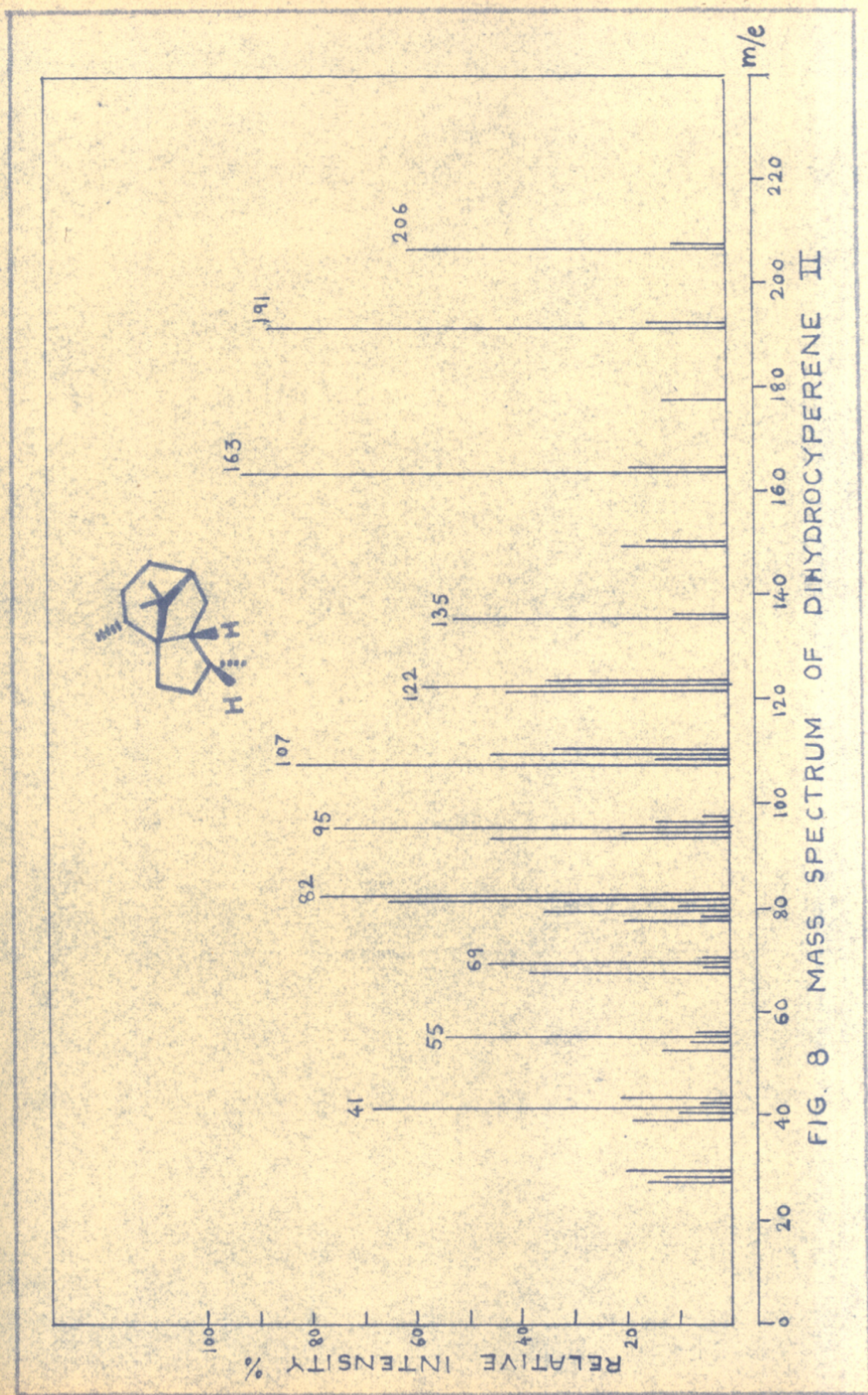


FIG. 7 NMR SPECTRUM OF DIHYDROCYPERENE II (IN CCl₄).



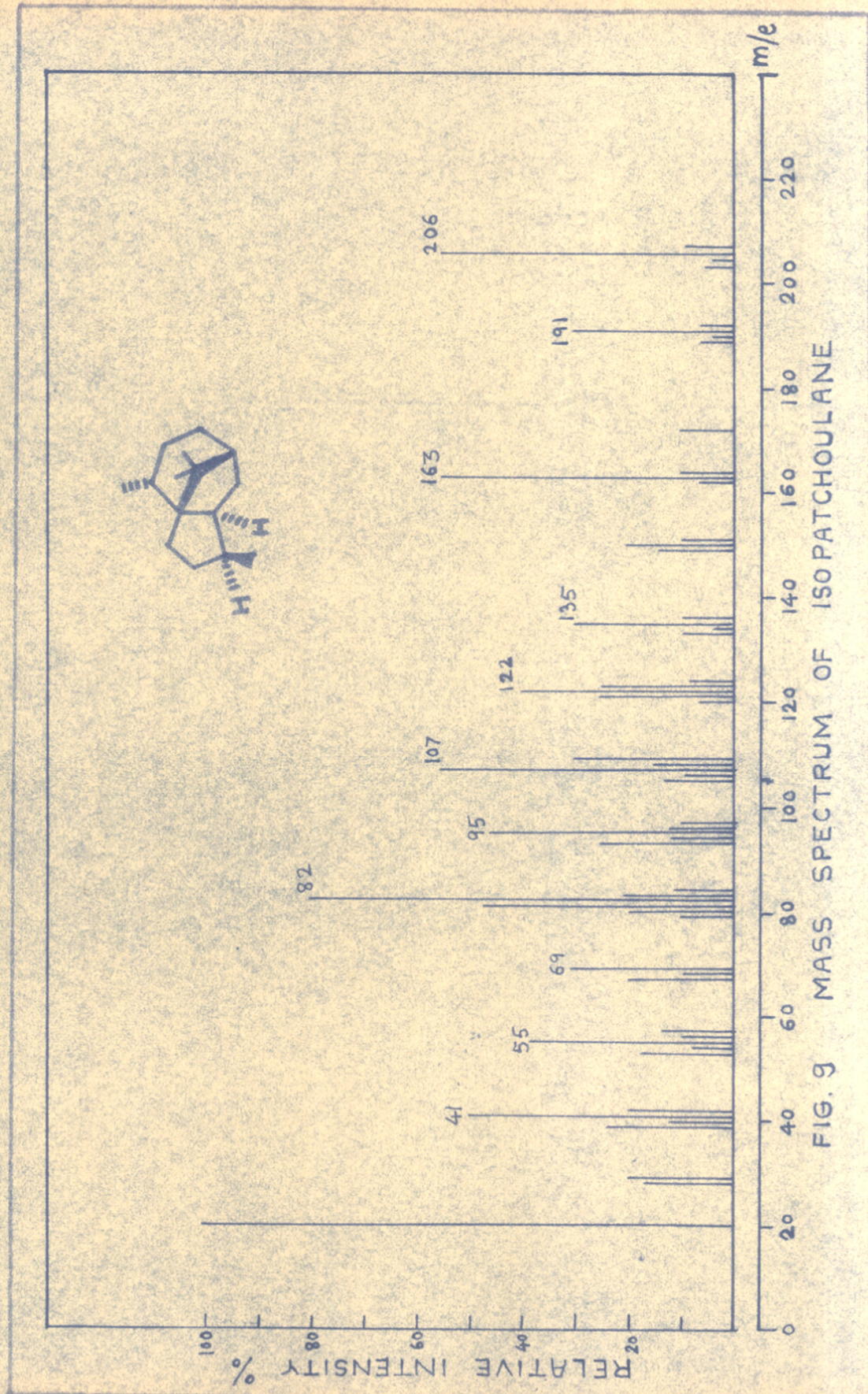


FIG. 9 MASS SPECTRUM OF ISOPATCHOULANE

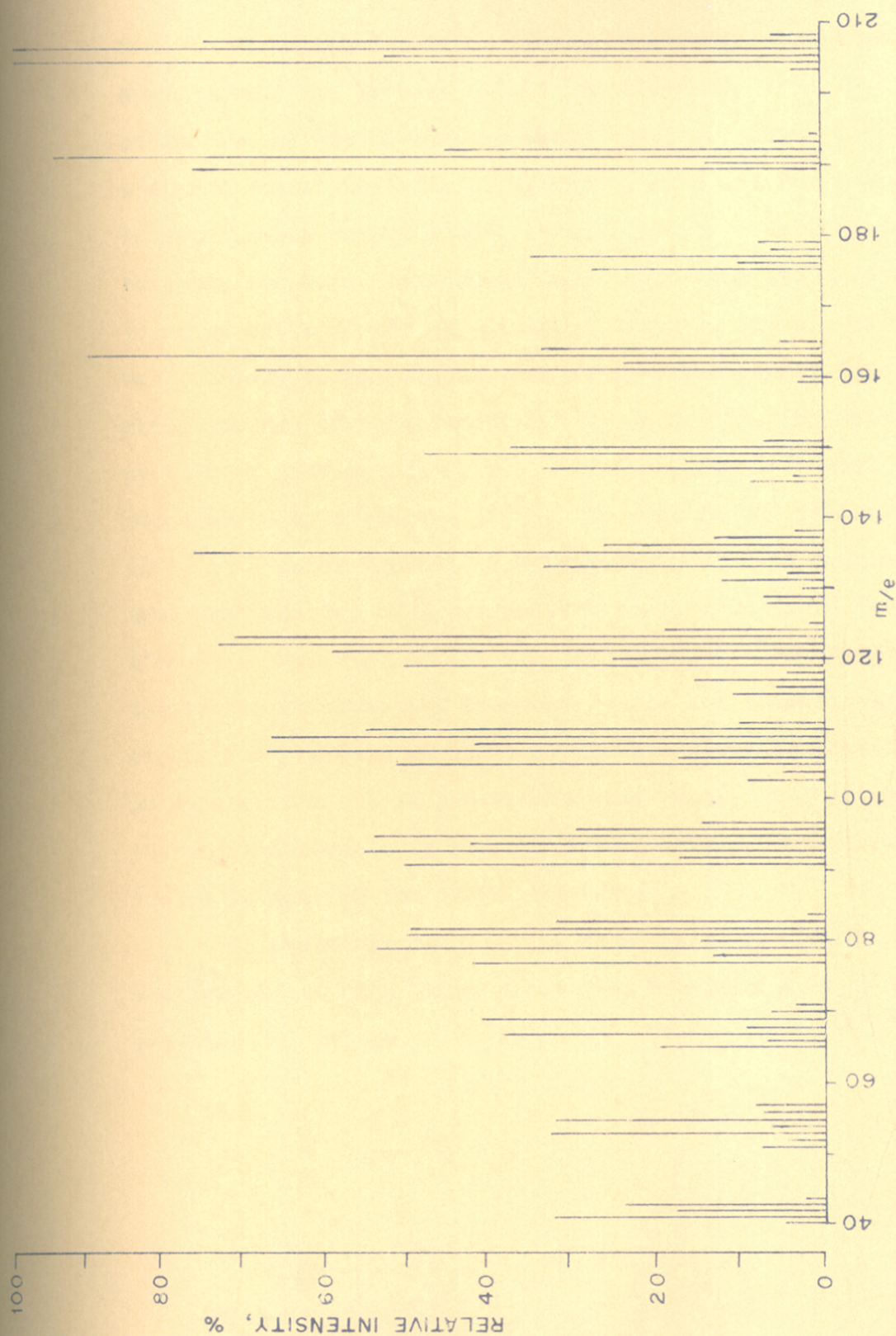


FIG. 10 MASS SPECTRUM OF THE HYDROGENATION PRODUCT OF CYPERENE
(MIXTURE OF THREE COMPONENTS)

from α and γ -patchoulene¹². Thus, if above conclusions are correct, the stereochemistry of dihydrocyperene-I is automatically established as shown in (VI). Since the assigned stereochemistry at C₄ and C₅ of α and γ -patchoulenes is established beyond doubt, it will not be unsafe to assume that the addition of hydrogen took place from the α -face (side opposite of the gem-dimethyl bridge). This observation was contrary to our expectation as dihydrocyperene-I is the minor product of hydrogenation. Consequently, the major product of hydrogenation, i.e. dihydrocyperene-II must be represented by structure (XII). To explain the formation of (XII) as a major product of hydrogenation, it is concluded that the addition of hydrogen took place from the β -face (from the same side of gem-dimethyl bridge). The reasons for the preferential attack from the β -face are immediately not clear. Examination of molecular models indicate that there is no appreciable steric hinderance from the β -face. It is therefore desirable to prepare (VI) and (XII) by different routes to confirm the above conclusions.

It should be pointed out that hydrogenation of α - and γ -patchoulenes also takes place from the same side as the gem-dimethyl bridge.

Isolation and structural features of the new sesquiterpene

It was mentioned earlier that the GLC analysis of the hydrogenated cyperene indicated the presence of three components. The mass spectroscopic molecular weight determination of the hydrogenated mixture indicated the presence of a substance having molecular formula $C_{15}H_{24}$ (M^+ 204, Fig.10). Since the hydrogenation of cyperene proceeds smoothly in acetic acid over platinum oxide catalyst, presence of an impurity which resists hydrogenation was considered. Unfortunately, this minor constituent could not be isolated by preparative GLC. A close examination of the GLC data on various cyperene fractions indicated the presence of a trace constituent, now designated as C. scariousus hydrocarbon-A (CSHY-A) eluting earlier than cyperene on a polyester column. It was considered that this hydrocarbon may be resistant to hydrogenation and hence responsible for the m/e 204 in the mass spectrum of the hydrogenated product (Fig. 10). Attempts were then made to isolate this substance (CSHY-A) by extensive chromatography over $AgNO_3$ impregnated silica gel. Its isolation was finally attained by this procedure, though the yield was extremely small. Its IR and mass spectra are reproduced in Figures 11 and 12. Comparison of the mass spectra of pure CSHY-A and hydrogenated cyperene clearly substantiated our earlier assumption that cyperene is contaminated with a trace impurity which is resistant to catalytic hydrogenation. This is reminiscent of β -patchoulene (XIII)⁹ and patchouli hydrocarbon 'E'

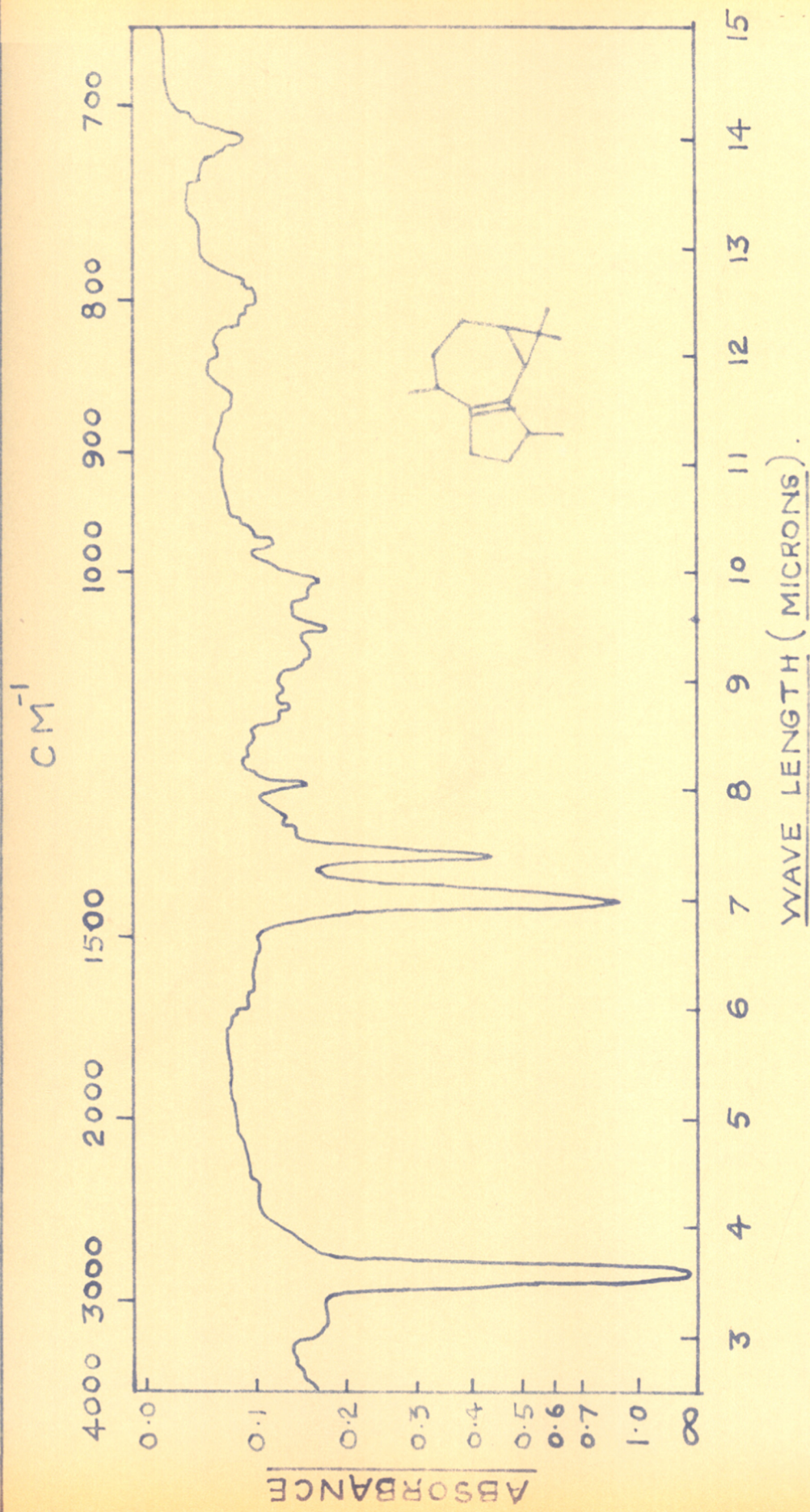
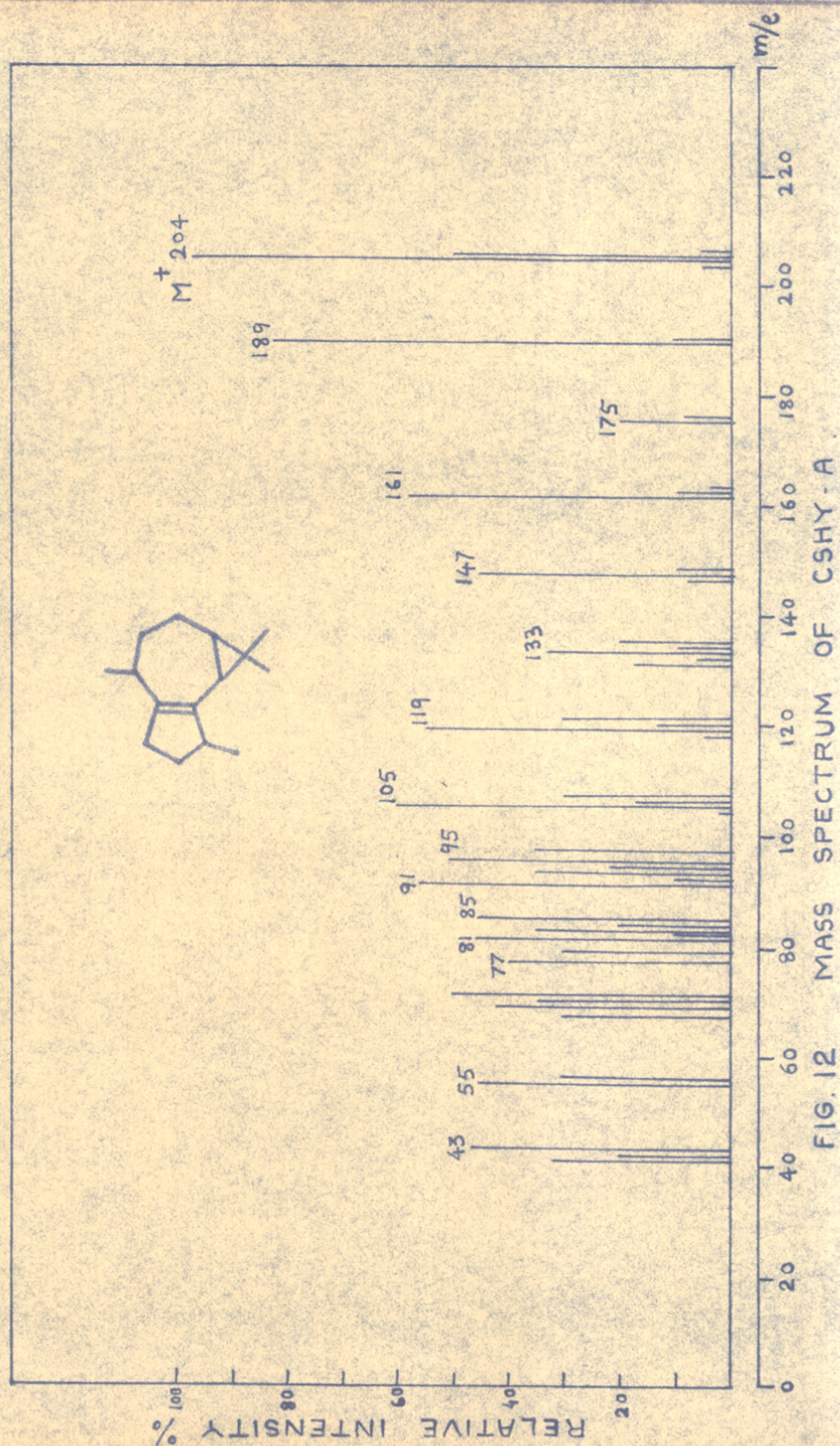
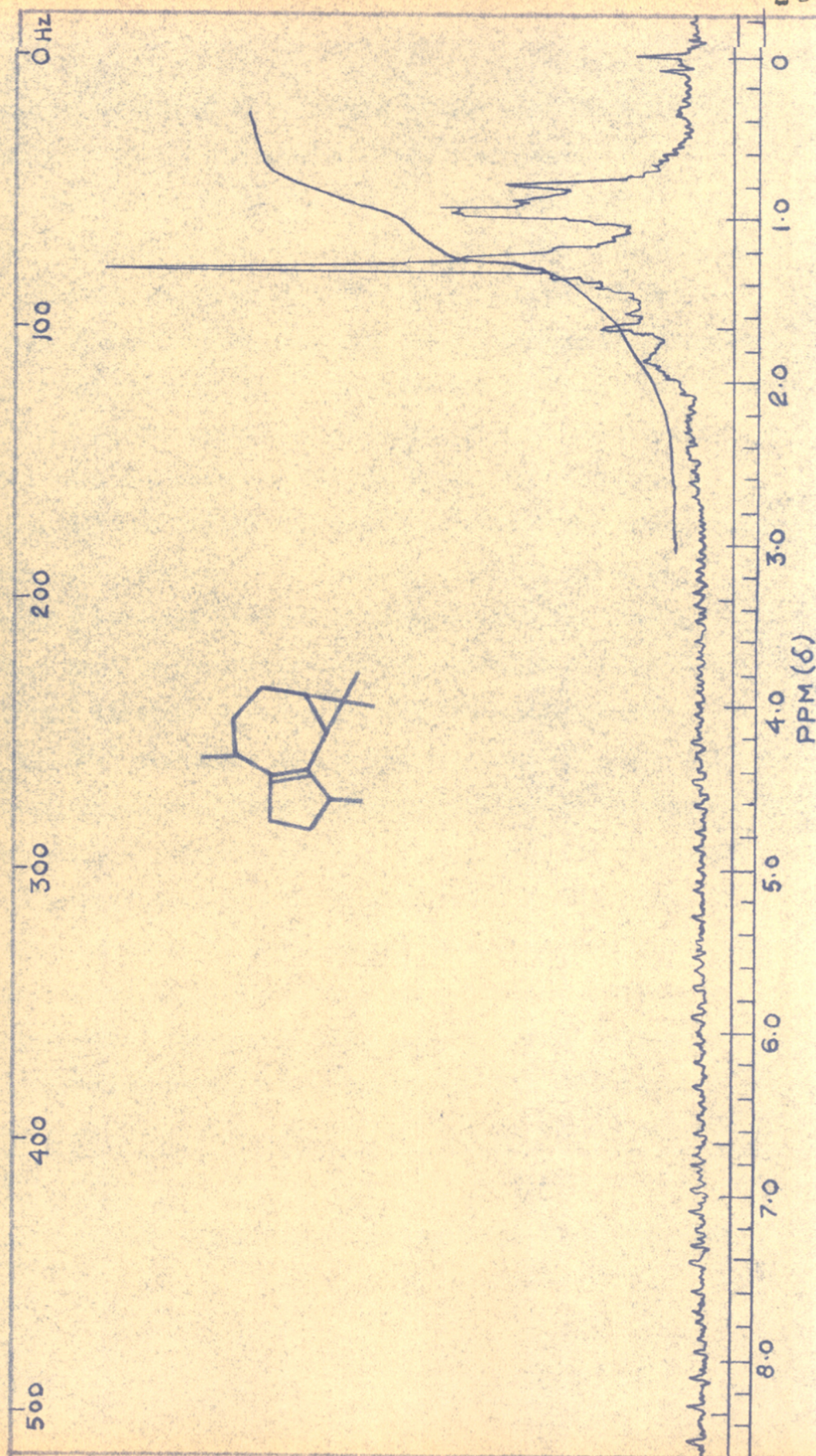


FIG. II IR SPECTRUM OF C. SCARIOSUS HYDROCARBON-A
(CSHY-A) Liq. Film.



FIG. 13 NMR SPECTRUM OF CSHY-A (IN CCl_4).

of unknown structure⁹. It seemed likely that CSHY-A might be identical with β -patchoulene (XIII) or patchouli-hydrocarbon 'E' of Tsubaki et al.⁹ Their non-identity was, however, evident on the basis of spectral data (IR, NMR, mass)*.

The NMR spectrum of CSHY-A (Fig. 13) showed the presence of two $-\text{CH}-\text{CH}_3$ and two $>\text{C}-\text{CH}_3$ groups and absence of any vinyl proton. In fact, there was no absorption above 1.5 μ . Due to the paucity of material further structural studies were not possible but the formation of the solid diketone (X) and liquid diketone** on ozonolysis of cyperene can now be explained. Solid diketone (X) is the only ozonolysis cleavage product of cyperene and the liquid diketone must be resulting from the ozonolysis of CSHY-A. The liquid diketone gives a crystalline semicarbazone m.p. 206-207° and shows in its NMR spectrum (Fig. 14) absence of $-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}-\text{CH}_3$ grouping. This conclusion was further supported by the negative haloform reaction. C. scariousus hydrocarbon-A must therefore contain a tetrasubstituted double bond. On the basis of spectral and chemical data discussed above and its co-occurrence with cyperene and rotundene a tricyclic structure (XIV) looks very attractive for this hydrocarbon.

However, it should be mentioned that this is only a tentative assignment and further work is highly desirable.

*We are thankful to Dr. Y. Hirose for supplying us with the spectral data on patchouli hydrocarbon 'E'.

**This experiment was repeated and both the diketones were obtained.

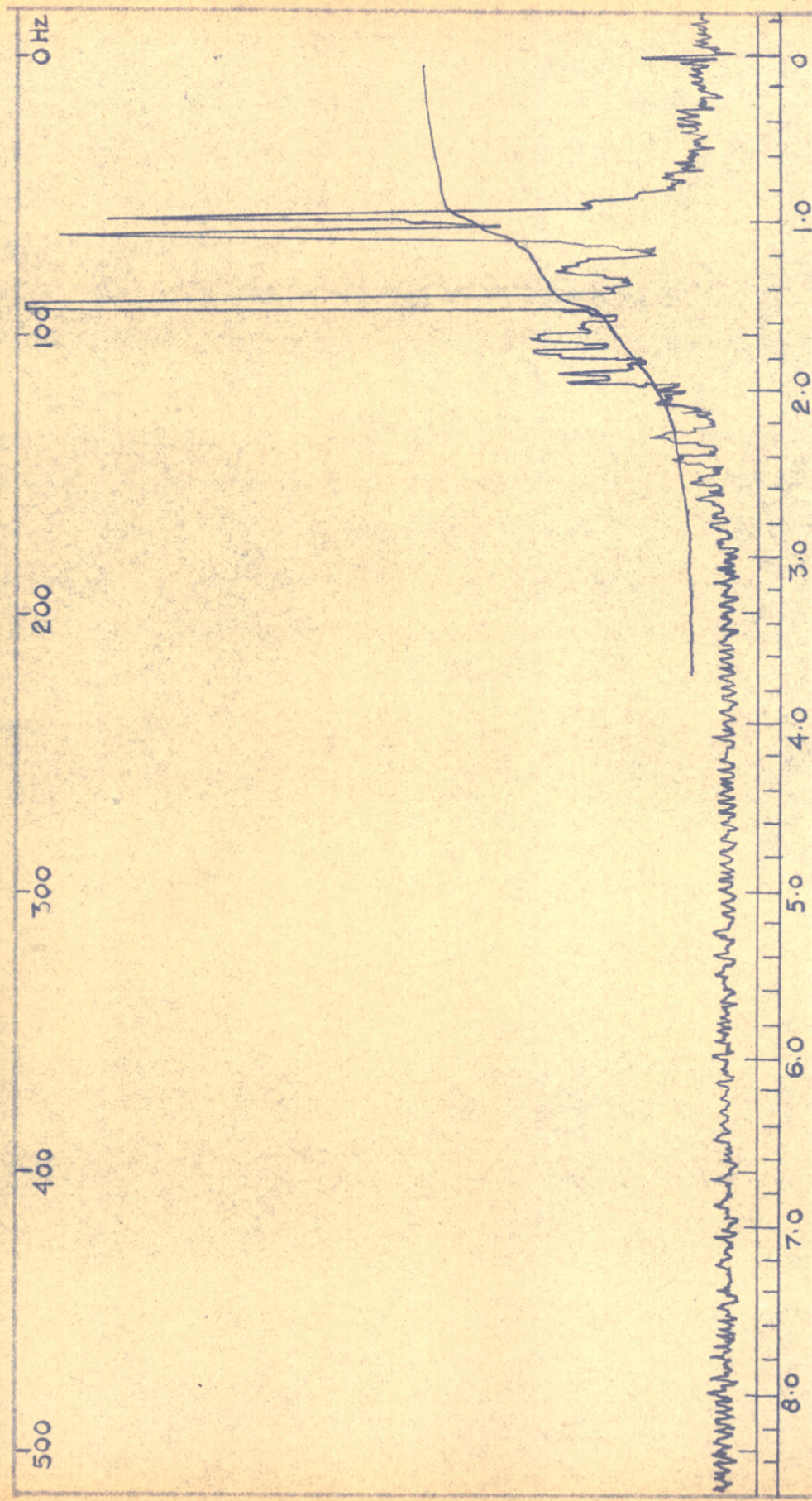
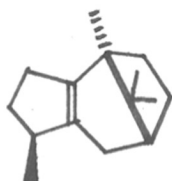
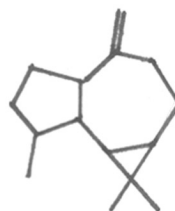
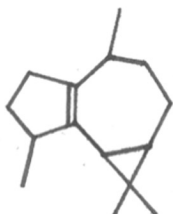
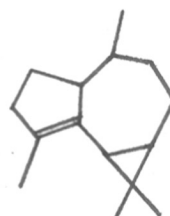


FIG. 14 NMR SPECTRUM OF LIQUID DIKOTONE (IN CCl_4).

It is of interest to note that while the natural occurrence of aromadendrene¹³ (XV) and α -gurjunene¹⁴ (XVI) is well established, there is no information about the natural existence of (XIV).

During the course of this investigation, rotundene could be obtained in pure form. Its IR and NMR spectra are reproduced in figures 15 and 16 respectively. Identity was established by direct comparison with an authentic sample. Catalytic hydrogenation gave a saturated dihydro derivative. The GLC retention time of dihydrorotundene was found to be identical with one of the components of dihydrocyperene. This was in complete agreement with earlier observations. However, this was just due to coincidence and not due to structural identity.

XIIIXVXIVXVI

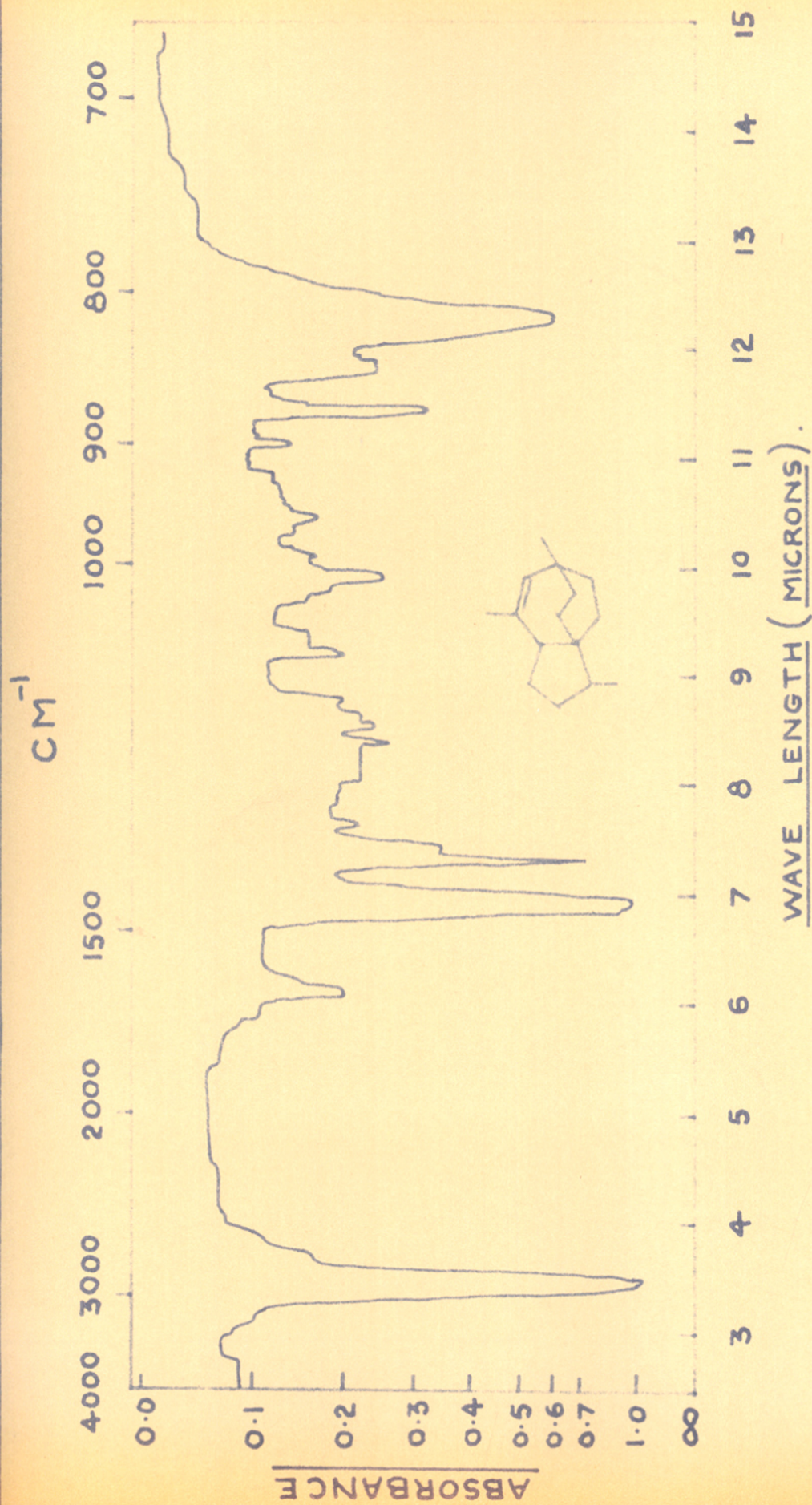


FIG. 15. IR SPECTRUM OF ROTUNDENE (LIQUID CELL).

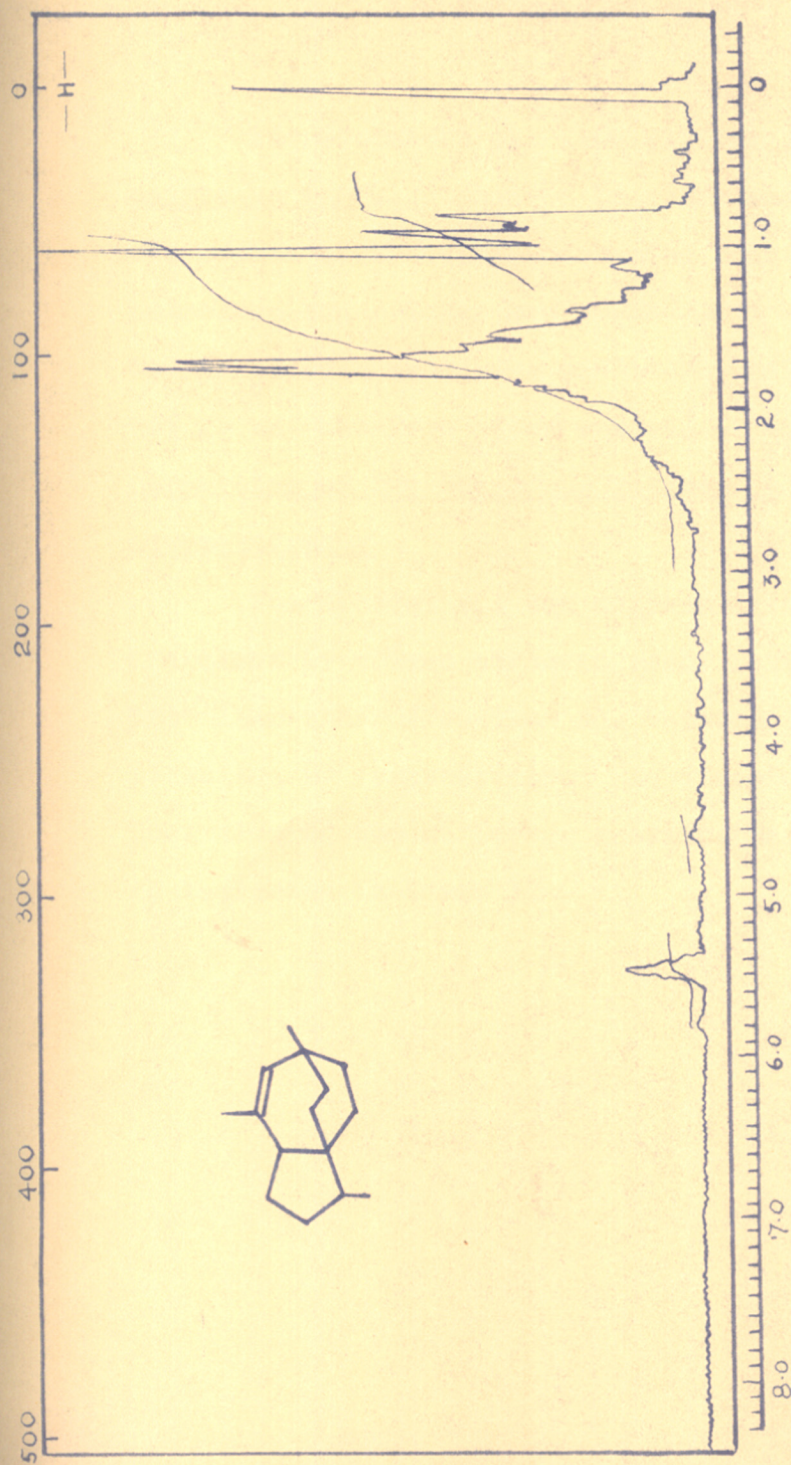
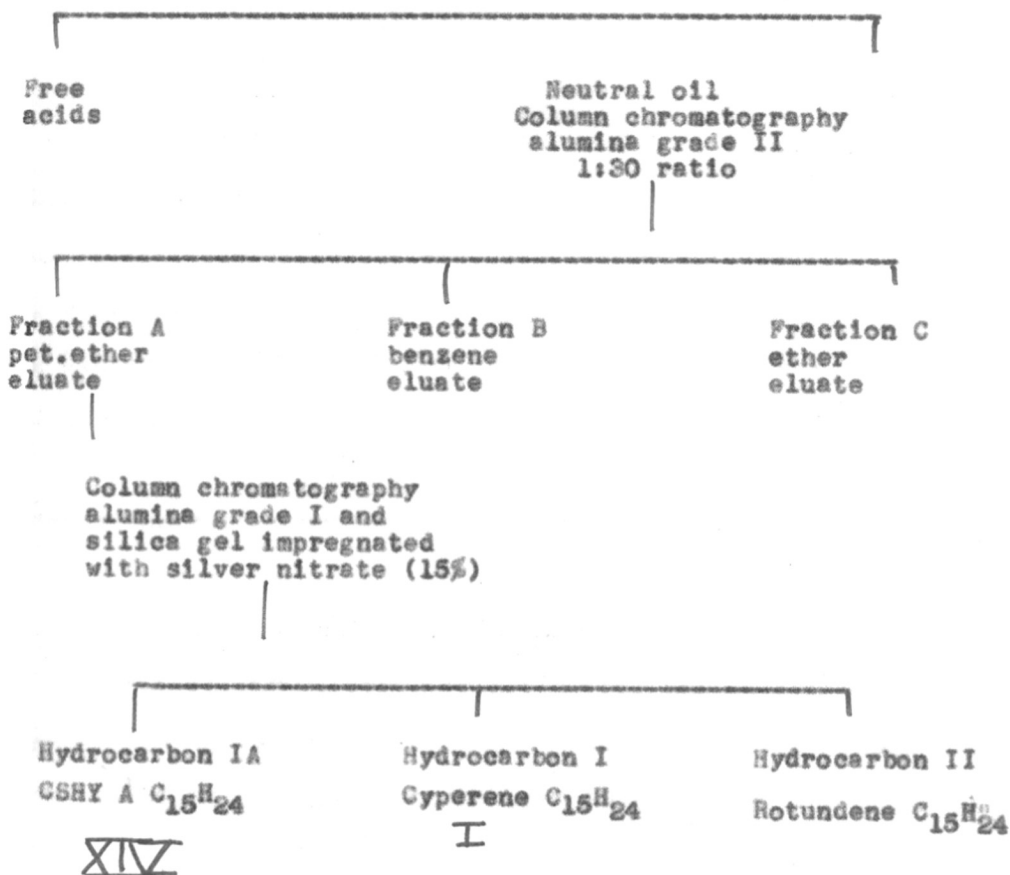


FIG. 16 NMR SPECTRUM OF ROTUNDENE (IN CCl₄).

EXPERIMENTAL

The essential oil (100 g) from Cyperus scariosus R.Br., dissolved in petroleum ether (500 ml), was washed with sodiumbicarbonate solution (10%). The petroleum-ether layer was washed with brine (2 x 50 ml) and dried over anhydrous sodium sulphate. The neutral oil (95 g) was obtained after removal of solvent by distillation. Separation of hydrocarbons, ketones and alcohols by column chromatography

The neutral oil was separated into three major fractions by column chromatography (Table 1), eluting with petroleum-ether (Fraction A), benzene (Fraction B) and ether (Fraction C) respectively. The procedure adopted for the isolation of the different constituents from these fractions is indicated in Chart 1.

CHART 1Cyperus scariousus oil

The acid free oil was chromatographed over neutral alumina (Gr. II) and the details of the chromatography are described below:

Table I

Weight of Neutral oil - 50 g

Weight of Neutral alumina Gr.II 1500 g

S.No.	Eluent	Vol. of Eluent (ml)	Weight of Fractions (g)
1.	Pet. ether A	2000	23.7920
2.	Benzene B	1000	15.3200
3.	Ether C	1000	8.5320
			47.6440

Recovery 92%

Isolation of Hydrocarbons Fraction A

The petroleum ether fraction from the column chromatography (Chart I and Table I) contained only hydrocarbons (GLC analysis) and was rechromatographed over neutral alumina. Several fractions were collected by eluting with petroleum ether.

Table II

Weight of Fraction A 20 g.

Weight of alumina Gr. I 1000 g.

Sr.No.	Eluent	Vol. of eluent (ml)	Wt. of fractions (g)	GLC of Hydrocarbons		
				IA	I	II
1.	Pet.ether	100 ml	2.3150	40 :	60 :	20
2.	"	"	6.7900	10 :	60 :	30
3.	"	"	4.5320		60 :	40
4.	"	"	2.0870		60 :	40
5.	"	"	1.8550			
6.	"	"	0.6400			
7.	"	"	0.4380		30 :	70
8.	"	1000 ml	0.9800			
			19.6370	Recovery 98.5%		

Hydrocarbon IA and Hydrocarbon I

The Petroleum ether fractions (No.1 and 2, Table II) were mixed and rechromatographed over silica gel impregnated with silvernitrate (15%).

Table III

Weight of Fraction	8 g.
Weight of silica gel impregnated with AgNO_3 (15%)	400 g.

SNo.	Eluent	Volume of eluent (ml)	Wt. of fraction (g)	GLC of hydro- carbons		
				IA	I	II
1.	Pet.ether	50	0.0650	100%		
2.	"	50	1.3200	10 : 90		
3.	"	100	3.1050	100		
4.	"	100	2.0680	10 : 90		
5.	"	100	1.3200	10 : 90		

7.8780

Recovery 99%

The fractions No.1 and No.3 (Table III) were separately distilled over sodium under reduced pressure and hydrocarbon IA and hydrocarbon I were obtained
Hydrocarbon II

The petroleum ether fraction No.4 (Table III) was chromatographed over silica gel impregnated with silver-nitrate (15%).

Table IV

Weight of Fraction 2 g.
Weight of silica gel
impregnated with AgNO₃(15%) 100 g.

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fraction (g)	GLC of hydrocarbon	
				I	II
1.	Pet. ether	25	0.5023	5 :	95
2.	"	25	0.8902		100
3.	"	25	0.2030	1	
4.	"	50	0.0600	2 :	98
5.	"	50	0.0500		
6.	"	100	0.2500		

1.9555

Recovery 98%

The fraction No.2 (Table IV) was distilled over sodium under reduced pressure when hydrocarbon II was obtained.

Hydrocarbon I $C_{15}H_{24}$ (cyperene) I

The hydrocarbon I had the following properties and was shown to be identical with cyperene (I)

b.p. 95-96°(bath)/2 mm

$(\alpha)_D^{23}$ - 19.29° (c, 4.0)

Mol. wt. - 204 (Mass spectrum)

Analysis

Found: C, 88.30; H, 11.80%

Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84%

IR band (Fig. 1, liquid film) 1699, 1389, 1366, 1333, 1316, 1269, 1250, 1222, 1205, 1181, 1163, 1141, 1070, 1037, 988, 934, 892, 877 and 832 cm^{-1} .

Hydrogenation of cyperene

Cyperene (500 mg) dissolved in glacial acetic acid (30 ml) was stirred in an atmosphere of hydrogen with Adam's platinum oxide catalyst (60 mg). The equivalent of one mole of hydrogen was absorbed in 30 minutes. The catalyst was filtered and the solvent was removed under vacuum. The hydrogenated product was taken up in ether and washed with water to remove traces of acetic acid. The ether extract was dried over anhydrous sodium sulphate and solvent was removed under vacuum. The product on chromatography over alumina Gr. I followed by vacuum distillation gave dihydrocyperene having the following properties.

b.p. 93-94° (bath)/2 mm, $(n_D^{26}) - 50.7^\circ$

(c, 3.7), GLC*. Two peaks in 1:3 ratio. Mol. weight 206 (mass spectrum Fig. 10).

Analysis

Found: C, 87.40; H, 12.68

Calculated for $\text{C}_{15}\text{H}_{26}$: C, 87.30; H, 12.70%

IR bands (Fig. 2, liquid film) 1460, 1387, 1360, 1342, 1312, 1294, 1251, 1205, 1178, 1139, 1114, 1076, 995, 982, 965, 948, 930, 921, 893, 880 and 852 cm^{-1} .

*Dr. Ohloff and Dr. F. Gutschi have confirmed the presence of three components (two major and one minor by GLC from hydrogenated cyperene from our sample.

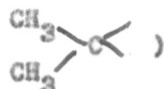
The dihydrocyperene stereoisomers (GLC two peaks in 1:3 ratio) could not be separated by column chromatography. The two ~~pan~~ isomers were separated by preparative GLC*. The two stereoisomers (dihydrocyperene I and dihydrocyperene II) showed the following properties.

Dihydrocyperene I $C_{15}H_{26}$ VII

Mol. weight 206 (mass spectrum Fig. 5)

IR bands (Fig. 3 liquid film) 1475, 1460, 1385, 1360, 1330, 1290, 1205, 1170, 1145, 1110, 1064, 1025, 975, 930, 860, and 780 cm^{-1} .

NMR spectrum (Fig. 4)

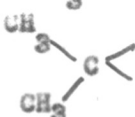
δ 0.71 and δ 0.82 (2 CH_3 - )
 δ 0.91 and δ 0.95 (6H, )

Dihydrocyperene II $C_{15}H_{26}$ XII

Mol. weight 206 (mass spectrum, Fig. 8)

IR bands (Fig. 6, liquid film) 1475, 1460, 1385, 1360, 1330, 1290, 1205, 1170, 1145, 1110, 1064, 1025, 975, 930, 860 and 780 cm^{-1} .

NMR spectrum (Fig. 7)

δ 0.71 and δ 0.82 (6H 2 CH_3 - )
 δ 0.91 and δ 0.99 (6H, )

Ozonolysis of cyperene solid diketone $C_{15}H_{24}O_2$

A stream of dry ozone was passed into a solution of cyperene (1 g) in ethyl acetate (50 ml) cooled to 0°C. The solution turned yellow after 30 minutes. Ethyl acetate was removed under reduced pressure and the ozonide was decomposed

by refluxing with distilled water (25 ml) for four hours and extracted with ether (4 x 50 ml). The combined ether extract was washed with sodium hydroxide solution (5%) to remove any acid formed during ozonolysis. The neutral portion was dried and evaporation of solvent yielded a colourless crystalline compound (860 mg). It was recrystallised from pet. ether, m.p. 77° . The filtrate was preserved for isolation of liquid diketone.

Analysis

Found: C, 75.90; H, 9.84%

Calculated for $C_{15}H_{24}O_2$: C, 76.54; H, 10.30%.

Solid diketone

IR spectrum (nujol) 1724, 1412, 1329, 1300, 1245, 1224, 1190, 1171, 1157, 1105, 1075, 1058, 1031, 980, 972, 943, 840 and 790 cm^{-1} .

NMR spectrum (in CCl_4)

δ 0.72 (3H, $\text{J} = 6\text{ cps } CH_3 - C \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix} H$), δ 0.94 (3H, $CH_3 - C \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$). δ 1.16 (3H $CH_3 - C \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$), δ 2.07 (3H, $CH_3 - CO -$) and δ 1.94 (2H, $-CH_2 - CO -$).

Semicarbazone derivative of solid diketone ($C_{17}H_{30}O_2N_6$)

A solution of semicarbazide hydrochloride (500 mg) and sodium acetate (700 mg) in distilled water was added to a solution of solid diketone (200 mg) in petroleum ether.

The mixture was turbid and alcohol was added till solution was clear. The solution was shaken and warmed on water bath and allowed to cool in ice bath. Solid

crystals of semicarbazone derivative (380 mg) was separated. It was crystallised from petroleum ether, m.p. 211° .

Analysis

Found: N, 23.88%

Calculated for $C_{17}H_{30}O_2N_6$ = N, 23.98%.

Liquid diketone $C_{15}H_{24}O_2$

The filtrate from the crystallisation of solid diketone gave a gum which was chromatographed over silica gel and eluted with pet.ether, benzene ethyl acetate and ether respectively.

The details of chromatography are shown below:

Weight of liquid gum	125 mg.
Weight of silica gel	100 g.

S.No.	Eluent	Volume of Eluent (ml)	Wt. of fraction (g)	TLC
1.	Pet. ether	200	-	
2.	Pet.ether benzene (50:50)	200	-	
3.	Benzene	200		
4.	Benzene-ethylacetate (80:20)	200	0.0425	two spot
5.	Benzene-ethylacetate (50:50)	200	0.0310	single spot
6.	Benzene ethyl acetate (20:80)	200	-	
7.	Ethyl acetate	200	-	
8.	Ether	200	0.025	

The liquid diketone obtained from the fraction No.5 after removal of solvent showed single spot on TLC.

Analysis

Found: C, 75.60; H, 9.70%

Calculated for $C_{15}H_{24}O_2$ C, 76.54; H, 10.3%

IR band (liquid film) 2950, 1720, 1470, 1420, 1400, 1360, 1340, 1290, 1240, 1165, 1100, 1075, 1030, 1010, 990, 960 and 830 cm^{-1} .

NMR spectrum (Fig. 14)

δ 0.94 and δ 1.06 (3H, each 2 CH_3 - $\overset{CH}{\text{C}}$)

δ 1.48 (6H, $\overset{CH_3}{\text{C}} \diagup \text{C} \diagdown \overset{CH_3}{\text{C}}$). The signal for methyl ketone ($-\overset{O}{\underset{||}{C}}-CH_3$) was absent.

Semicarbazone derivative of liquid diketone ($C_{17}H_{30}O_2N_6$)

A solution of semicarbazide hydrochloride (300 mg), sodiumacetate (500 mg) in distilled water (10 ml) was added to the solution of liquid diketone in benzene. The solution was turbid and alcohol was added to make the solution clear. The solution was shaken and warmed on water-bath and allowed to cool in ice bath. Solid crystalline semicarbazone derivative separated. It was recrystallised from pet.ether m.p. 206-207°.

Analysis

Found: N, 22.80%

Calculated for $C_{17}H_{30}O_2N_6$ N, 23.98%

Hydrocarbon IA $C_{15}H_{24}$ (CSHYA) XIV

A new sesquiterpene hydrocarbon isolated from the Cyperus scariosus oil (Table III Fraction No.1) was distilled over sodium. It showed the following properties:

b.p. $104-105^{\circ}$ (bath)/2 mm

Mol. weight - 204 (mass spectrum, Fig. 12)

Analysis

Found: C, 88.30, H, 11.80%

Calculated for $C_{15}H_{24}$: C, 88.16, H, 11.84%

*

Hydrogenation of CSHY-A

Hydrocarbon CSHY-A (20 mg) dissolved in glacial acetic acid (20 ml), was stirred in on atmosphere of hydrogen with Adam's platinum oxide (30 mg) catalyst for 30 minutes. It was observed that there was no uptake of hydrogen. The catalyst was filtered and solvent was removed under vacuum. The product was taken up in ether and ether extract was washed with water and dried over anhydrous sodium sulphate. After removal of solvent, the product (18 mg) was found to be the starting material. Hydrocarbon CSHY-A, resist catalytic hydrogenation.

Hydrocarbon II $C_{15}H_{24}$ (Rotundene)

A sesquiterpene hydrocarbon isolated from cyperus scariosus oil (Table IV fraction No.2) was distilled over sodium. It showed the following properties

b.p. 122° (bath)/2 mm

Mol. weight 204 (mass spectrum)

IR bands (liquid film, Fig. 11) 2860, 1465, 1385, 1270 and 1070 cm^{-1} .

NMR spectrum (Fig. 13) δ 0.77 and δ 0.9 (6H, CH_3 C)

δ 1.28 (6H, $2CH_3-CH$) CH_3

Analysis

Found: C, 88.05; H, 11.9%

Calculated for $C_{15}H_{24}$: C, 88.10; H, 11.84%

IR band (Fig. 15 liquid film) 2860, 1630, 1460, 1440, 1340, 1320, 1205, 1190, 1155, 1070, 1020, 965, 880 and 815 cm^{-1} .

NMR spectrum (Fig. 16)

δ 0.85 (3H, CH_3-CH) δ 0.99 (3H CH_3-C)
 δ 1.7 (3H $CH_3-CH=CH-$) δ 5.45 $C=CH-$

Dihydrorotundene ($C_{15}H_{26}$)

Rotundene (50 mg) was hydrogenated in ethyl alcohol (10 ml) with platinum oxide (40 mg) as catalyst at room temperature. After the uptake of one mole hydrogen (1/2 hour), the solution was filtered and the filtrate was evaporated to give dihydro product (49 mg). The product was chromatographed over alumina (Gr. I) when dihydrorotundene was obtained.

Analysis

Found: C, 87.34; H, 12.8%

Calculated for $C_{15}H_{26}$: C, 87.3; H, 12.7%

IR bands (liquid film) 1380, 1320, 1270, 1165, 1160, 1130, 1110, 1020, 855, 800, 770 and 750 cm^{-1} .

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Chapter-II

**Isolation & Characterization of
Hydrocarbons From**

a] Singapdre Patchouli Oil

b] Gurjun Balsam Oil

Sesquiterpene hydrocarbons of Singapore patchouli oil

Commercial patchouli oil is mainly obtained from the leaves of Pogostemon cablin Benth (Syn. P. patchouli Pallet var. suavif Hook)¹ belonging to the family labiateae. The oil is one of the most important raw material for composition of fancy perfumes. The oil contains a large percentage of sesquiterpene compounds² viz. β -patchoulene (I), α -guaiene (II), α -bulnesene (III) and patchouli alcohol (IV). Recently, a detailed analytical study of patchouli oil has been carried out³. The chemical composition and biogenetic relationship of patchouli oil constituents are shown in Charts I and II respectively. Besides the already known constituents^{4,5,6} (I to IV), β -elemene (V), caryophyllene (VI), α -patchoulene (VII), alloaromadendrene (VIII), α -gurjunene (IX) and two new hydrocarbons patchouli HC-E and patchouli HC-G have been characterised. A new sesquiterpene hydrocarbon, seychellene (X) has been isolated by Ourisson and co-workers⁷ and shown to be identical with patchouli HC-G of Tsubaki et al. Among sesquiterpene alcohols, besides the well-known constituent of patchouli oil, patchouli alcohol (IV), a bicyclic tertiary alcohol, pogostol (XI) has been isolated by Hikino et al.⁸ Very recently Henderson et al.⁹ have described techniques for the microanalysis of free volatile sesquiterpenes by direct loading GLC.

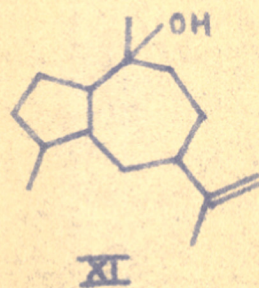
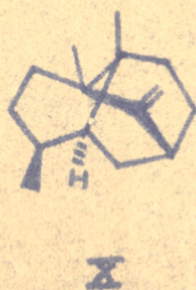
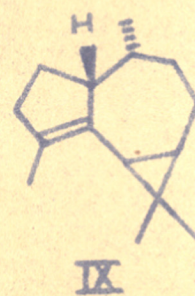
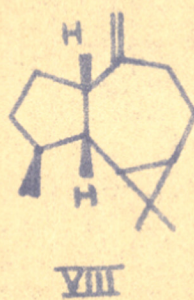
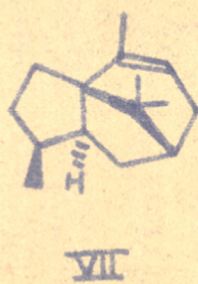
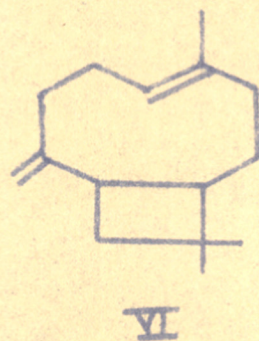
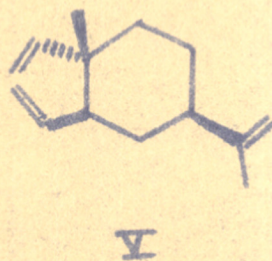
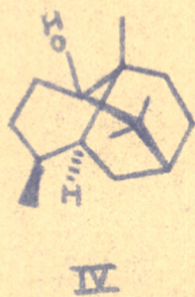
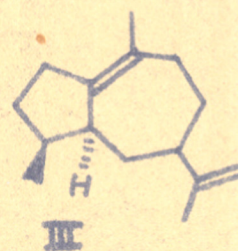
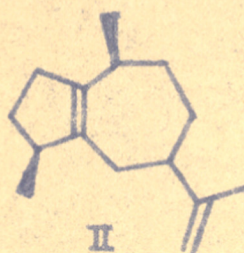
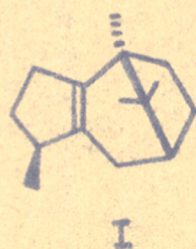


CHART - I

Present Study

Isolation of rotundene and rotundenol having an unknown skeleton was reported from this laboratory¹⁰. On the basis of the chemical and spectral data collected so far, it was considered that the above sesquiterpenes could be correlated with pogostol (XI). After screening few commercially available samples of patchouli oil, it was found that Singapore patchouli oil contains the desired substance. Singapore patchouli oil (Pogostemon cablin Benth) was therefore processed by the usual techniques. During this study a complete analysis of the hydrocarbon fraction has been carried out and the details are presented in this chapter. The chromatographic tail fractions contains pogostol (XI), which is being processed further*

Isolation of hydrocarbons

Singapore patchouli oil was procured from a local dealer (Amrut Industrial Products), Bombay. IR spectrum of the oil (Fig. 1) shows the presence of hydroxyl and carbonyl groups. The oil was freed from acidic components by standard procedure and the neutral oil was resolved into three main fractions (fractions A,B,C) by column chromatography over alumina (activity III). The IR spectra of fractions A,B and C are

*The correlation of pogostol with rotundene is in progress. This work is being carried out by another research worker and hence omitted in the following discussion.

reproduced in figures 2,3,4 respectively. The petroleum-ether eluted fraction 'A' was further subjected to repeated chromatography over alumina and silica gel impregnated with silvernitrate.

The procedure adopted for the isolation of individual components of fraction 'A' is outlined in Chart III (see experimental). The course of separation was monitored by GLC and TLC on silica gel and silvernitrate impregnated silica gel plates.

During the present study, seychellene (X), α -gurjunene (IX), β -patchoulene (I) have been isolated in pure form and identified by direct comparison of spectral data (IR, UV, NMR, Mass) and physical constants with authentic samples.

While the presence of the above hydrocarbons in Singapore patchouli oil could be established beyond doubt, presence of caryophyllene (VI), β -elemene (V), α -guaiene (II), α -bulnesene (III) is suggested on the basis of spectral data on certain chromatographic fractions and TLC/GLC comparison with reference samples. However, it has not been possible to isolate them in pure form. The results which are recorded below are in agreement with literature.

The GLC of the petroleum ether eluate (Fig. 5) showed it to be a mixture of several hydrocarbons. By careful combination of column and preparative TLC following hydrocarbons, designated as Pat-HC-I, II, III and IV were isolated.

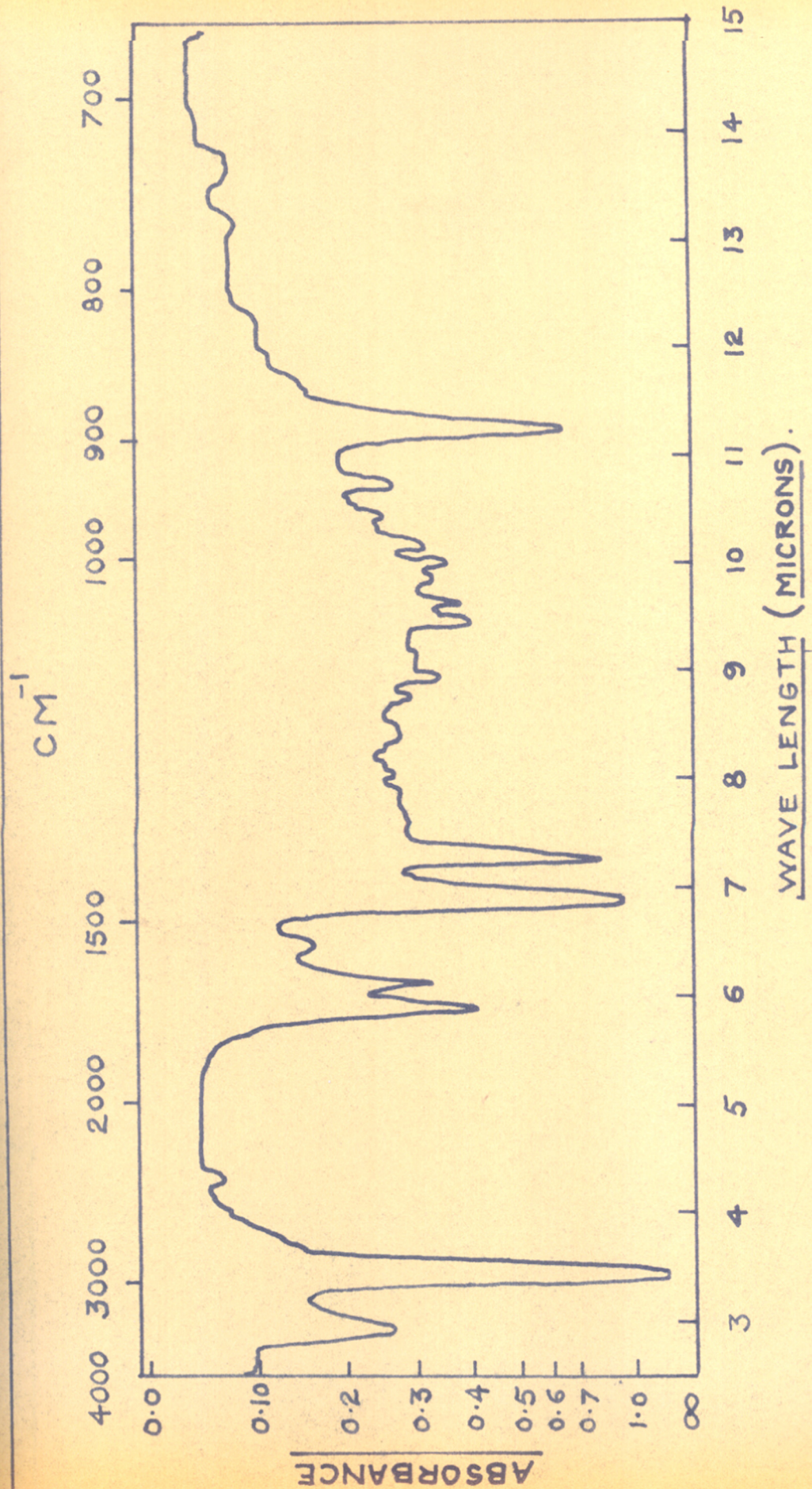


FIG. 1. IR SPECTRUM OF PATCHOULI OIL (LIQUID FILM).

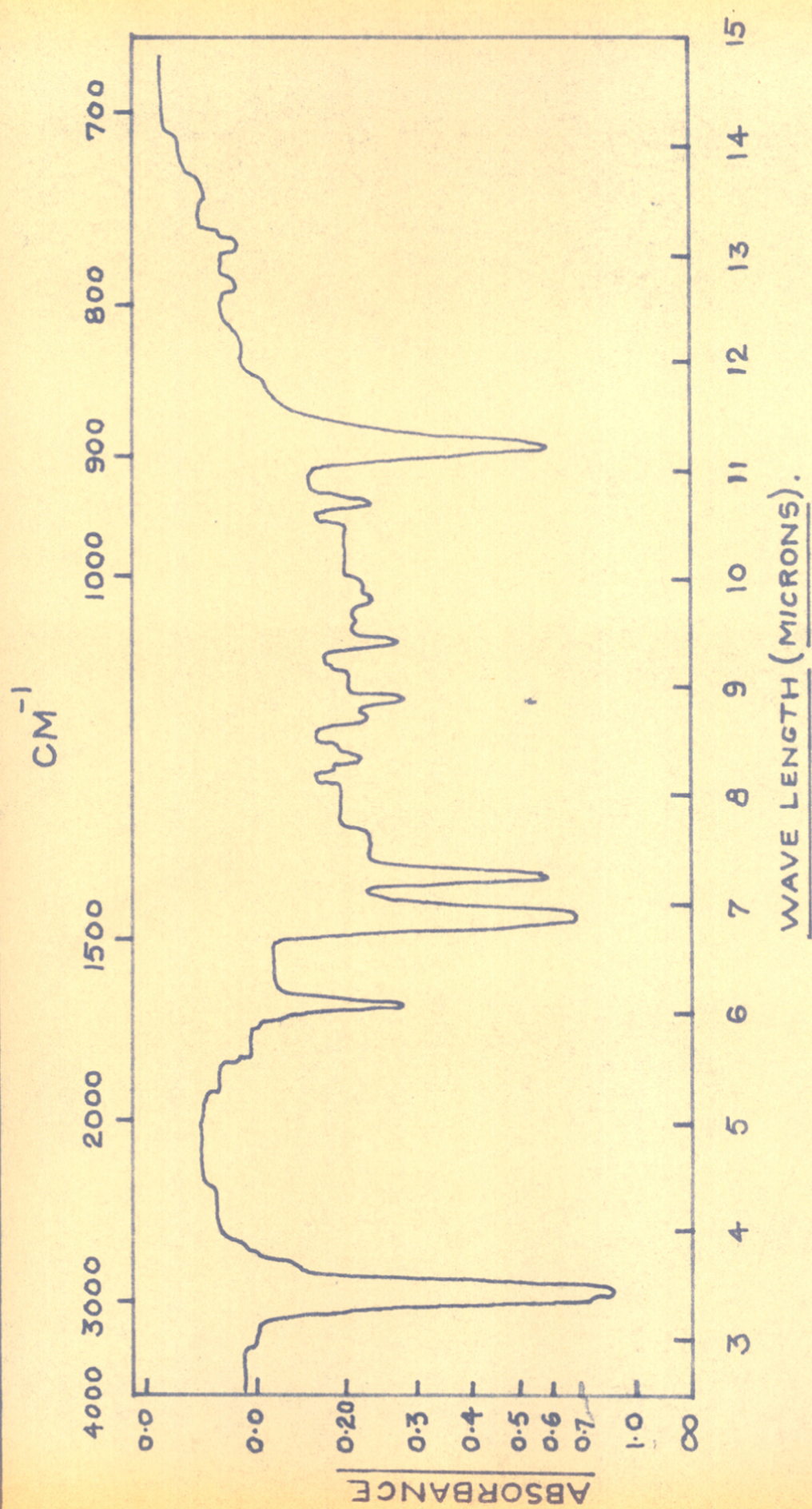


FIG. 2 IR SPECTRUM OF PETROLEUM ETHER FRACTION

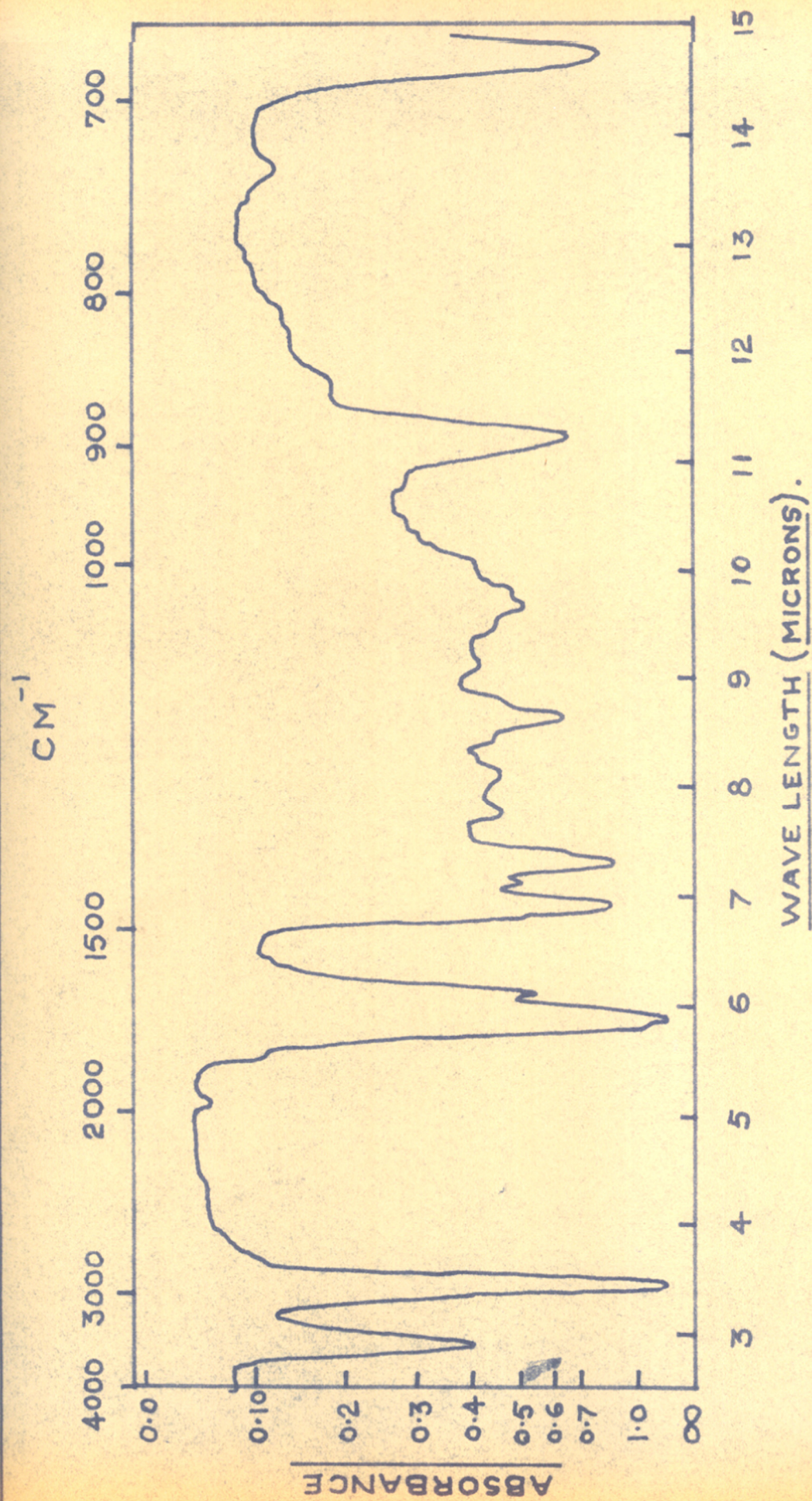


FIG. 3 IR SPECTRUM OF BENZENE FRACTION

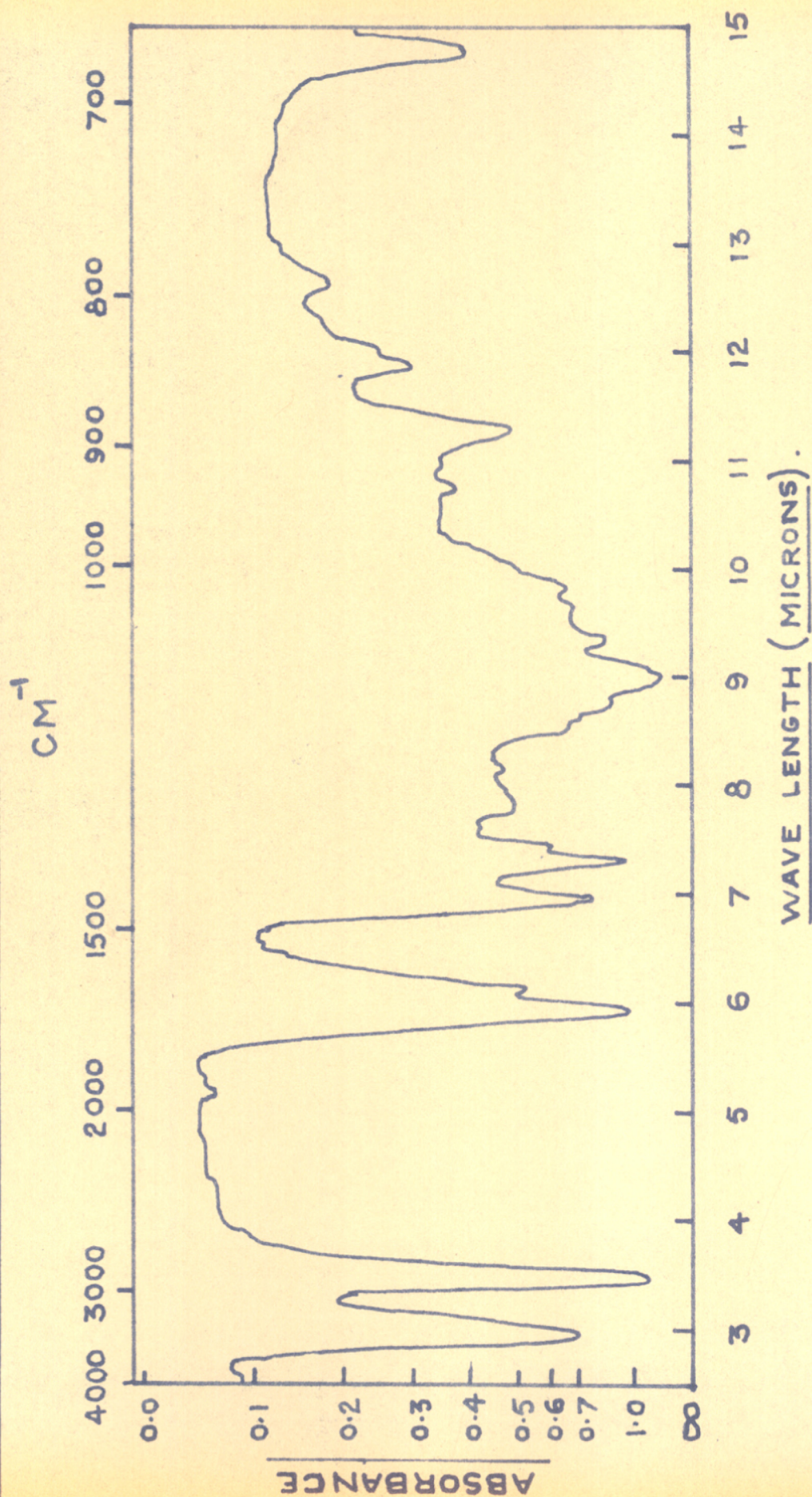


FIG. 4 IR SPECTRUM OF ETHER FRACTION

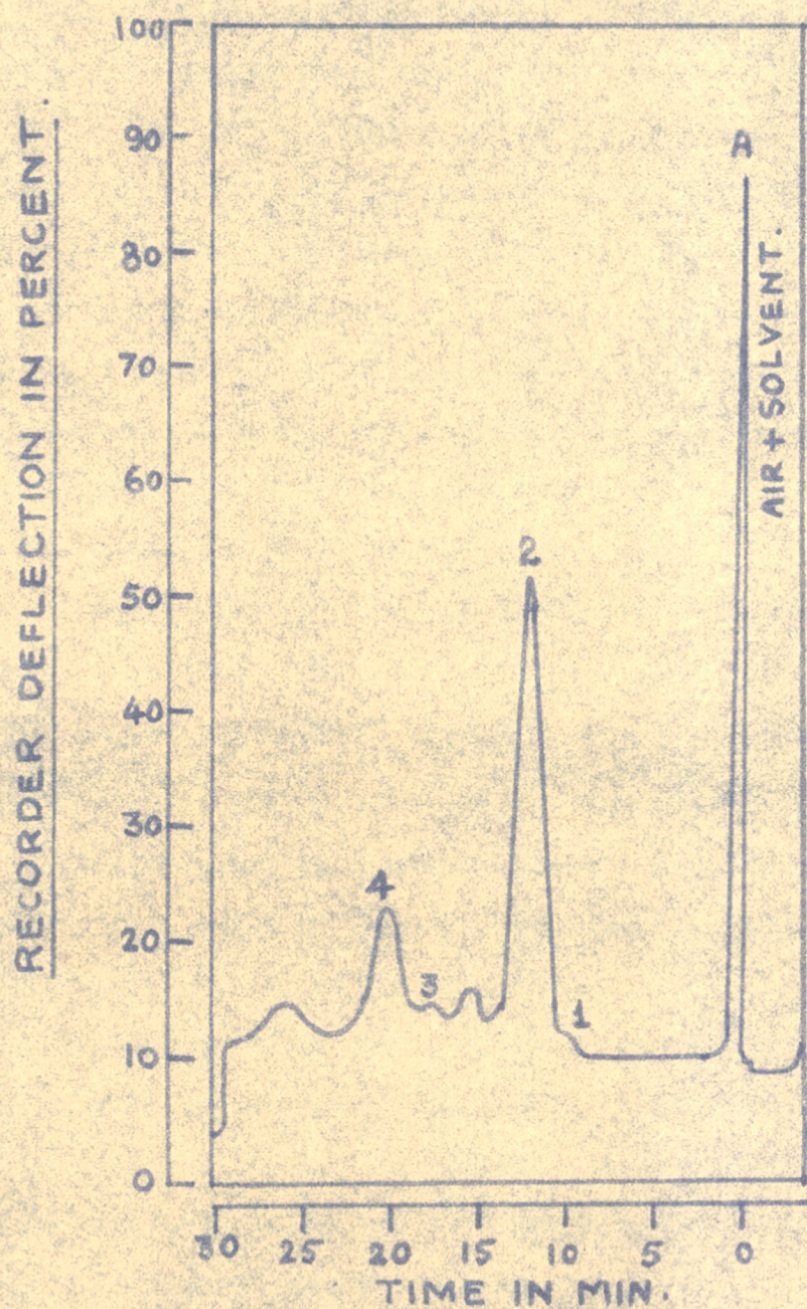


FIG. 5 GLC ANALYSIS OF PET-ETHER FRACTION

- | | | | |
|---|-----------------|---|----------------|
| ① | HYDROCARBON I | ② | HYDROCARBON II |
| ③ | HYDROCARBON III | ④ | HYDROCARBON IV |

Pat-HC-I

The initial chromatographic fractions yielded a sesquiterpene hydrocarbon, $C_{15}H_{24}$ (Pat-HC-I, M^+ 204) in pure form. The IR spectrum (Fig. 6) did not show any characteristic bands for $-C=C-$ stretching frequency. In further support of the above conclusion the NMR spectrum (Fig. 7) showed the absence of any vinyl proton signal. Moreover, all the methyl groups are attached to saturated carbon atoms. Pat-HC-I was resistant to catalytic hydrogenation. This information suggested its identity with β -patchoulene (I). An authentic sample of β -patchoulene, prepared from patchouli alcohol by the literature procedure¹¹ exhibited identical spectral data and TLC/GLC behaviour. It was therefore confirmed that Pat-HC-I is β -patchoulene (I).

Pat-HC-II

This hydrocarbon having molecular formula $C_{15}H_{24}$ (M^+ 204), $(\alpha)_D - 197.2^\circ$ gave a saturated dihydroderivative. Pat-HC-II must therefore be tricyclic.

Its NMR spectrum (Fig. 9) shows two tertiary methyl singlets (δ 0.84; 3H and δ 0.88; 3H), a secondary methyl doublet (δ 1.08; 3H; $j = 6.5$ Hz), a broad singlet due to a vinylic methyl (δ 1.64) and no vinyl protons. Its IR spectrum is reproduced in (Fig. 8).

Considering the physical constants and the spectral data mentioned above, the above sesquiterpene appeared to be

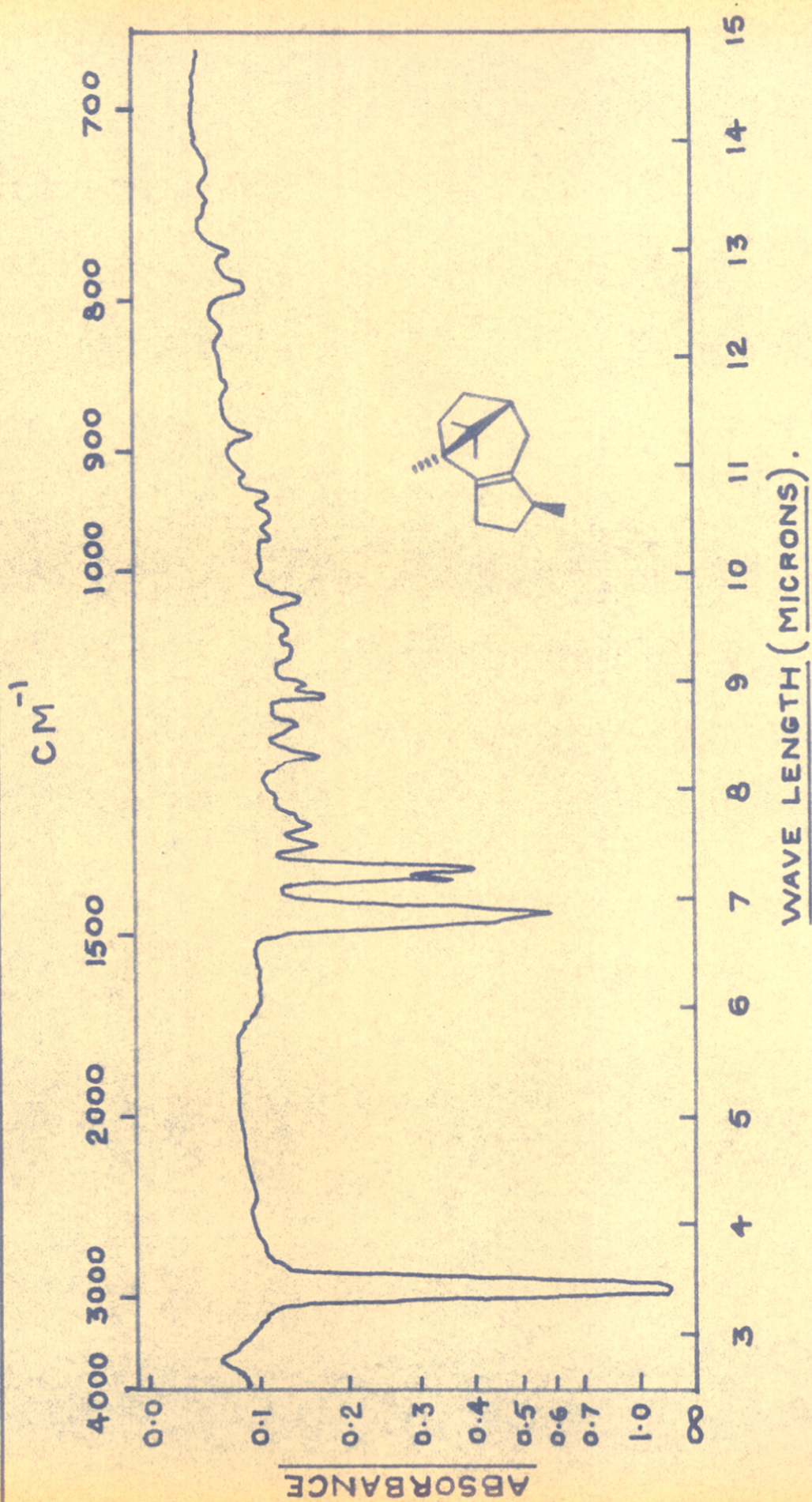
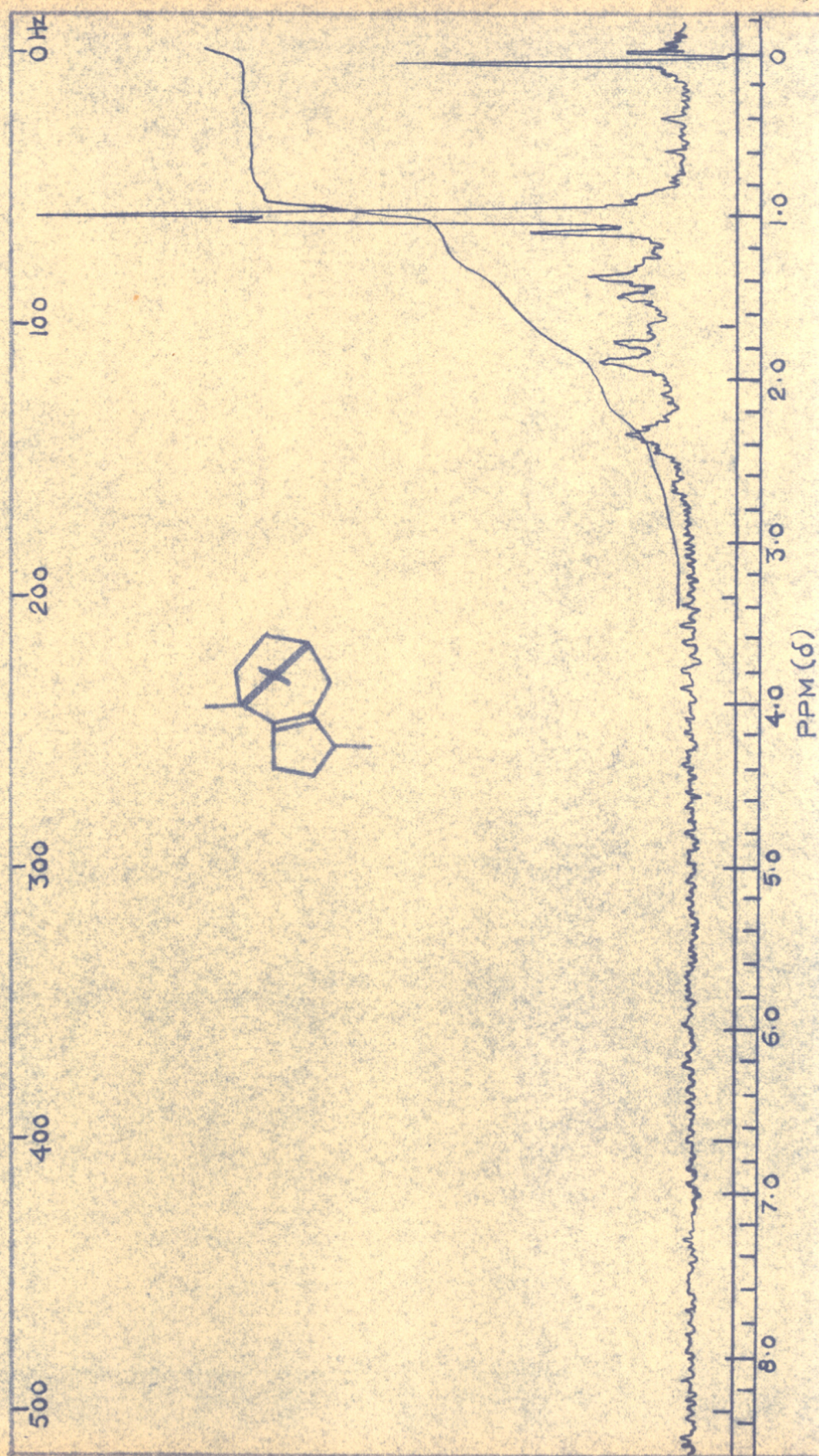


FIG. 6 IR SPECTRUM OF β PATCHOULENE (LIQUID FILM).

FIG. 7 NMR SPECTRUM OF β PATCHOULENE (IN CCl_4).

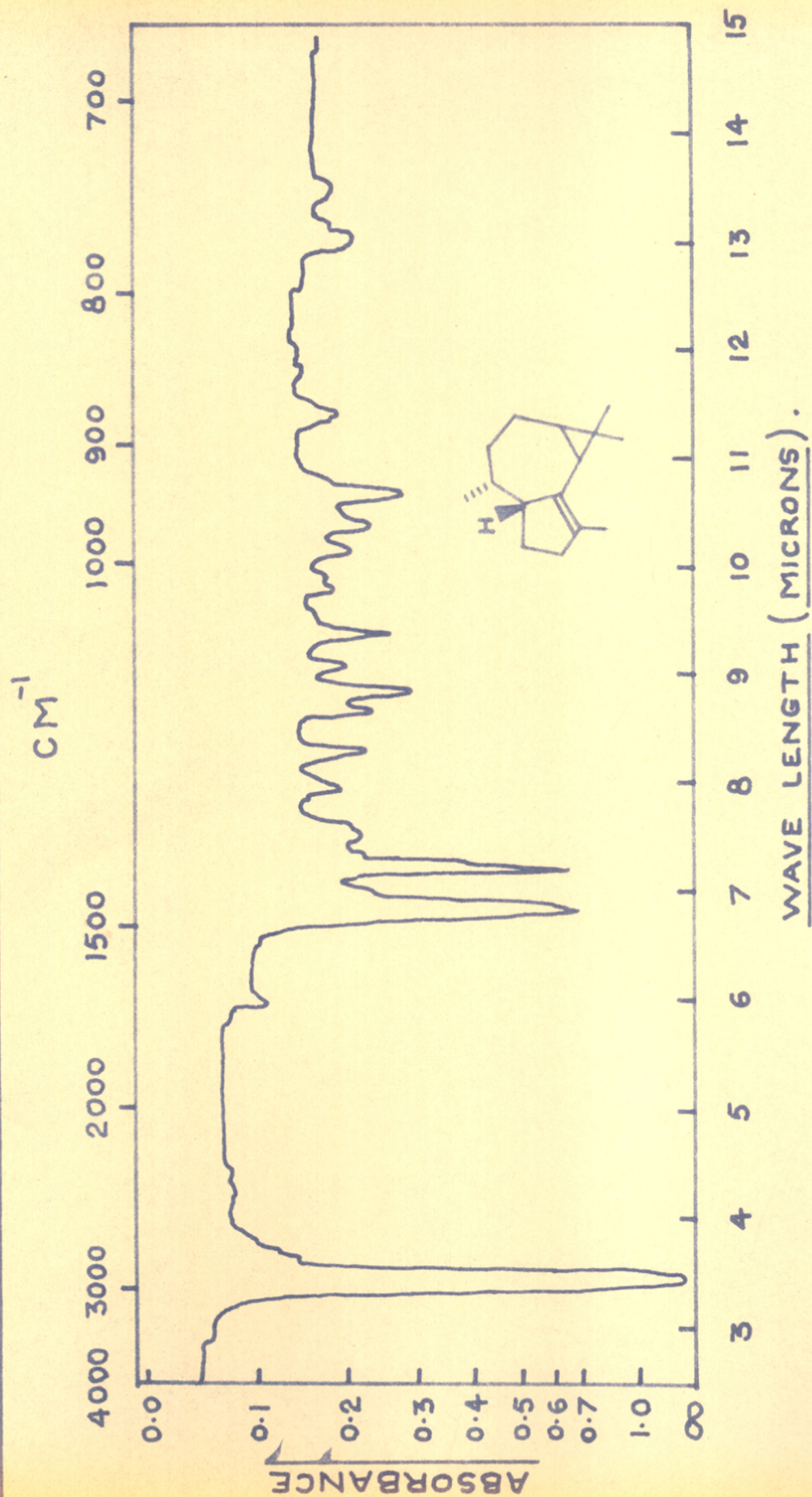
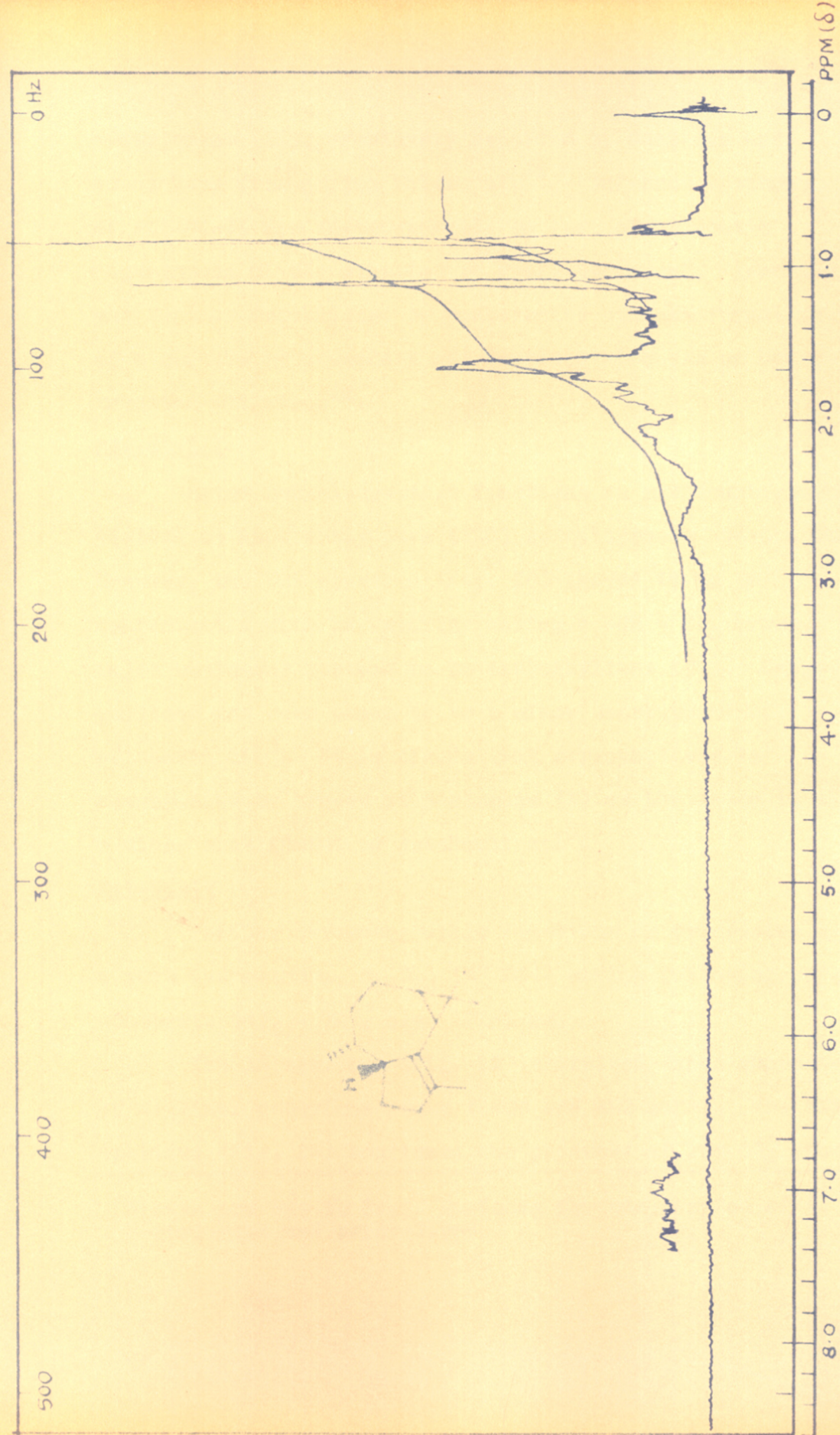


FIG. 8 IR SPECTRUM OF α GURJUNENE (LIQUID FILM).

FIG. 9 NMR SPECTRUM OF α GURJUNENE (IN CCl_4).

α -gurjunene (IX), previously reported by Ourisson and co-workers from gurjun balsam oil¹². Infrared spectrum of Pat-HC-II was superimposable on that of authentic α -gurjunene kindly supplied by Prof. Ourisson*. Identity was further established by comparative GLC and formation of a mixture of secondary and tertiary alcohols on hydroboration oxidation¹⁹.

Pat-HC-III

Later petroleum-ether fractions on rechromatography yielded an apparently pure (TLC) sesquiterpene hydrocarbon. However, the NMR spectrum (Fig. 10) showed it to be contaminated with an impurity having a $-\text{CH} = \text{CH}_2$ group. The major component appears to be caryophyllene (VI). Caryophyllene has been shown to be a minor constituent of patchouli oil³. It is likely that caryophyllene is accompanied by traces of β -elemene (V) as the later is also a minor constituent of patchouli oil³.

Pat-HC-IV

Further chromatographic fractions on processing gave a pure hydrocarbon, $\text{C}_{15}\text{H}_{24}$ (M^+ 204) having following physical constants (α)_D - 72°, b.p. 101/1 mm.

The IR spectrum (Fig. 12) exhibited bands characteristic of terminal methylene group (1640 and 880 cm^{-1}). The NMR spectrum (Fig. 13) confirmed the presence of $>\text{C} = \text{CH}_2$ group

*Thanks are due to Prof. Ourisson for the samples of α -gurjunene and seychellene.

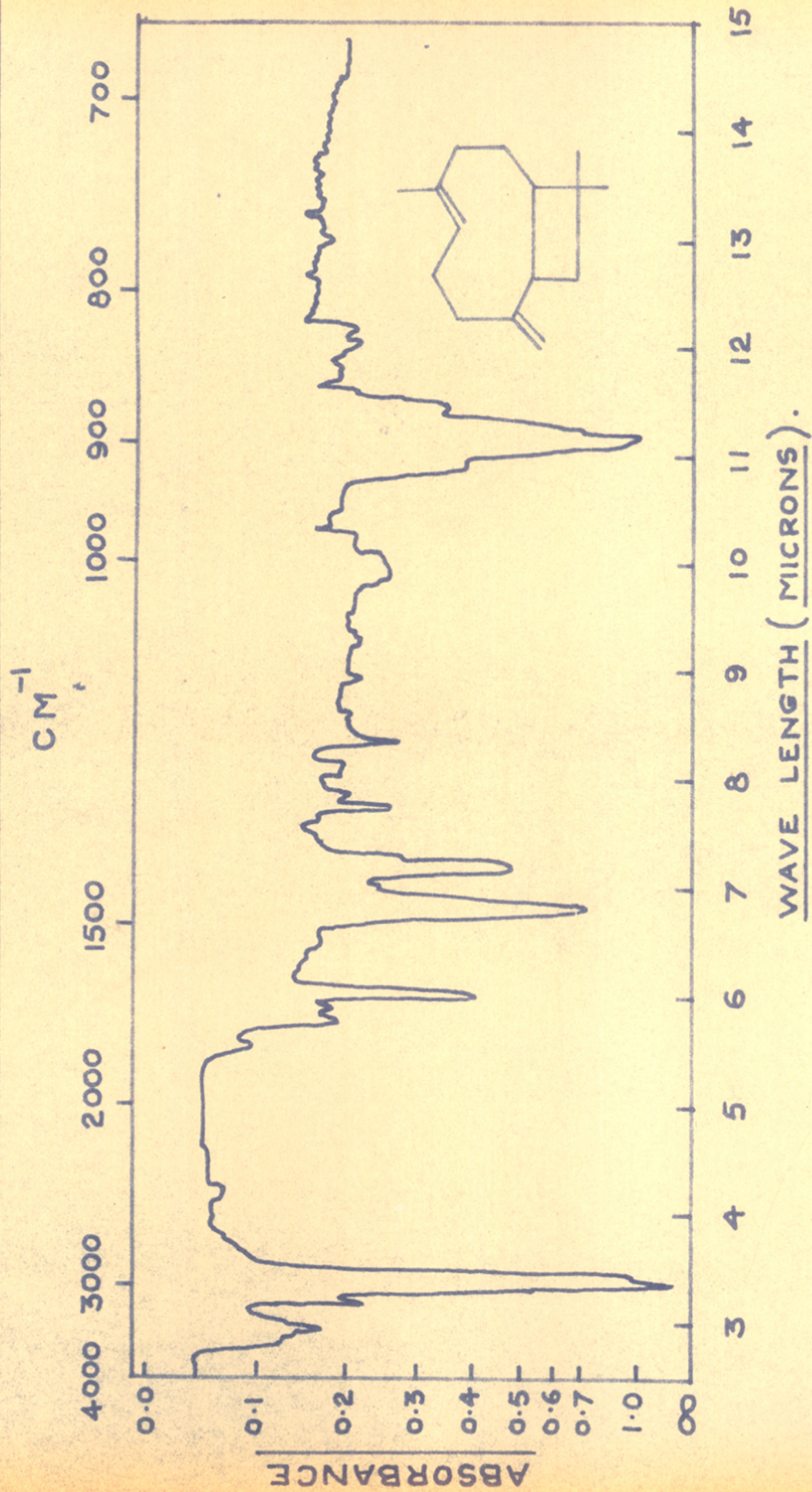


FIG. 10 IR SPECTRUM OF CARYOPHYLLENE (LIQUID FILM).

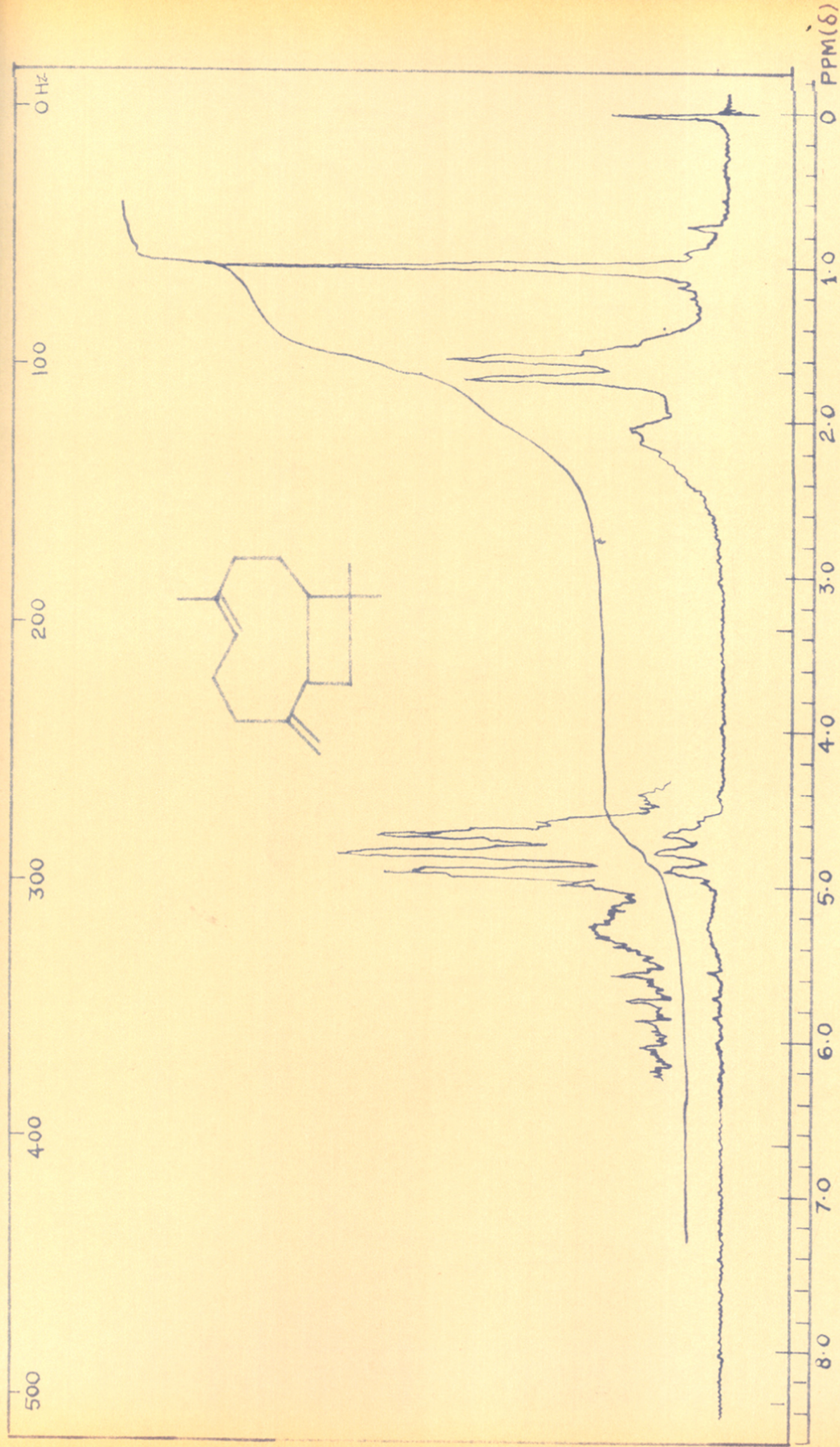


FIG. 11 NMR SPECTRUM OF CARYOPHYLLENE (IN CCl_4).
CONTAMINATED WITH β ELEMENE .

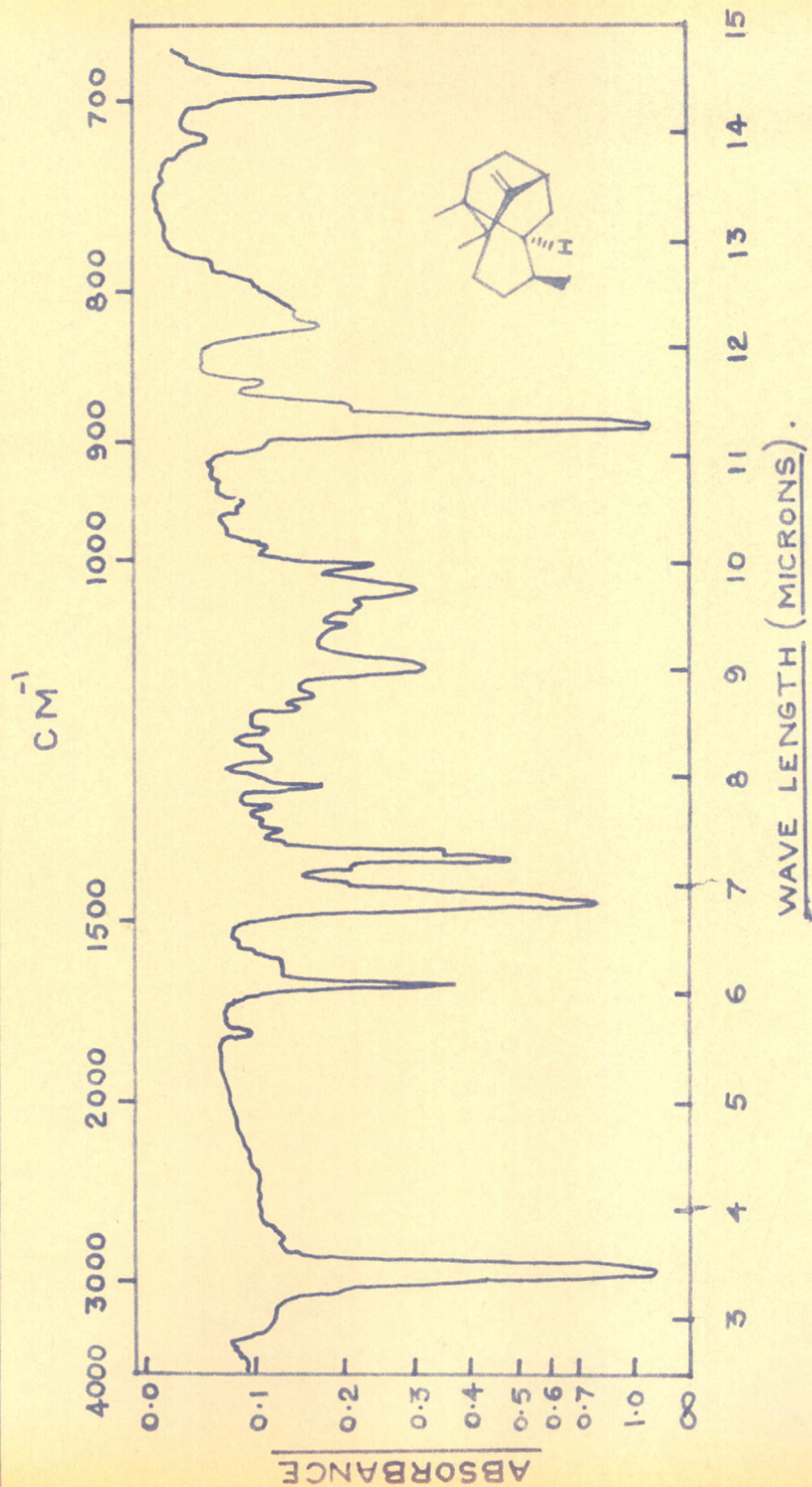
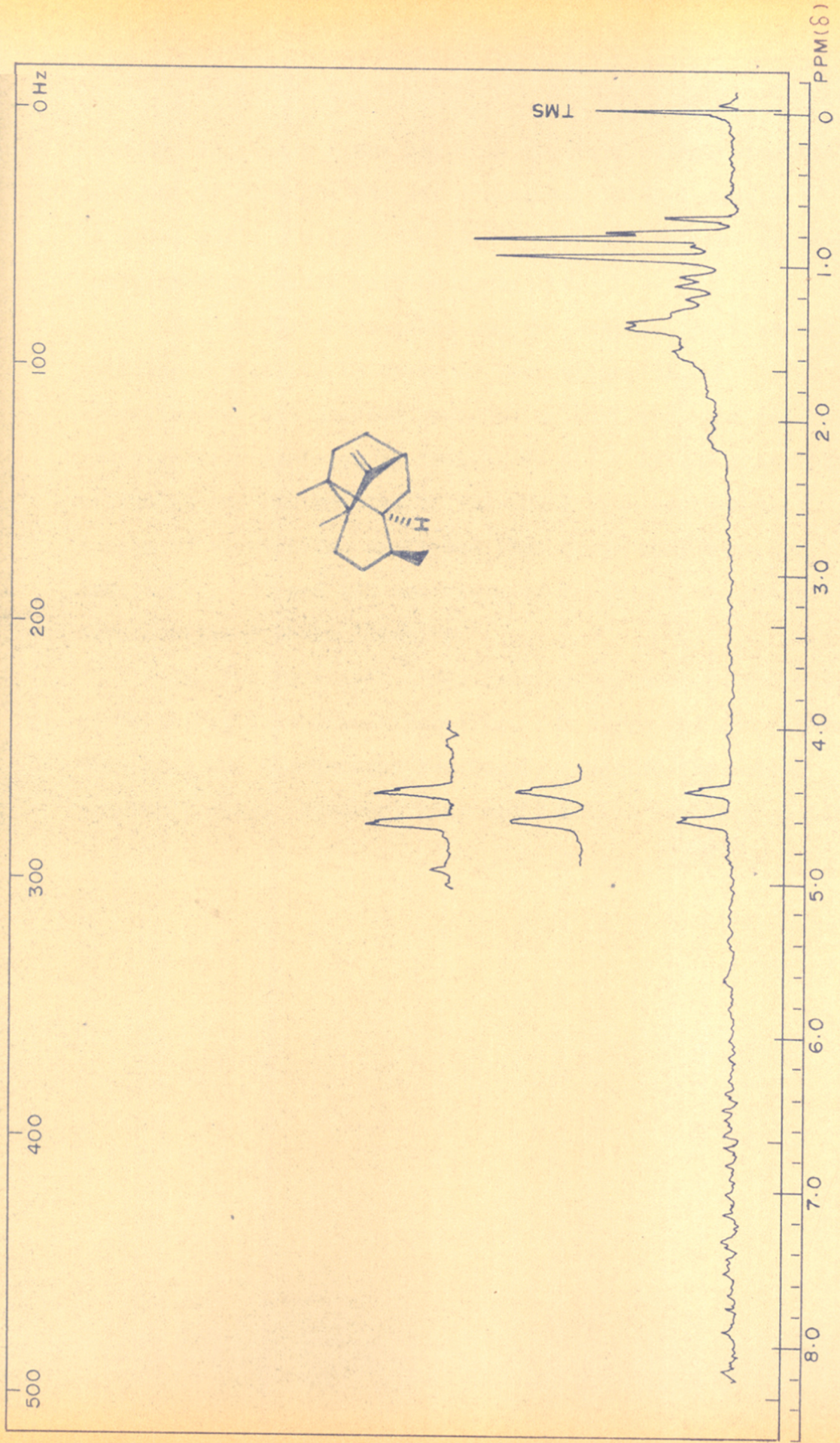


FIG. 12 IR SPECTRUM OF SEYCHELLENE (LIQUID FILM).

FIG.13 NMR SPECTRUM OF SEYCHELLENE (IN CCl₄)



(δ 4.63; 1H, and δ 4.98; 1H), two tertiary methyl singlets (δ 0.85; 3H, δ 0.98; 3H) and a secondary methyl doublet (δ 0.76; 3H; $J = 6$ Hz). Pat-HC-IV gave a saturated dihydro-derivative and hence must be tricyclic.

The physical constants and the spectral data showed striking similarity to those of seychellene (X), a sesquiterpene hydrocarbon reported by Curisson⁷ and patchouli hydrocarbon-G isolated by Tsubaki et al.³ Direct comparison of the IR and NMR spectra of Pat-HC-IV with an authentic sample of seychellene confirmed that these compounds are identical. TLC and GLC behaviour of Pat-HC-IV and seychellene also confirmed their identity.

The spectral data (NMR) obtained on certain individual chromatographic subfractions (Fig. 14) suggested the presence of α -guaiene and α -bulnesene previously reported from other patchouli oils. However, this could not be verified so far.

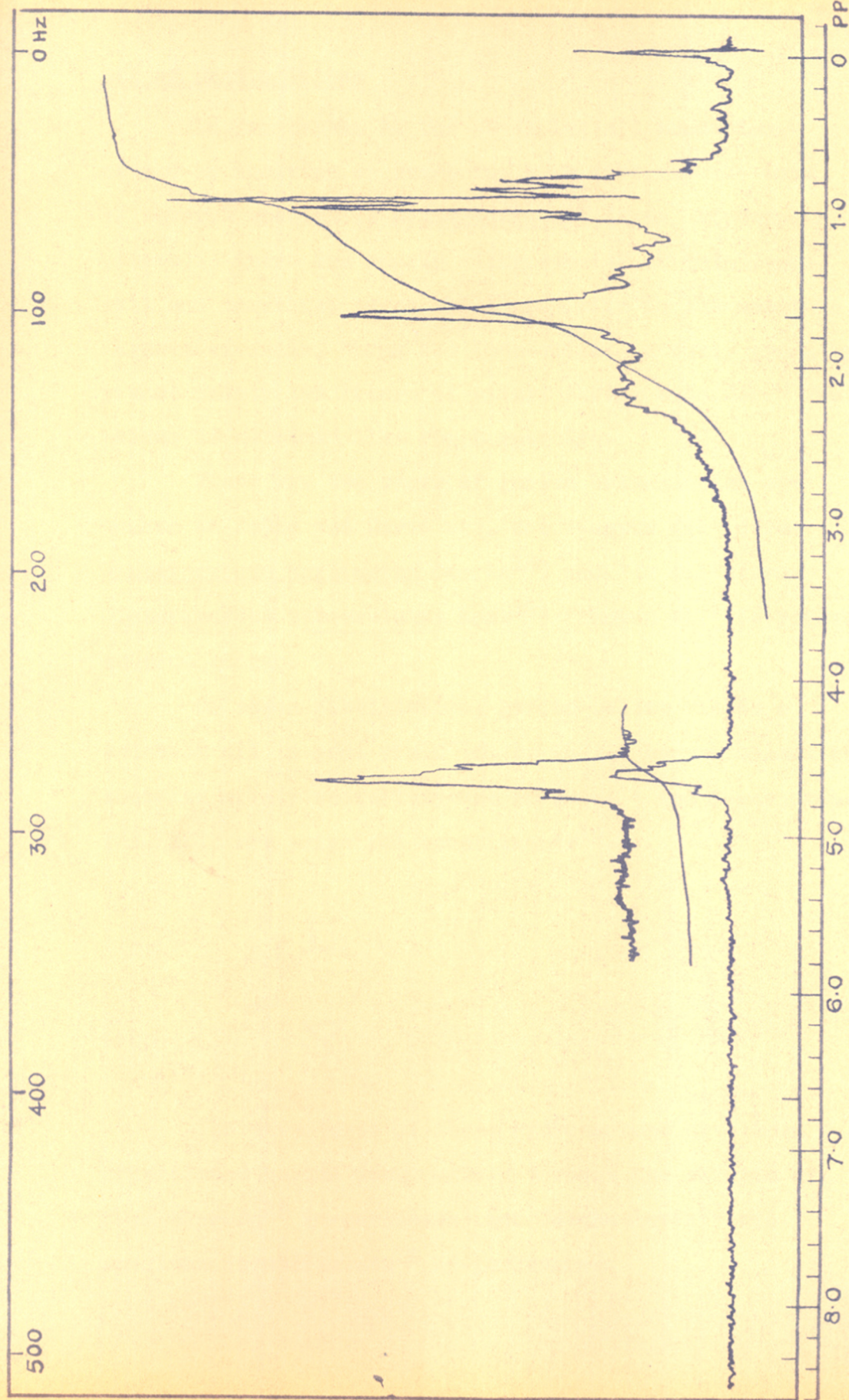


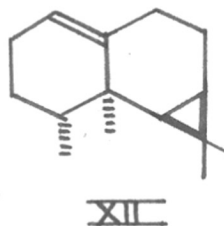
FIG. 14 NMR SPECTRUM OF α GUAIENE CONTAMINATED WITH
 α BULNESENE (IN CCl_4).

Oil of Gurjun Balsam

Gurjun balsam is the pathological exudation, caused by incision or scorching with fire, of the wood of several species of Dipterocarpus¹³ (Fam: Dipterocarpaceae). There are nearly fifty species of this genus, tall and beautiful trees which grow wild in the mountain forests of India, Burma and Indo-china. A fully grown tree yields nearly 180 liters of balsam during a summer. The balsam is obtained like gum turpentine.

There are two types of gurjun balsam which are common to India and Burma viz. the 'Kanyin Oil' (from Dipterocarpus turbinatus Gaertn¹³) and 'In Oil' (from Dipterocarpus tuberculatus Roxb¹³) 'Kanyan Oil' is regarded as true balsam.

On steam distillation, gurjun balsam yields a volatile oil in good yield (60 to 75%). Gurjun balsam oil contains mainly sesquiterpenes of which α and β -gurjunenes (IX, XII) are principal constituents¹⁴.



The structures of these hydrocarbons have been established beyond doubt mainly through the efforts of French group¹² (α -gurjunene) and Czechoslovak¹⁵ and American investigators¹⁶ (β -gurjunene),

Present Study

The present investigation was undertaken with a view to make an authentic sample of α -gurjunene (IX) available for comparison purposes. Secondly, in connection with other work, α -gurjunene was required in large quantity. It was therefore considered worthwhile to isolate this substance from the steam distilled oil of gurjun balsam*.

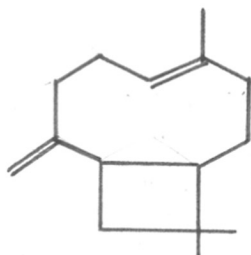
The steam distilled oil used in the present investigation is a pale yellow coloured mobile liquid. Chromatographic (column) behaviour on different grades of alumina, coupled with GLC/TLC indicated that it contains mainly two hydrocarbons. Chromatography of the oil over alumina (activity I) resulted in about 80% recovery by elution with petroleum ether (60-80°). GLC analysis showed the presence of mainly two components. These two hydrocarbons could be obtained in pure form by careful combination of column chromatography over alumina and silvernitrate impregnated silica gel. The course of separation was monitored by GLC and TLC on silica gel and silvernitrate impregnated silica gel plates.

The individual hydrocarbons (hydrocarbon-I and hydrocarbon-II) have been obtained in the pure form.

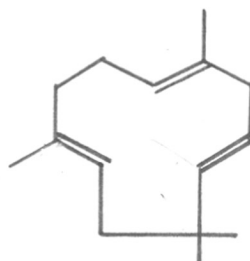
On the basis of physical constants, spectral data and direct comparison with authentic samples, hydrocarbons I

*The oil was obtained by steam distillation of gurjun balsam resin sometime during 1963.

and II have been identified as caryophyllene (VI) and humulene (XIII)¹⁷.



VI



XIII

Surprisingly, presence of α or β -gurjunene could not be detected. It appears, therefore, that the oleoresin used for steam distillation may not be genuine gurjun balsam*. It is of interest to note that the steam distilled oil obtained from copaiba balsam is a rich source of caryophyllene and humulene¹⁸. It seems likely that the commercially supplied sample of gurjun balsam may actually be copaiba balsam or closely related oleoresin.

It has been mentioned earlier that the authenticity of commercially supplied sample of 'gurjun balsam' is doubtful. A further literature survey indicates that the volatile oil obtained from the oleo-resin of Dipterocarpus pilosus is a rich source of caryophyllene and humulene²⁰. Besides these two hydrocarbons, 9,10 epoxyhumulene has been isolated from this source²¹. It appears, therefore, that the commercially supplied sample is of 'gurjun balsam' from D. pilosus and not D. tuberculatus or D. turbinatus.

*On the basis of the results described above, we have developed some doubt regarding the authenticity of the commercially supplied sample of gurjun balsam.

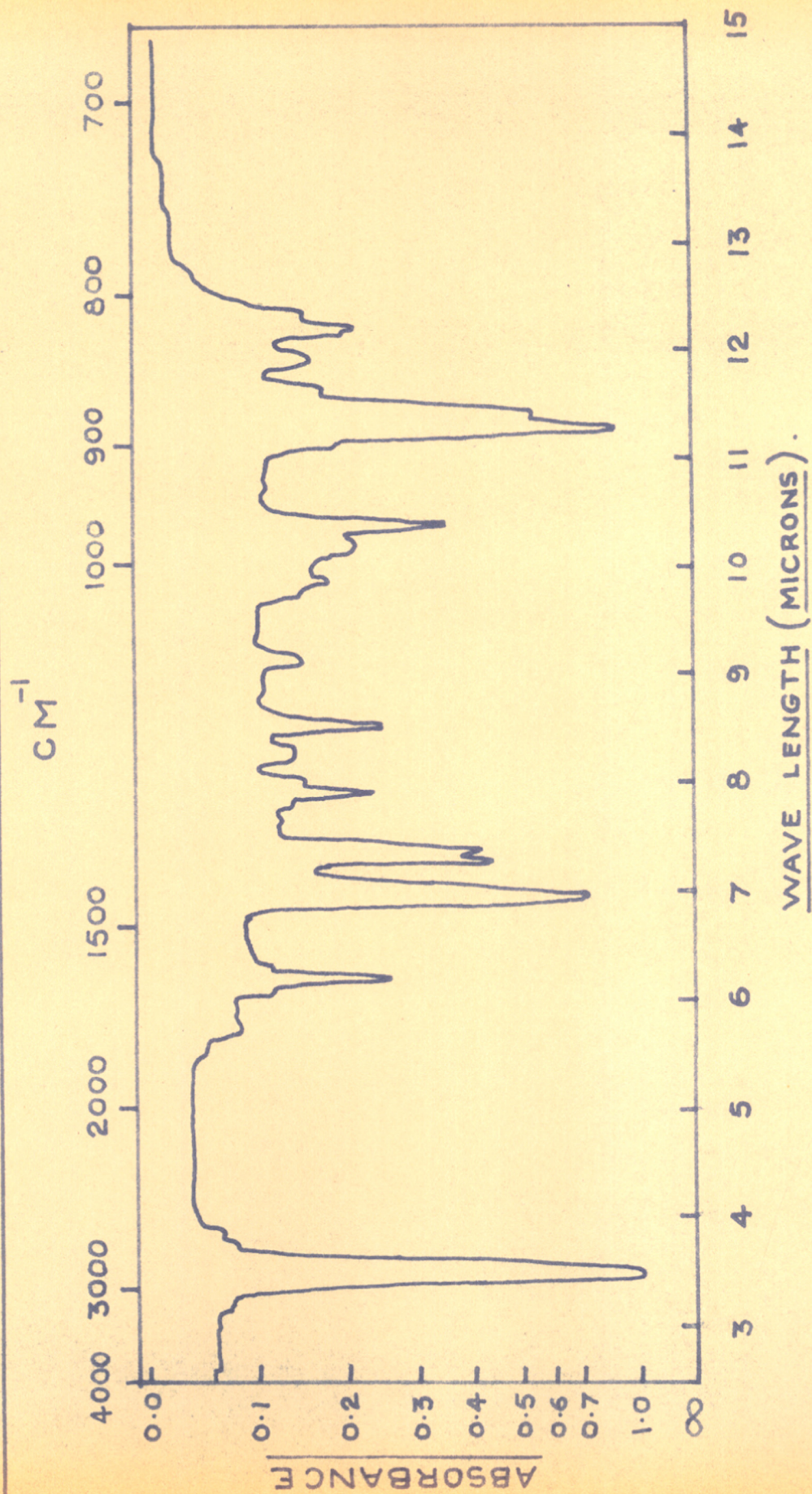


FIG. 15 IR SPECTRUM OF GURJUN BALSAM OIL (PET-ETHER FRACTION)

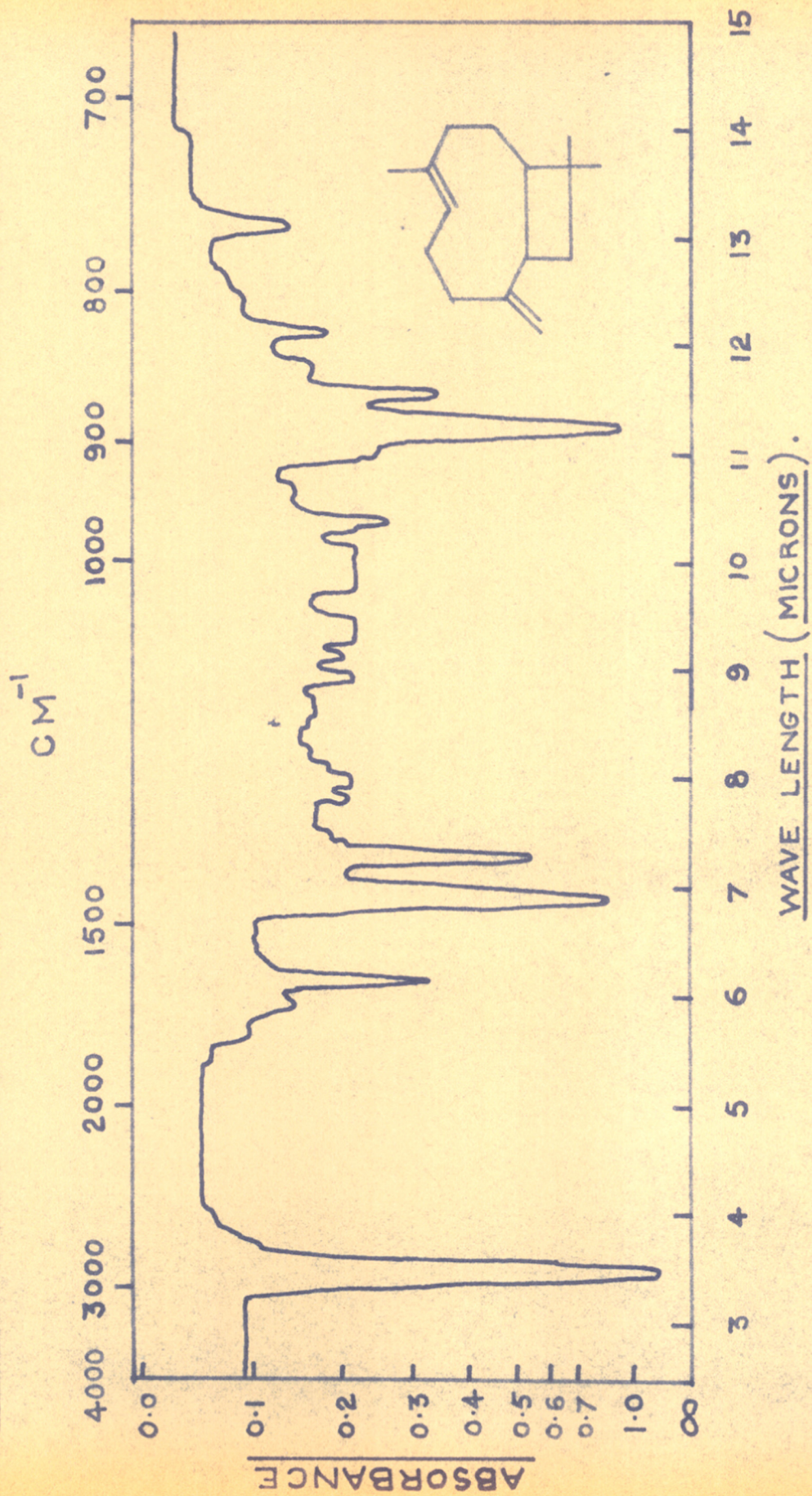


FIG. 16 IR SPECTRUM OF CARYOPHYLLENE (LIQUID FILM).

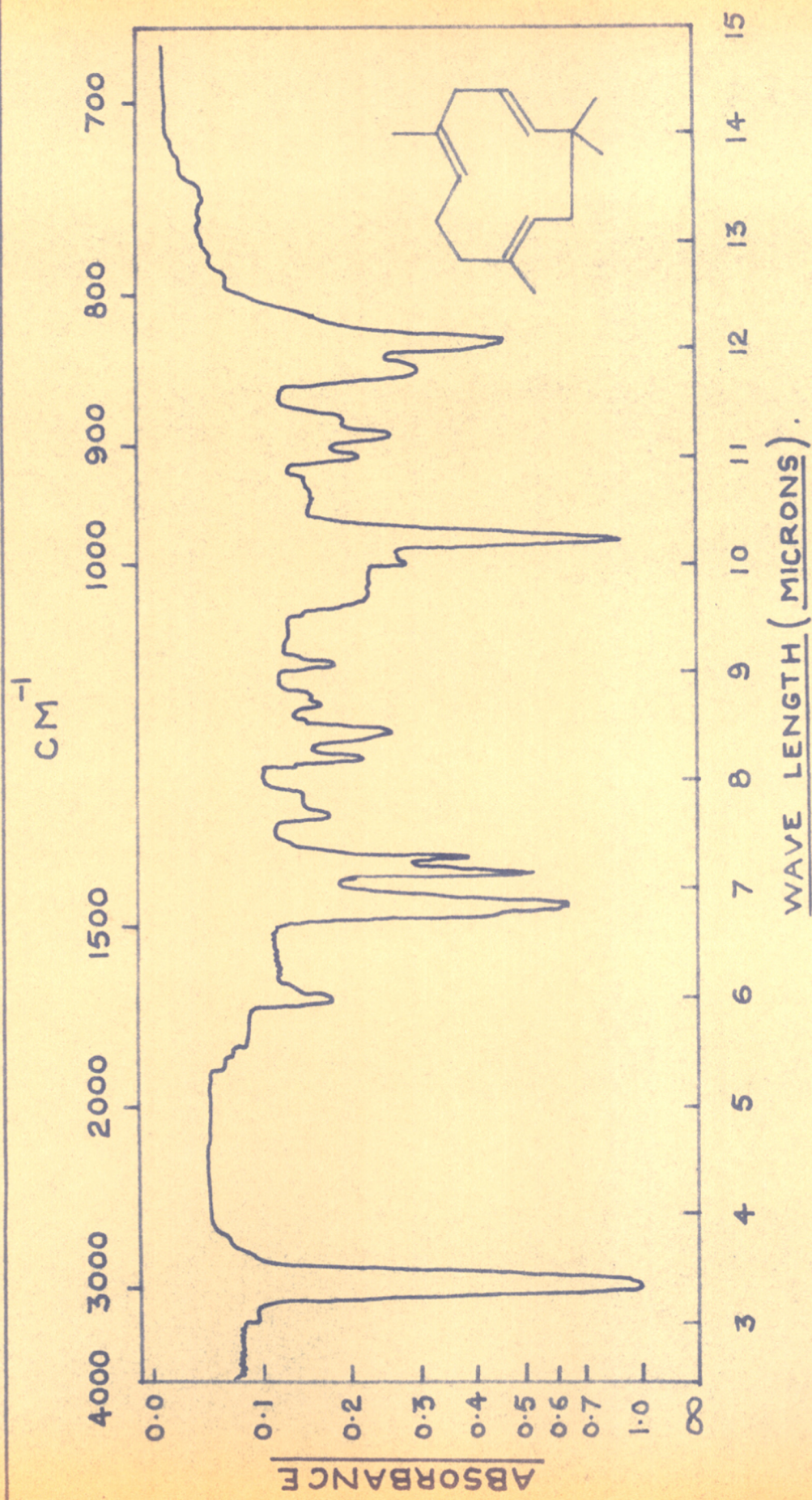


FIG.17 IR SPECTRUM OF HUMULENE (LIQUID FILM).

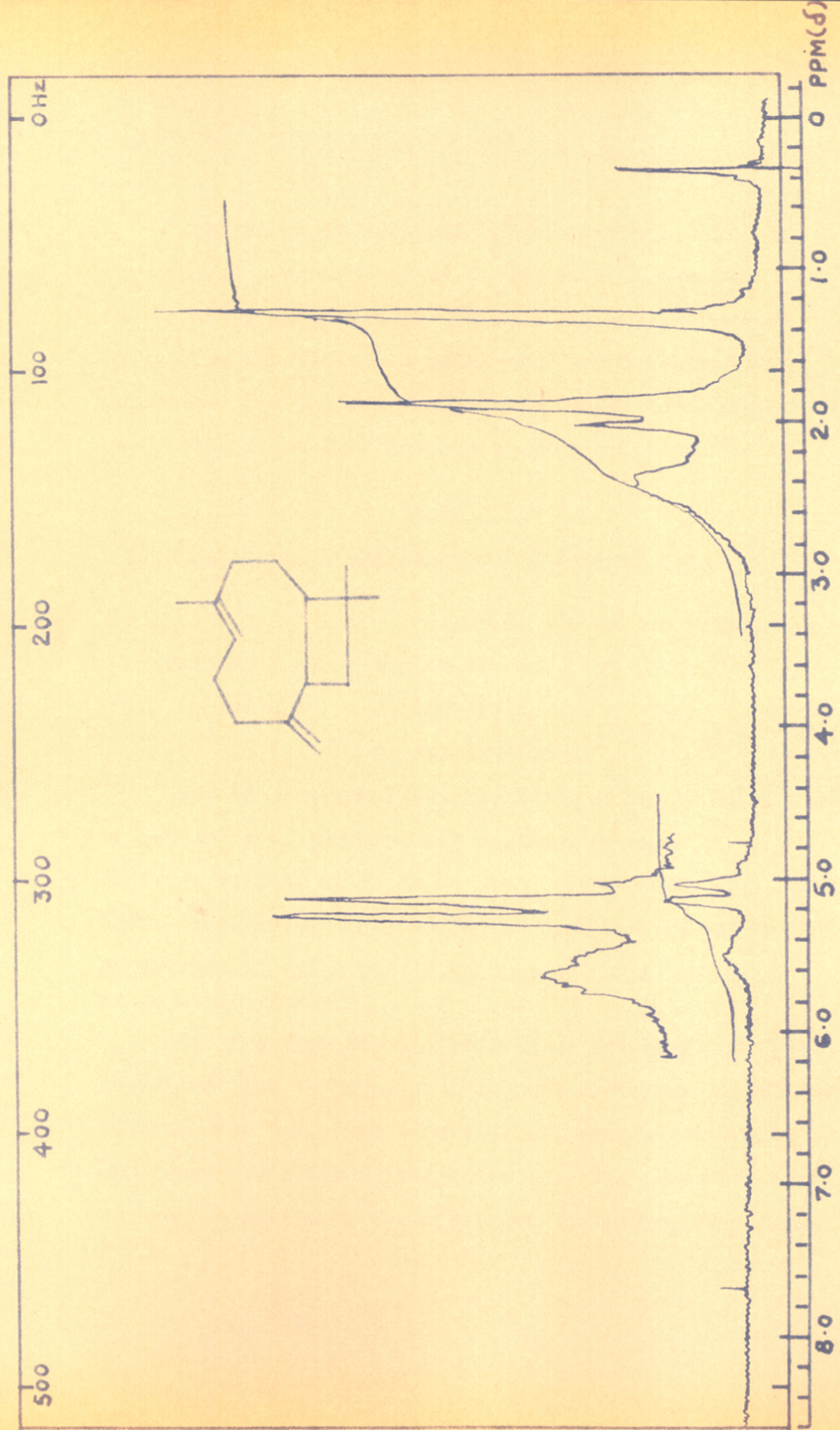


FIG. 18 NMR SPECTRUM OF CARYOPHYLLENE (IN CCl_4).

EXPERIMENTAL

The free acids present in the patchouli oil (Singapore) were removed by treatment with aqueous sodium bicarbonate (10%). The neutral oil was washed with brine and water and dried over anhydrous sodium sulphate. The oil was fractionated under reduced pressure when three major fractions were often obtained.

Table I

Fractional Distillation of Patchouli oil

	b.p.	Wt. of fraction
Patchouli oil	1 110°/10 mm	70 g
100 g	2. 115°/10 mm	10 g
	3. 120-130°/10 mm	5 g.

The IR spectrum (Fig. 1) of the first fraction showed the presence of hydroxyl and carbonyl groups.

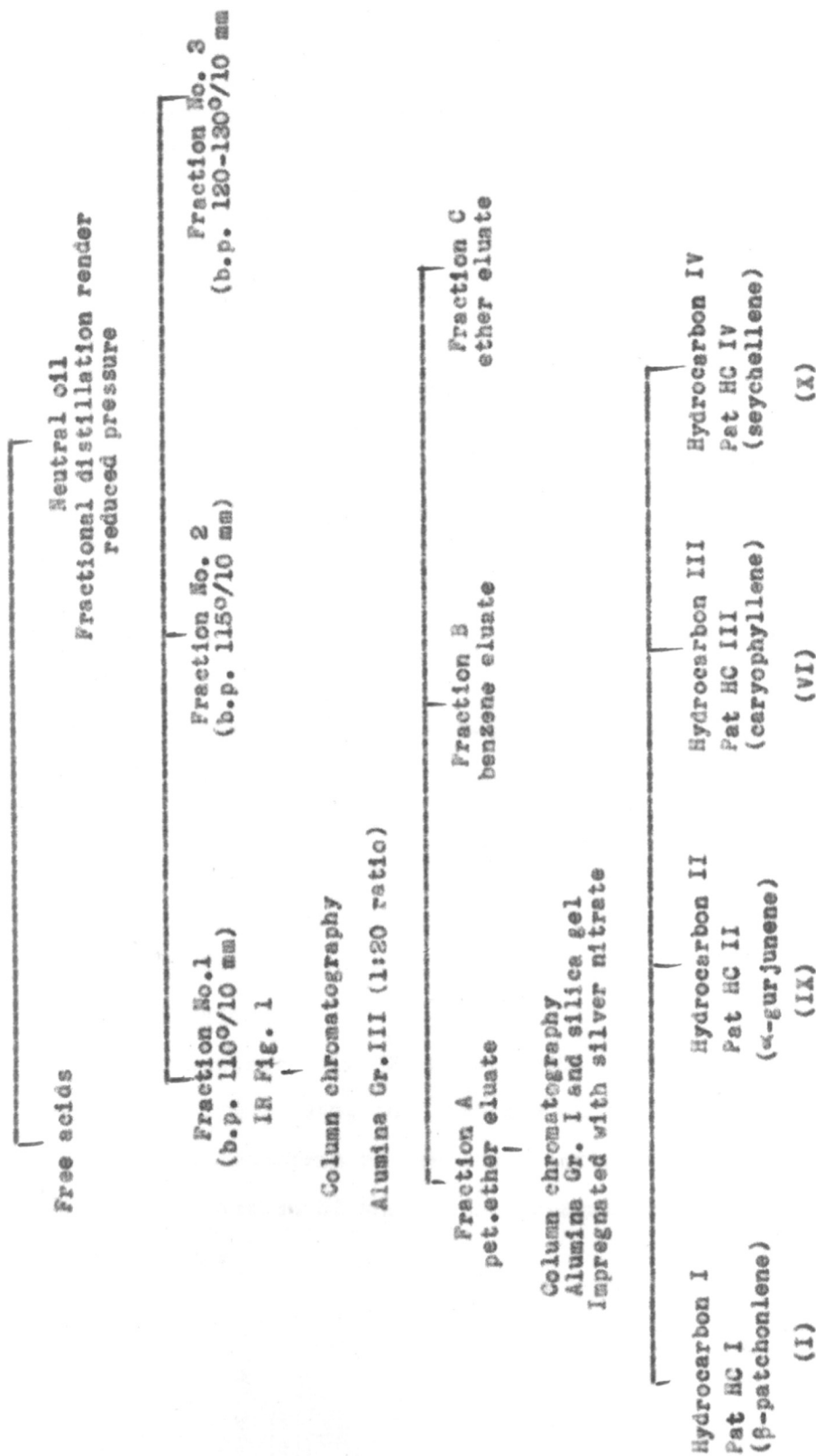
IR bands (liquid film) 3450, 2950, 1720, 1640, 1540, 1460, 1360, 1120, 1070, 1055, 1005, 980, 935 and 890 cm^{-1} .

Separation of hydrocarbons ketones and alcohols by column chromatography

The fraction No. 1 (Table I) of patchouli oil was separated into three major fractions by column chromatography, eluting with pet.ether (Fraction A), benzene (Fraction B), and ether (Fraction C) respectively. The procedure adopted for the isolation of the different components from these

CHART III

Patchouli oil (Singapore)



fractions is indicated in Chart III

Table II

Wt. of distilled fraction No.1 25 g

Wt. of neutral alumina Gr. III 500 g.

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fraction (g)
A	Pet.ether	1000	14.3085
B	Benzene	1000	5.2200
C	Ether	1000	23.2485
Recovery 95%			

Fraction A Hydrocarbons

IR spectrum (Fig. 2) 2900, 1640, 1450, 1380, 1220, 1150, 935 and 890 cm^{-1} .

The IR spectrum of the pet. ether fraction (Fig. 2, Table II Fraction A) showed the absence of hydroxy and carbonyl groups. The GLC analysis (Fig. 5) showed the presence of four components.

Fraction B Ketones

IR spectrum (Fig. 3) 3460, 2850, 1925, 1710, 1680, 1440, 1360, 1290, 1240, 1040 and 890 cm^{-1} .

The IR spectrum of benzene fraction (Fig. 3) (Table II, Fraction B) showed the presence of alcoholic and carbonyl bands.

Fraction C Alcohol

IR spectrum (Fig. 4) 3400, 2890, 1930, 1690, 1640, 1440, 1360, 1170, 1120, 1070, 1020, 930, 860 and 840 cm^{-1} .

The IR spectrum of ether fraction (Fig. 4, Table II Fraction C) showed the presence of alcoholic bands. The ether fraction contained pogostol* and patchouli alcohol.

Isolation of hydrocarbons

The IR spectrum (Fig. 2) of the fraction A (Table II) showed the presence of hydrocarbons (GLC analysis Fig. 5). On chromatography over alumina Gr. I, several fractions were collected eluting with pet. ether.

Table III

Wt. of fraction A 20 g.

Wt. of neutral alumina Gr. I 800 g.

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fractions (g)	GLC of hydrocarbons			
				I	II	III	IV
1.	Pet. ether	100	1.4560	60 :	20 :	5 :	15
2.	"	100	6.3200	5 :	60 :	5 :	30
3.	"	100	5.500		60 :	:	40
4.	"	250	3.8400		60 :	:	40
5.	"	250	0.5930		50 :	:	50
6.	"	250	1.2890		50 :	:	50
			18.9980				
			Recovery 95%				

*The correlation of pogostol with rotundene is in progress, the work being carried out by another research worker.

Hydrocarbon I

Fractions No.1 and No. 2 (Table III) were mixed and rechromatographed over silica gel impregnated with silver nitrate (15%). The details of chromatography are shown in Table IV.

Table IV

Weight of fraction 7.5 g
 Wt. of silicagel impregnated with AgNO_3 (15%) 250 g.

S.No.	Eluent	Vol. of Eluent (ml)	Wt. of Fraction (g)	GLC of hydrocarbons			
				I	II	III	IV
1.	Pet.ether	25	0.4379	100%			
2.	"	25	3.3700		80:	5 :	15
3.	"	25	1.2300		90:	:	10
4.	"	50	1.0787		60:	:	40
5.	"	50	0.8580		20:	:	80
6.	"	100	0.3100		20:	:	80

7.2846

Recovery 98%

The fraction No.1 (Table IV) was distilled over sodium under reduced pressure when hydrocarbon I was obtained in pure form.

Hydrocarbon II

Fractions No.2 and No.3 (Table IV) were mixed and rechromatographed over silica gel impregnated with silver nitrate (15%).

Table V

Weight of fraction 4.5 g.

Wt. of silicagel impregnated with AgNO_3 (15%) 200 g.

S.No.	Eluent	Vol of eluent (ml)	Wt. of fractions (g)	GLC of hydrocarbons		
				II	III	IV
1.	Pet.ether	25	1.0431	100%		
2.	"	25	0.9392	40 :	5 :	55
3.	"	25	0.8579	10 :	5 :	85
4.	"	25	0.6500		5 :	85
5.	"	50	0.4250		5 :	85
6.	"	100	0.3270			
			4.2422			
		Recovery	98%			

The pet.ether fraction No.1 (Table V) gave hydrocarbon W when distilled over sodium under reduced pressure.

Hydrocarbon IV

The fractions No.4 and 5 (Table V) were mixed and rechromatographed over silica gel impregnated with silver nitrate (15%)

Table VI

Weight of fraction

0.8 g

Wt. of silicagel impregnated with AgNO_3 (15%)

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fraction (g)	GLC of hydrocarbons		
				III	IV	V
1	Pet.ether	20	0.0490	90		
2.	"	20	0.409 ⁰		100%	
3.	"	20	0.2030		90 :	10
4.	"	50	0.065 ⁰			80%

Hydrocarbon IV was obtained after distillation of the fraction No.2 (Table VI) with sodium under reduced pressure.

Hydrocarbon I $C_{15}H_{24}$ (Pat HC I β -patchoulene) I

Hydrocarbon I (Table IV fraction No.1) had the following properties and was shown to be identical with β -patchoulene

b.p. 99⁰(bath)/2 mm

Mol. weight 204 mass spectrum

Analysis

Found: C, 87.94; H, 12.06%

Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84%

IR bands (Fig. 6) 2950, 1460, 1395, 1380, 1360, 1315, 1200, 1130, 1030, 890 and 780 cm^{-1} .

NMR spectrum (Fig. 7)

δ 0.94 and δ 0.99 (6H, 2 CH_3 -CH)

δ 1.02 (6H $\begin{array}{c} CH_3 \\ \diagup \\ C \\ \diagdown \\ CH_3 \end{array}$)

β -Patchoulene from patchouli alcohol

A solution of patchouli alcohol, m.p. 56⁰ (50 mg) in ether (30 ml) was refluxed with sulphuric acid (1 ml) for

30 minutes on water bath. At the end of the reaction the mixture was poured into water and the ether layer separated and washed with water, dilute sodium bicarbonate solution and water and dried over anhydrous sodium sulphate. The ether was removed and the resulting product was chromatographed over silicagel impregnated with silver nitrate (15%). The first petroleum ether fraction contain β -patchoulene (28 mg).

β -Patchoulene and Pat. HC I exhibited identical spectral and TLC/GLC behaviour.

Hydrogenation of hydrocarbon I $C_{15}H_{24}$ (Pat. HC I)

Hydrocarbon I (30 mg) dissolved in ethanol (20 ml) was stirred in an atmosphere of hydrogen with Adam's platinum oxide (20 mg) catalyst for half an hour. It was observed that there was no uptake of hydrogen. The catalyst was filtered and solvent was removed under vacuum. The reaction product was found to be starting hydrocarbon (18 mg) after chromatography.

Hydrocarbon II $C_{15}H_{24}$ (Pat. HC II α -gurjnone) IX

Hydrocarbon II isolated (Table V Fraction No.I) had the following properties.

b.p. 99-100°(bath)/2 mm

$(\alpha)_D^{28} - 197.2$ (c, 4.0)

Mol. weight 204 (mass spectrum)

Analysis

Found: C, 88.52; H, 11.48%

Calculated for $C_{15}H_{24}$: C, 88.16; H, 11.84%

IR bands (Fig. 8 liquid film) 2950, 1640, 1460, 1360, 1255, 1220, 1150, 1125, 1100, 960, 935 and 870 cm^{-1} .

NMR Spectrum (Fig. 9)

δ 0.84 (3H), δ 0.87 (3H) (2 CH_3 - CH),

δ 1.08 (3H, J, 6.5 Hz)

a secondary methyl, δ 1.64 (3H) (vinyllic methyl)

Hydrocarbon III $\text{C}_{15}\text{H}_{24}$ (Pat. HC III carvophyllene) VI

Hydrocarbon III (Table VI Fraction No.1) is accompanied by traces of β -elemene was distilled over sodium.

IR bands (Fig. 10) (liquid film) 2940, 1710, 1650, 1455, 1380, 1280, 1190 and 890 cm^{-1} .

NMR spectrum (Fig. 11)

δ 1.05 (6H, $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{C} \\ \diagdown \\ \text{CH}_3 \end{array}$), δ 1.6 (3H CH_3 - CH),

δ 1.7 (2H, $\text{CH}_2 = \text{C} <$)

δ 4.6 (1H, vinyl proton)

Hydrocarbon IV $\text{C}_{15}\text{H}_{24}$ (Pat. HC IV is seychellene) X

Hydrocarbon IV (Table VI, Fraction No.2) had the following properties:

b.p. 101° (bath/2 mm)

$(\alpha)_D^{20}$ 72° (c, 4.0)

Mol. weight 204 (mass spectrum)

Analysis

Found: C, 88.10; H, 11.90%

Calculated for $\text{C}_{15}\text{H}_{24}$ C, 88.16; H, 11.84%

IR bands (Fig. 12) (liquid film) 2850, 1640, 1460, 1365, 1260, 1105, 1025, 1000 and 880 cm^{-1} .

NMR spectrum (Fig. 13)

δ 0.85 and δ 0.98 (3H $2 \text{CH}_3 - \text{CH}$)

δ 0.76 (3H; $J = 6\text{Hz}$) δ 4.63 and δ 4.98

(2H, $>\text{C} = \text{CH}_2$)

Isolation of hydrocarbons from oil of gurjun balsam

The gum gurjun balsam (steam distilled) oil was dried over anhydrous sodium sulphate and traces of moisture was removed by heating under reduced pressure. The oil (25 g) was separated into three major fractions by column chromatography eluting with pet. ether (Fraction A), benzene (Fraction B) and ether (Fraction C) respectively. The details of chromatography are shown below:

Table I

Wt. of gurjun balsam	25 g
Wt. of alumina Gr. II	500 g.

S.No.	Eluent	Vol. of eluant (ml)	Wt. of fractions (g)
A	Pet. ether	500	17.6280
B	Benzene	500	3.5630
C	Ether	500	2.8750
			24. 061
Recovery 96%			

Fraction A

IR band (Fig. 15 liquid film) 2900, 1640, 1450, 1370, 1355, 1280, 1175, 1100, 1015, 955, 880, 870, 840 and 830 cm^{-1} .

IR spectrum (Fig. 15) of pet. ether fraction (Table I fraction No.A) showed the absence of alcoholic and carbonyl groups. GLC analysis showed the presence of two hydrocarbons.

Isolation of hydrocarbons

The pet.ether fraction A (Table I) was rechromatographed over alumina Gr. I and several fractions were collected.

Table II

Weight of fraction A	15 g
Weight of alumina Gr. I	300 g.

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fractions (g)	GLC of hydrocarbons	
				I	II
1.	Pet.ether	100	3.7200	60 : *	40
2.	"	100	3.2500	60 :	40
3.	"	100	3.2600	60 :	40
4.	"	100	2.9800	50 :	50
5.	"	100	1.8400	50 :	50
			14.7200		

Isolation of hydrocarbon I and Hydrocarbon II

Fractions No.I and II (Table II) were mixed and rechromatographed over silica gel impregnated with silvernitrate

(20%).

Table III

Wt. of fraction 5 g
 Wt. of silica gel impregnated
 with AgNO_3 (20%) 200 g

S.No.	Eluent	Vol. of eluent (ml)	Wt. of fractions(g)	GLC of hydrocarbons	
				I	II
1.	Pet. ether	50	0.7790	95	
2.	"	50	1.0810	100%	
3.	"	50	0.8200	80 :	20
4.	"	50	0.6190	30 :	70
5.	"	50	0.5490	10 :	90
6.	"	50	0.5320		100
7.	"	100	0.2780	:	98
			4.6280		
Recovery 97%					

The fractions No.2 and No.6 (Table III) were distilled separately over sodium under reduced pressure.

Hydrocarbon I $\text{C}_{15}\text{H}_{24}$ (Caryophyllene) VI

Hydrocarbon I obtained from the fraction No.2 (Table III) had the following properties b.p. $87-88^\circ$ (bath)/2 mm
 IR bands (Fig. 16, liquid film) 2900, 1640, 1450, 1380, 1275, 1250, 1100, 960, 890, 860 and 830 cm^{-1} .

NMR spectrum (Fig. 17)

δ 0.99 (6H, dimethyl) δ 1.58 (3H vinyl methyl
 $\text{CH}_3 - \text{C} = \text{C}$) δ 2.1 (2H, exomethylene) 4.96 (H, vinyl proton)

Hydrocarbon II $C_{15}H_{24}$ (humulene)-XIII

Hydrocarbon II. The fraction No.6 had following properties b.p. 90° (bath)/2 mm.

IR band (Fig. 18 liquid film) 2900, 1650, 1380, 1355, 1280, 1210, 1180, 1090, 970, 885 and 825 cm^{-1} .

NMR spectrum

δ 1.1 (6H dimethyl) δ 1.2 (3H, secondary methyl

δ 1.66 (2H exomethylene)

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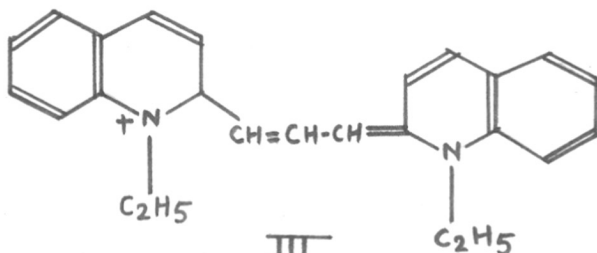
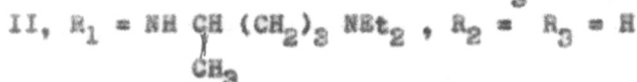
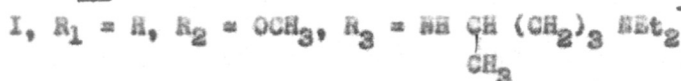
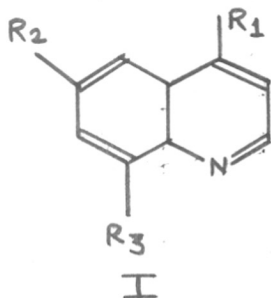
Chapter-III

a) Substituted Quinolines From Citral, Pyruvic Acid & Aromatic Amines

b) Attempted Preparation of 2-Methylene, 6,6-Dimethyl, Bicyclo [3.2.0] Heptane.

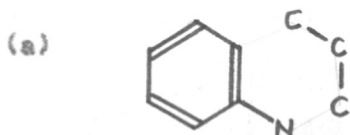
Substituted quinolines from citral, pyruvic acid and aromatic amines

Nitrogen heterocycles form an important group of compounds in the field of medicinal chemistry. Quinoline and substituted quinolines belong to this group¹. The quinoline nucleus is contained in several groups of alkaloids e.g. quinine, in a number of synthetic materials with important physiological properties, such as antimalarials plasmoquin (I) and Chloroquin (II), in the cyanin dye pinacyanol (III) and in the analytical reagent 8-hydroxyquinoline (IV).

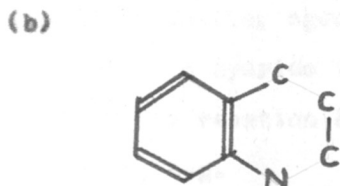


Synthetic Methods

The nucleus is formed in one of the two ways:



Skraup², Doebner-von Miller³,
Doebner (using pyruvic acid)⁴



Friedlaender⁵ and
Pfitzinger synthesis⁶

1. The Skraup synthesis

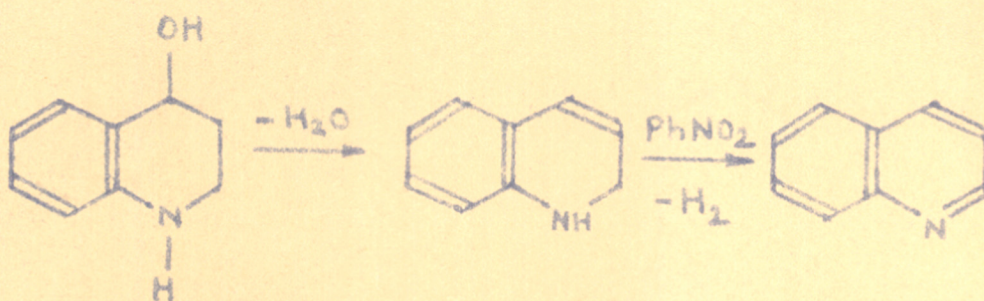
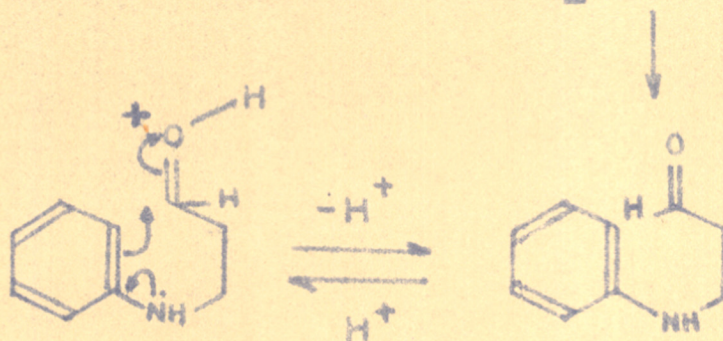
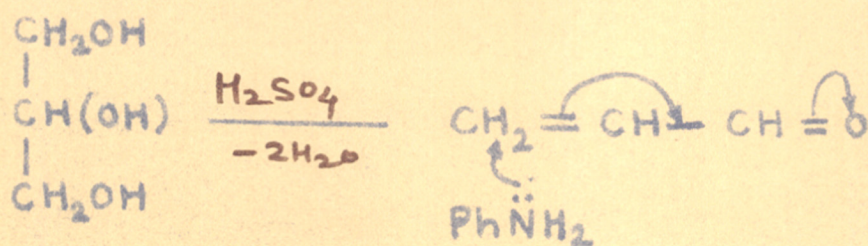
In the simplest case, aniline, glycerol, nitrobenzene, ferrous sulfate and sulfuric acid are mixed and gently heated. A vigorous exothermic reaction occurs which is completed by further heating. The reaction involves dehydration of glycerol to acrolein, Michael-type addition of aniline to acrolein, acid catalysed cyclisation and dehydrogenation by nitrobenzene. Ferrous sulfate acts to moderate the reaction. The mechanism involved is indicated below.

Synthetic applications

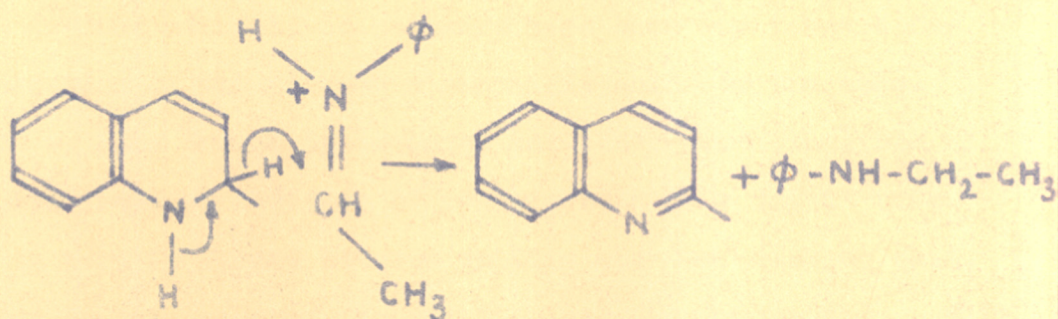
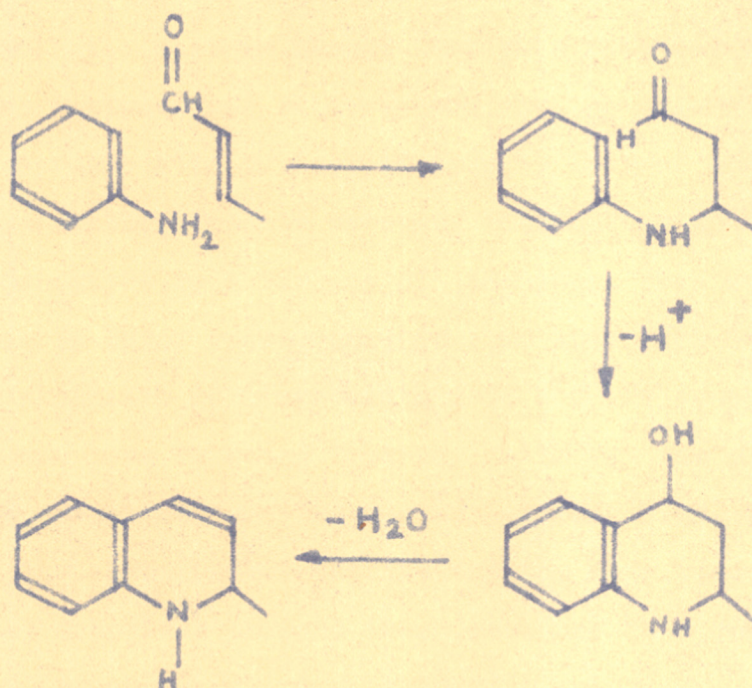
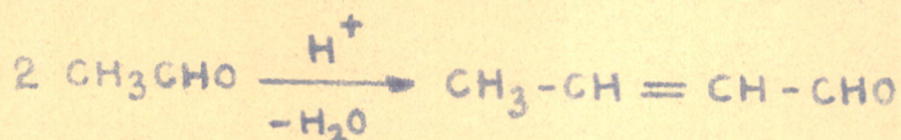
The aniline may contain nuclear substituents e.g. o and p substituted anilines give 8 and 6 substituted quinolines respectively and meta substituted anilines give a mixture of 5 and 7 substituted quinolines, the latter predominating with activating substituents such as methoxyl and the former predominating with deactivating substituents such as nitro group. Acrolein may be replaced by crotonaldehyde or methyl vinyl ketone, giving 2 and 4 methyl substituted quinolines respectively.

2. The Doebner- von Miller Synthesis

This differs from the Skraup method only in the following way. The three carbon fragment is formed in situ by an acid-catalysed aldol condensation without addition of a dehydrogenating agent. It is considered that the reaction involves a hydride transfer to the Schiff base which is formed by reaction between aniline and the aldehyde or ketone, e.g.



The Skraup Synthesis



The Döebner-von Miller Synthesis

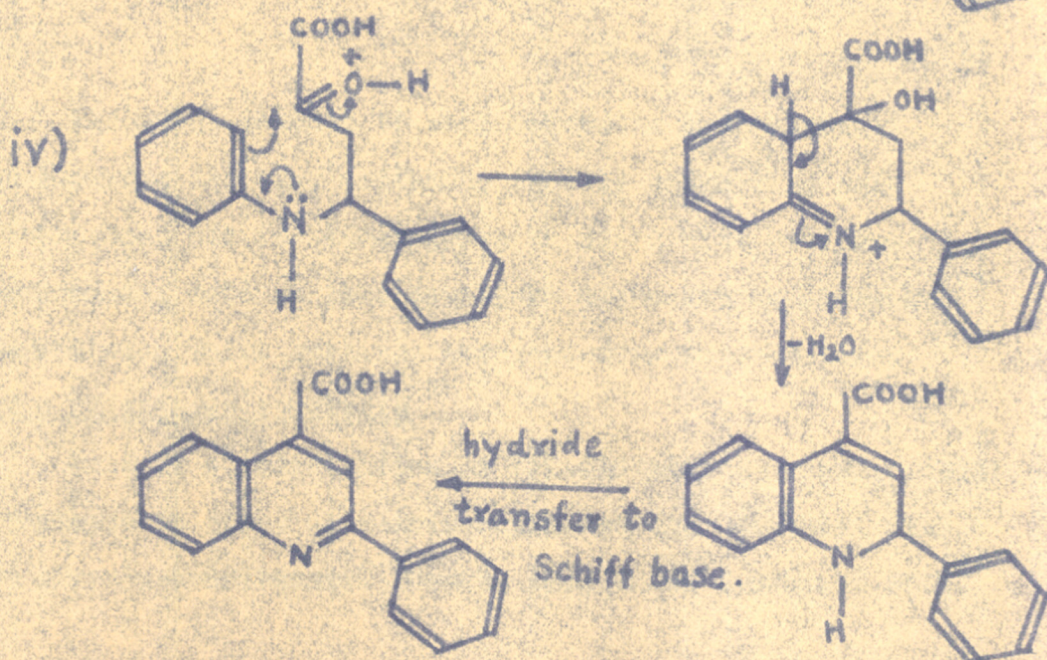
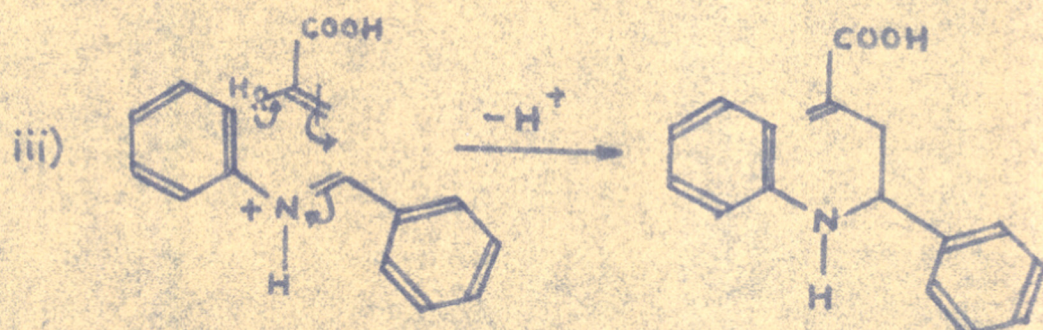
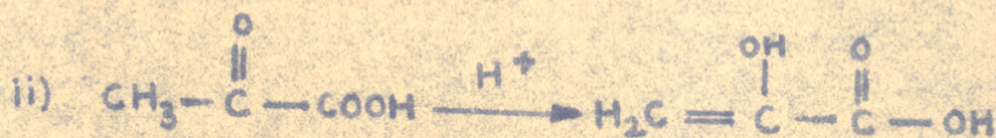
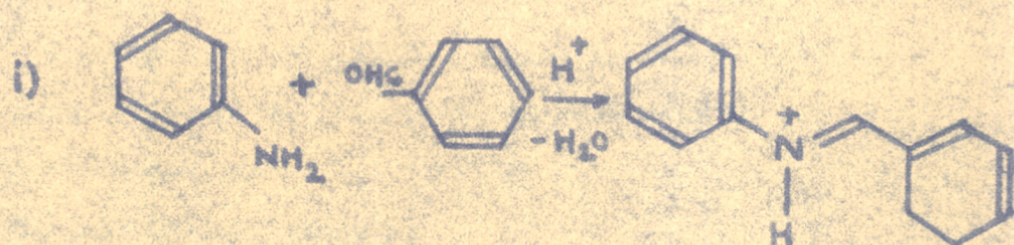


3. Doebner's Pyruvic acid synthesis

This modification of the above method employs the condensation of aniline with equimolecular mixture of pyruvic acid and acetaldehyde. The resulting product is 2-methyl, 4-carboxy quinoline. Doebner showed that this reaction in this particular instance can be accounted for, by partial conversion of pyruvic acid to acetaldehyde by loss of carbon-dioxide. Subsequently, Doebner extended this method by replacing acetaldehyde by a variety of aldehydes, both aromatic and aliphatic. In later years, considerable attention has been given to this synthesis of cinchoninic acid and the scope of this reaction now been extended further. Although the yields of substituted quinolines are frequently not very high, the simplicity of the experimental details and the easy accessibility of the starting materials commend this procedure as a convenient source of substituted quinoline 4-carboxylic acids.

Mechanistic considerations

Several mechanistic pathways have been put forward for the formation of substituted cinchoninic acids by the above procedure. The presently accepted and hence most plausible mechanism involves addition of pyruvic acid to a Schiff base, which in turn is formed between aniline and the aldehyde used, followed by ring closure, dehydration and dehydrogenation. This is further supported by the fact that addition of pyruvic acid

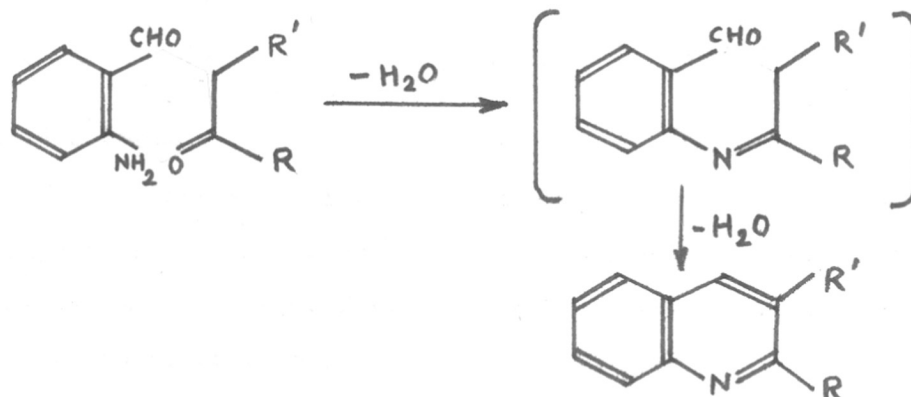


to a preheated mixture of aniline and aldehyde results in the formation of similarly substituted quinoline derivative.

Condensation of aniline, benzaldehyde and pyruvic acid gives cinchophen. The probable mechanism is presented in the following page. (p. 97)

4. The Friedlaender Synthesis

An o-aminobenzaldehyde is treated with an aldehyde or ketone; formation of a Schiff base is followed by dehydrative cyclisation.

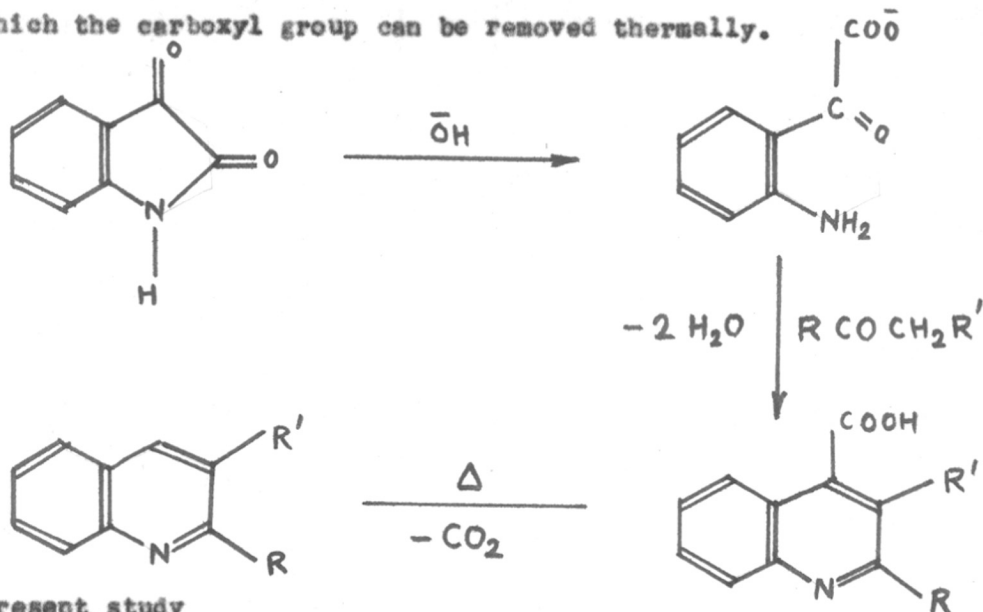


The main drawback of this method is that o-amino-benzaldehydes are very unstable, readily undergoing self condensation. It is advantageous to start with o-nitro-benzaldehyde, acid or base catalysed condensation gives an intermediate, which cyclizes spontaneously on reduction.

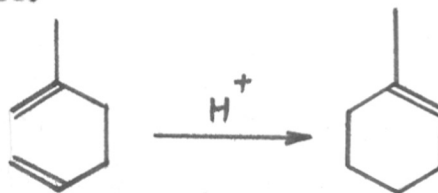
5. The Pfitzinger Synthesis

This is a modification of Friedlaender's method. Isatin is employed in place of o-aminobenzaldehyde. Reaction with base gives o-aminobenzoyl formate ion, which condenses with

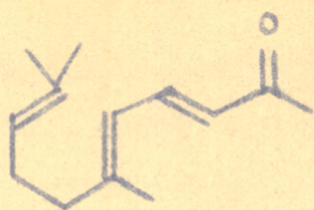
an aldehyde or ketone to give a 4-carboxyquinoline from which the carboxyl group can be removed thermally.



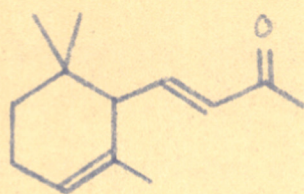
The formation of six membered rings by acid catalysed cyclisation of 1,5-dienes is a well-known reaction which has been observed to occur frequently with terpenoids. A typical example is given below.



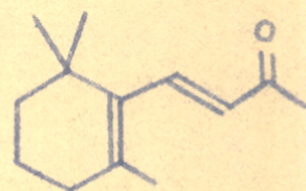
The structural requirements for such cyclisations have been discussed by Hibbit and Linstead⁷. Special mention may be made of the cyclisation of α -ionone (V) to α and β -ionones⁸ (VI and VII respectively), geranic acid (VIII) to



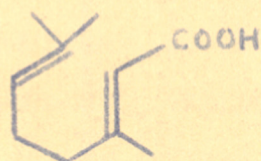
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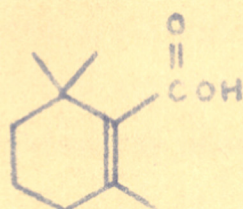
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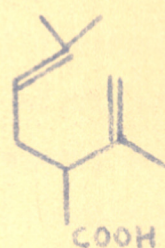
VII



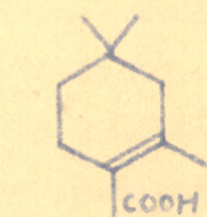
VIII



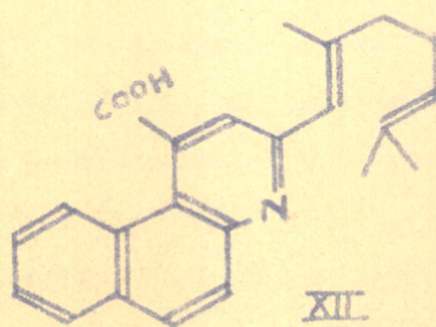
IX



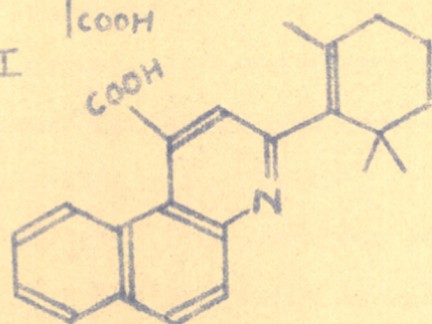
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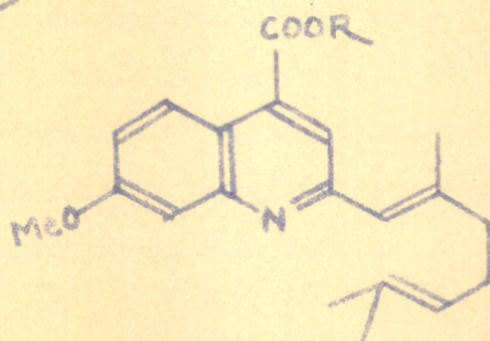
XI



XII



XIII



XIV

β -cyclogeranic acid⁹ (IX), lavandulic acid (X) to β -cyclo-lavandulic acid¹⁰ (XI).

Doebner reported that condensation of citral with β -naphthylamine in presence of pyruvic acid gives a crystalline product, m.p. 199-200°. Both citral 'a' and 'b' give the same product having structure¹¹ (XII).

On the basis of reported cyclisation of 1,5-dienes under acidic conditions, an alternative structure (XIII) seemed equally probable. The correctness or otherwise of the new structure can be inferred by NMR data, which, however, was not available in the literature. In order to learn whether the cyclisation of the 1,5-diene system is taking place or not, the condensation product reported by Doebner has been prepared by the literature procedure and studied by present-day standards. In the studies recorded below, we offer evidence in support of the structure (XII). We also describe the results obtained by replacing β -naphthylamine by *m* and *p*-anisidines.

By following Doebner's procedure, a mixture of citral and β -naphthylamine in absolute alcohol was added to freshly distilled pyruvic acid in absolute alcohol, and the contents were refluxed for three hours on a water-bath. Usual work up gave a solid, m.p. 190-95°. The crude product on recrystallisation from absolute ethanol gave yellow leaflets m.p. 201-2°. The substance is undoubtedly the citryl-naphtho-cinchoninic acid reported by Doebner¹¹. It analysed for

$C_{23}H_{23}O_2N$. This elemental composition was also clear from its mass spectrum (m/e 345, M^+). The methyl ester obtained by esterification with diazomethane also showed a molecular weight 359 (mass spectrum). The IR spectrum of citryl-naphthocinchoninic acid (Fig. 1), though not much informative, can be used for identification purposes. Its NMR spectrum in CF_3COOH (Fig. 2) revealed the presence of three methyl groups attached to unsaturated carbons (δ 1.72, 6H, 2.29; 3H). These features, in conjunction with the already accepted mechanism for Doebner's pyruvic acid synthesis of cinchoninic acids, are only explicable by the Doebner's formula¹¹ (XII). This only observation, clearly indicates that no cyclization of the 1,5-diene system of the side-chain has taken place. Citryl-naphthocinchoninic acid must therefore be represented by structure (XII).

Doebner recommended the preparation of the above derivative for characterization of citral. In view of the high cost and the non-availability of β -naphthylamine, we considered that preparation of other derivatives by replacing β -naphthylamine by other aromatic amines such as *m* and *p*-anisidines would be useful.

Under identical experimental conditions (*vide supra*), *m*-anisidine, citral and pyruvic acid gave a crystalline product *m.p.* 161° to which we assign structure (XIV $R = H$). Its IR spectrum is reproduced in Fig. 3. The 7-methoxy citryl-cinchoninic acid thus obtained was converted into its

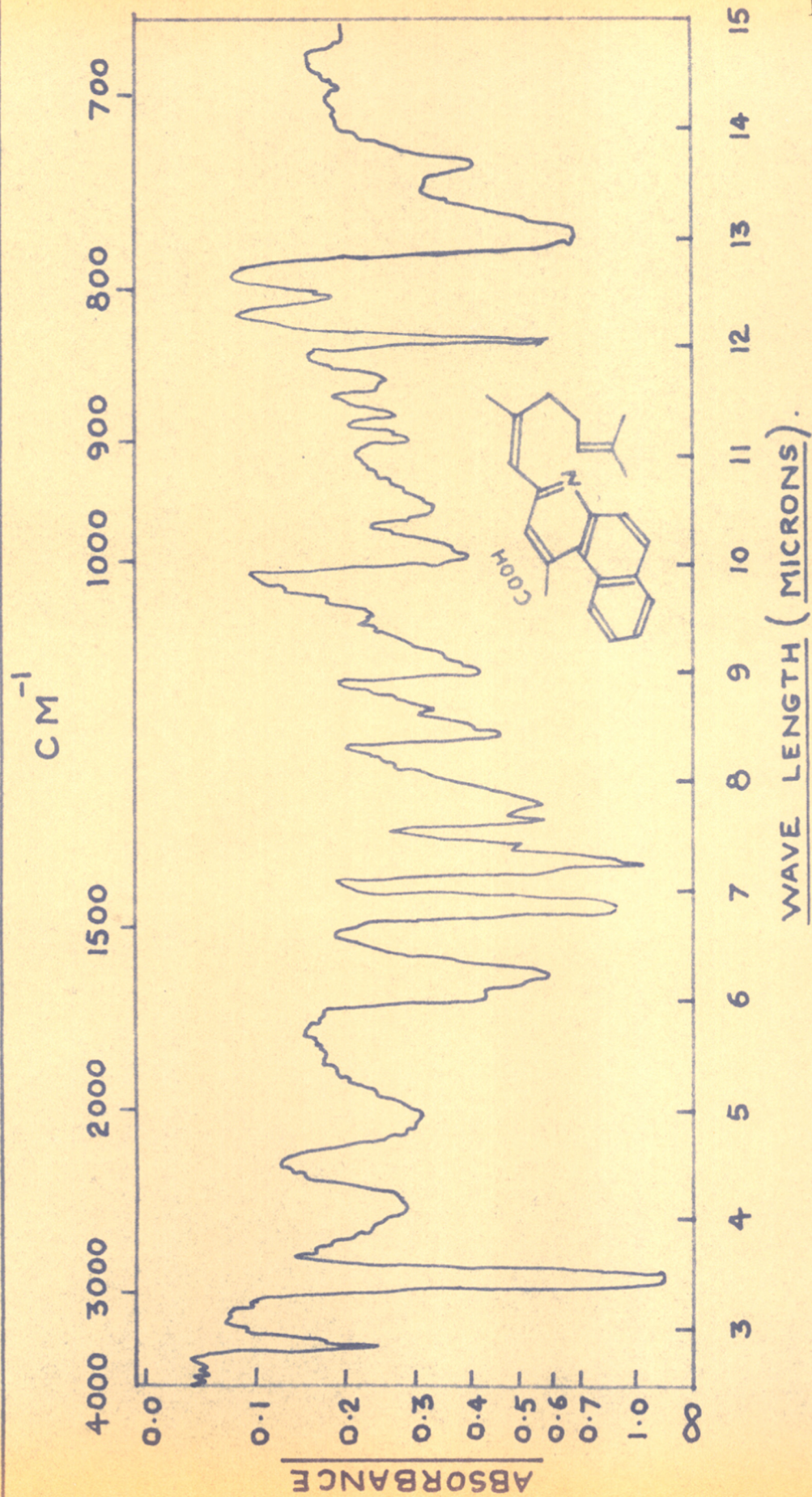
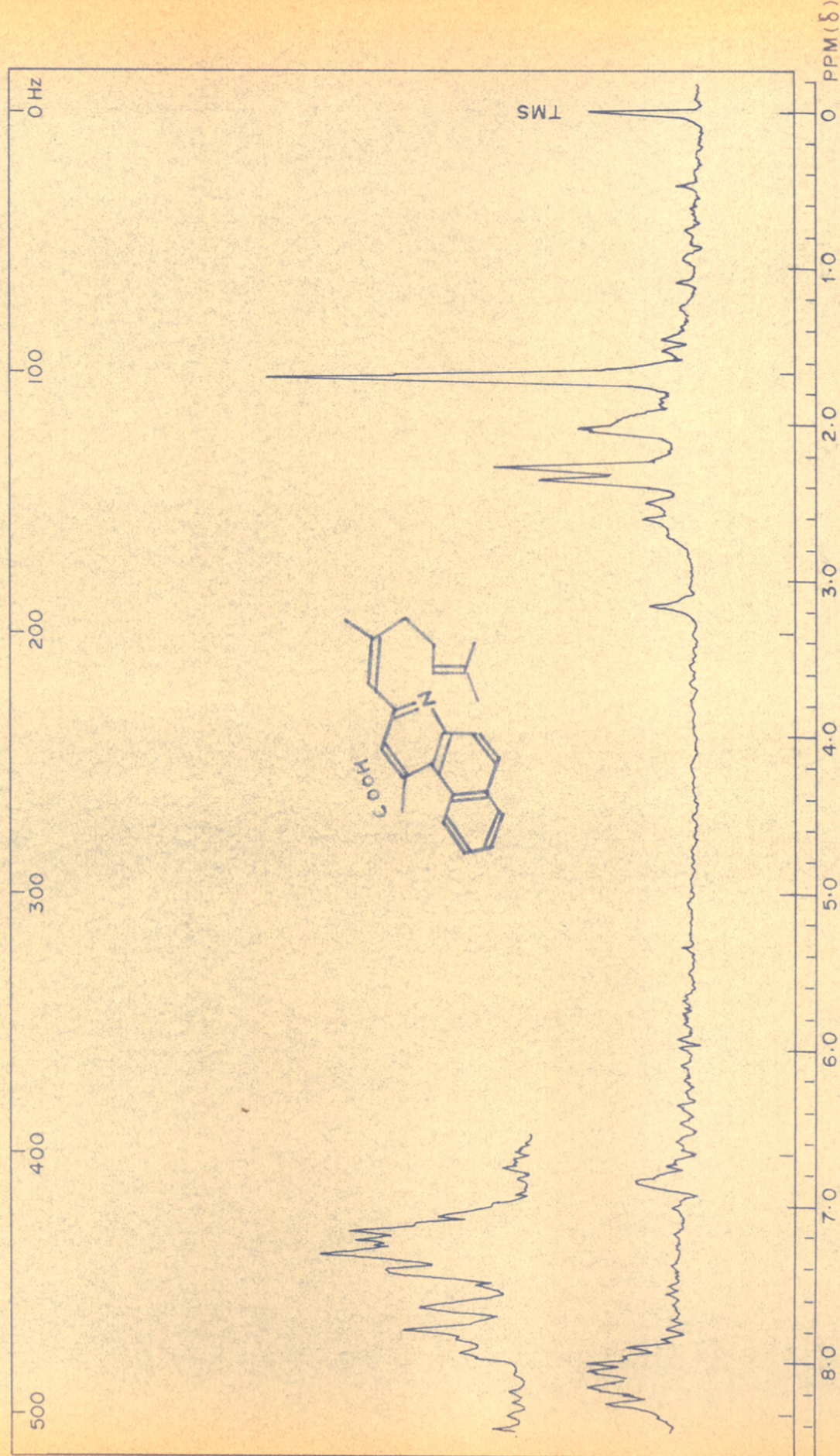


FIG.1 IR SPECTRUM OF CITRY-NAPHTHOCINCHONIC ACID (IN NUTOL)

FIG. 2 NMR SPECTRUM (CF_3COOH) OF CITRYLNAPHTHOCINCHONIC ACID

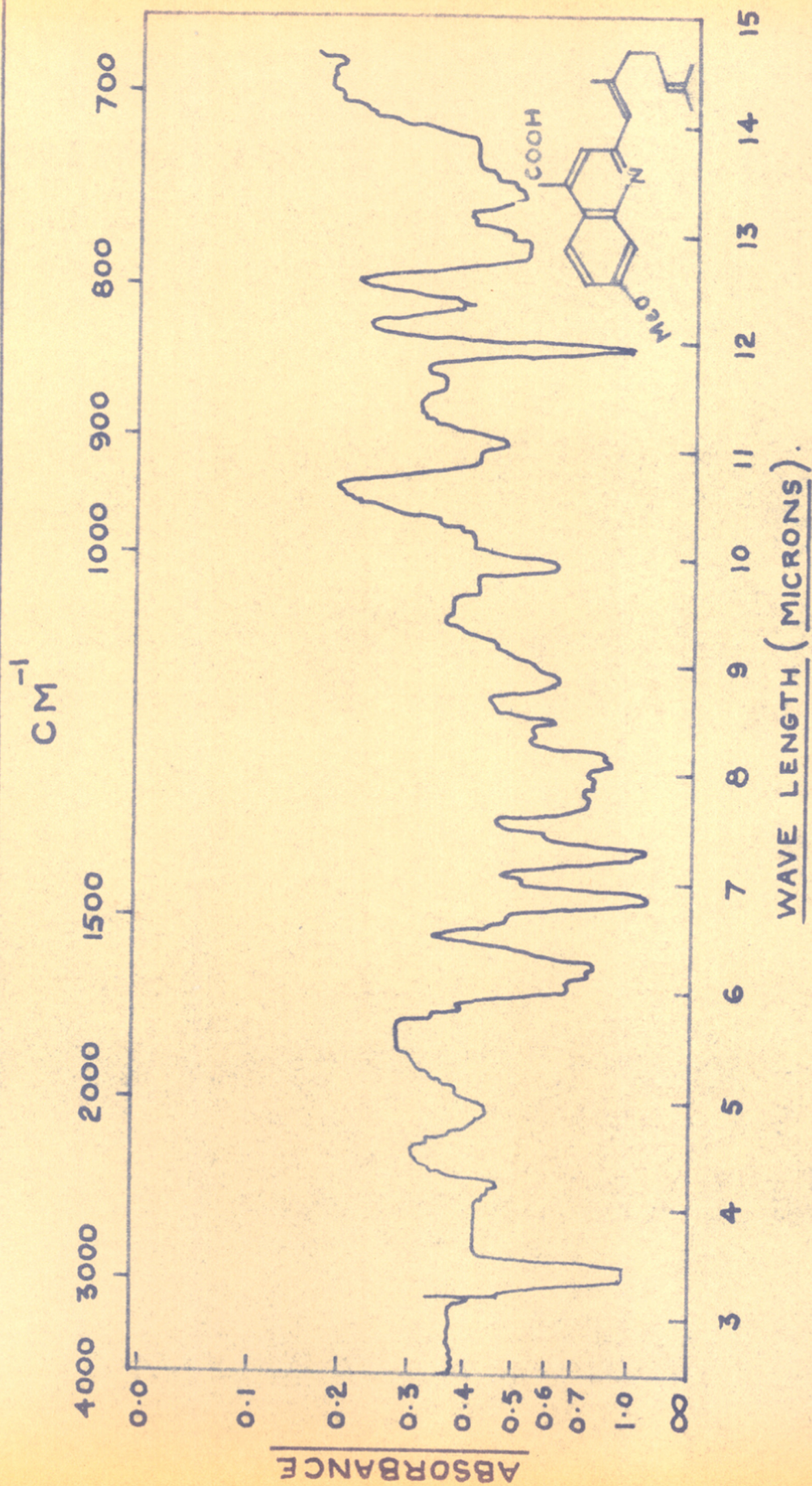


FIG. 3 IR SPECTRUM OF 7 METHOXY CITRYLCINCHONIC ACID (in nujol).

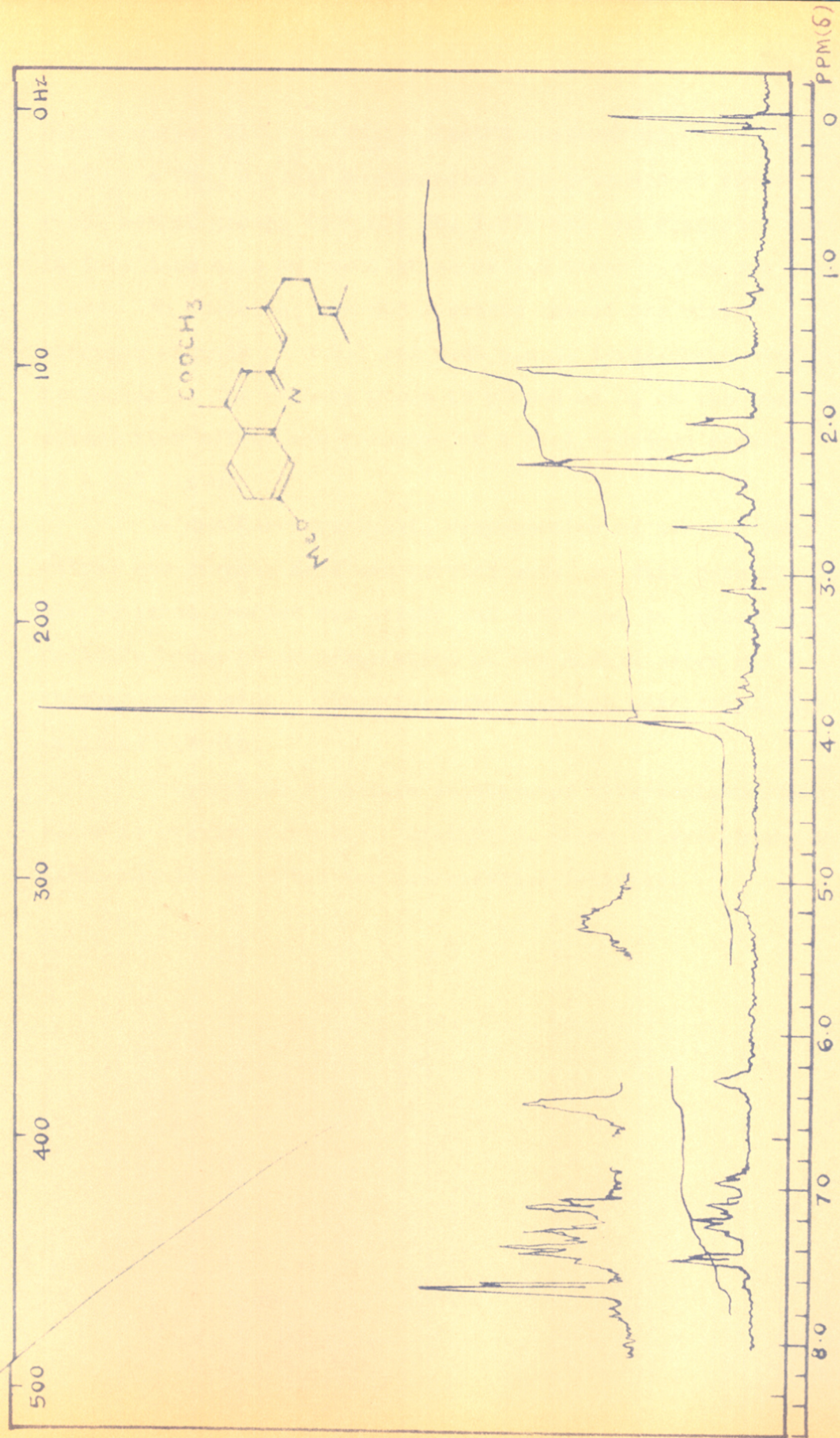


FIG. 4 NMR SPECTRUM OF 7 METHOXY CITRYL- GINCHONIC ACID METHYL ESTER (IN CCl_4).

methyl ester (XIV, $R = CH_3$). The NMR spectrum of (XIV, $R = CH_3$, Fig. 4) demonstrated the presence of three vinyl methyl groups (δ 1.67; 6H, 2.27; 3H) and suggested that, in this case also no cyclisation of 1,5 diene system has taken place. In addition, the NMR spectrum showed the vinyl hydrogen signals at δ 5.1 and 6.25 p.p.m. (side-chain olefinic hydrogens). The methoxy group is placed at C_7 on the basis of orientation effect and presence of a peri hydrogen signal at δ 7.5 p.p.m.

In another experiment, condensation of p-anisidine, citral and pyruvic acid was carried out. In this case however, the reaction product was sticky and could not be induced to solidify, even after preparation of the methyl ester and further processing. Chromatography also did not lead to any positive results.

Preparation of chloro substituted citryl-cinchoninic acids is highly desirable. Similarly, these citryl-cinchoninic acids should be tested for antimalarial activity.

Attempted preparation of 2-methylene, 6,6 dimethyl, bicyclo (3.2.0) heptane

Twenty or so years ago, the suggestion of an epoxide group as a structural feature in a natural product would have provoked immediate scepticism. It was a rarity to say the least. Since then many terpenoids have been found in nature which possesses this feature. One such monoterpene, Δ^3 -carene-5,6 epoxide (XV) was ~~pre~~ reported to occur in the Australian plant Ziria smithii¹² (Fam: Rutaceae). Commenting on the reactions of Δ^3 -carene-5,6 epoxide, Blanchard¹³ correctly reformulated as shown in (XVI), and suggested its identity with chrysanthenone. Subsequently, the validity of this interesting suggestion was confirmed by Bates et al.¹⁴ These investigators have established that Simonsen's sample of Δ^3 -carene-5,6 epoxide was a mixture of chrysanthenone (XVI, 70%), cyclobutanone* (XVII, 20%) and linalool (XVIII, 10%).

This observation was followed by isolation of (-) filifolone (XIX), an optical antipode of (XVII)¹⁴. This was the first report of the natural occurrence of a bicyclo-(3.2.0) heptane skeleton among monoterpenes.

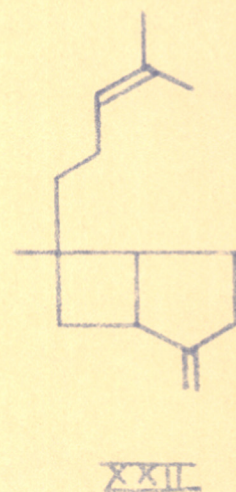
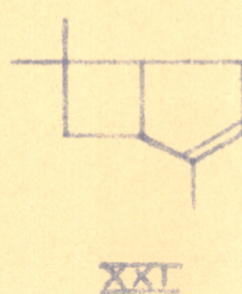
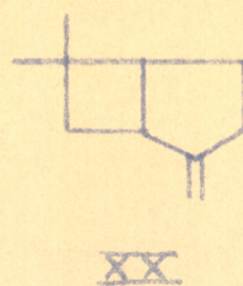
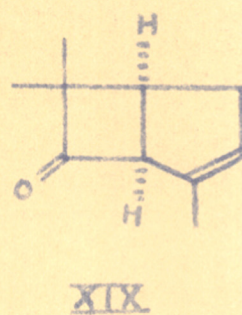
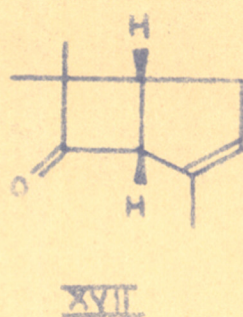
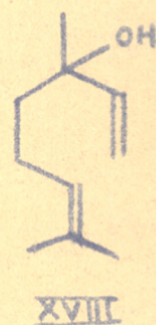
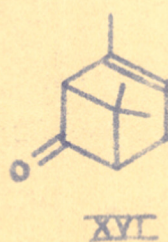
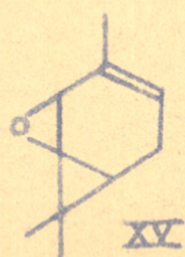
Present study

Although the natural occurrence of (XX) and/or (XXI) is not reported so far we anticipate such hydrocarbons on biogenetic considerations. We, therefore, considered it worthwhile to synthesise (XX) and (XXI). We were particularly interested in (XX), because, the spectral data on the synthetic sample would either support or reject the structure (XXII) assigned¹⁶ to one of the photo-products of β -farnesene**.

The four membered ring having a gem-dimethyl group, a characteristic feature in caryophyllene¹⁸ (XXIII) led us to consider it as a starting material for the synthesis of title

*The racemic product was obtained earlier by Beereboom¹⁵ by cyclisation of geranic acid.

**Photo- β -farnesene has now been shown to be identical with trans- β -bergamotene¹⁷.



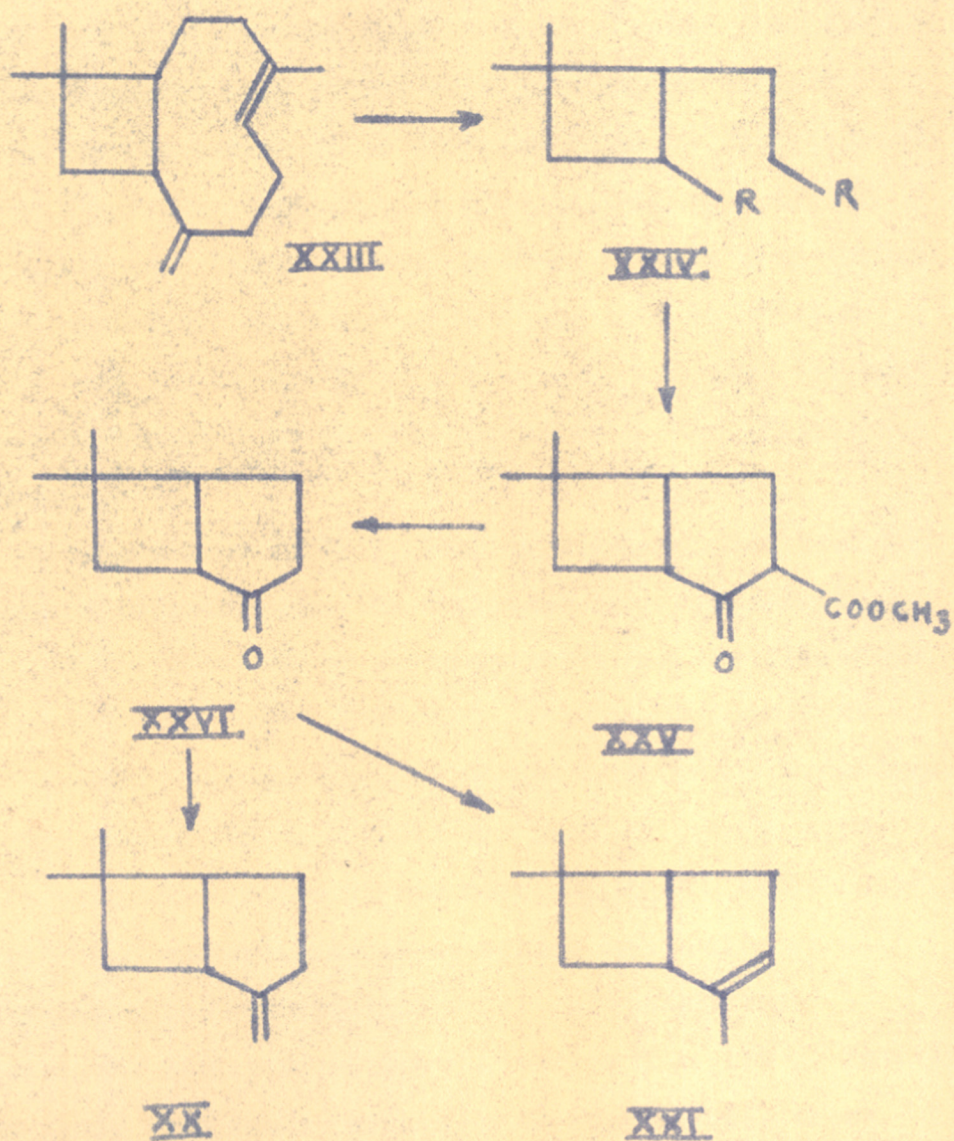


CHART - I

hydrocarbons having a bicyclo (3.2.0) heptane skeleton. The synthetic scheme which is outlined in Chart I, consists of conversion of homocaryophyllenic acid¹⁹ (XXIV, R = COOH), the dimethylester of which on Dieckmann cyclisation followed by hydrolysis and decarboxylation would give (XXVI). This five membered ring ketone (XXVI), ought to be readily converted to the hydrocarbons (XX) and (XXI) by the reported procedure²⁰ of the reaction with methyl lithium, followed by dehydration of the resultant tertiary alcohol with thionyl chloride-pyridine. Hydrocarbon (XX) can also be obtained directly from the ketone by reaction with methylene triphenylphosphorane-dimethyl sulphoxide according to the literature procedure²¹.

Caryophyllene (XXIII), the starting material used in the present investigation was available from the laboratory stock. It was purified by chromatography and distillation, b.p. 108-109°/6 mm, n_D^{30} 1.4970, $(\alpha)_D - 10^\circ$. The purified sample appeared homogeneous by TLC/GLC behaviour. Homocaryophyllenic acid (XXIV, R = COOH) was prepared by the hypobromite oxidation of the diketo acid, $C_{14}H_{22}O_4$, an ozonolysis product* of caryophyllene, as described by Jarolim et al.²² The dibasic acid thus obtained (XXIV, R = COOH) was characterized by its dianilide derivative m.p. 182°. However, in our hands this route proved to be impractical, since

*In several experiments, it was observed that only the trisubstituted double bond gets cleaved preferentially.

relatively large quantities of homocaryophyllenic acid could not be obtained. Besides, the yields were poor, and purification also offered many difficulties. The infrared spectrum (Fig. 5) showed the presence of carboxyl group (broad absorption between $3300-2700\text{ cm}^{-1}$). Its NMR spectrum (Fig. 6) showed a six six proton singlet (δ 1.00) due to two tertiary methyls and a two proton singlet (δ 10.83) assignable to $-\text{COOH}$ hydrogens.

With ethereal diazomethane, homocaryophyllenic acid gave a dimethyl ester (XXIV, $\text{R} = \text{COOCH}_3$), whose IR spectrum showed carbonyl absorption at 1750 cm^{-1} . The NMR spectrum indicated a diester (δ 3.68 s, 6H) and a gem-dimethyl group (δ 1.10 s, 3H, 1.07 s, 3H).

The desired diester, although available in small quantity, was used for the next step, viz. Dieckmann condensation.

In view of a very small amount of the diester (XXIV, $\text{R} = \text{COOCH}_3$) available for further reactions and different experimental conditions that can be used for Dieckmann cyclisations, it was decided to carry out this reaction on a model compound. While diethyl adipate gave a satisfactory yield of the desired product (Na and dry xylene, reflux)²³, the dimethyl ester of homocaryophyllenic acid, under identical experimental conditions failed to give the keto-ester (XXV)

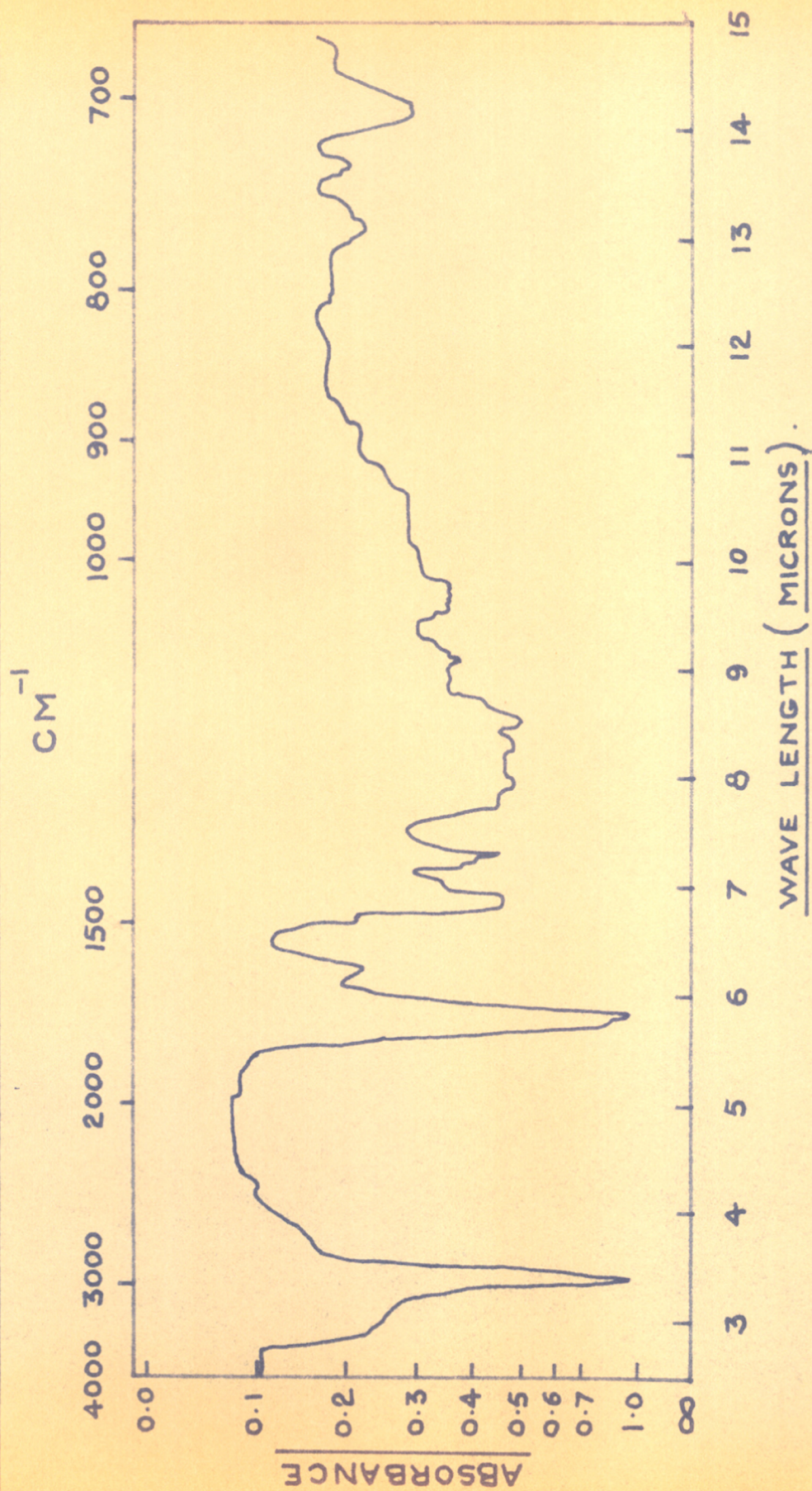


FIG. 5 IR SPECTRUM OF HOMOCARYPHYLLENIC ACID (NEAT).

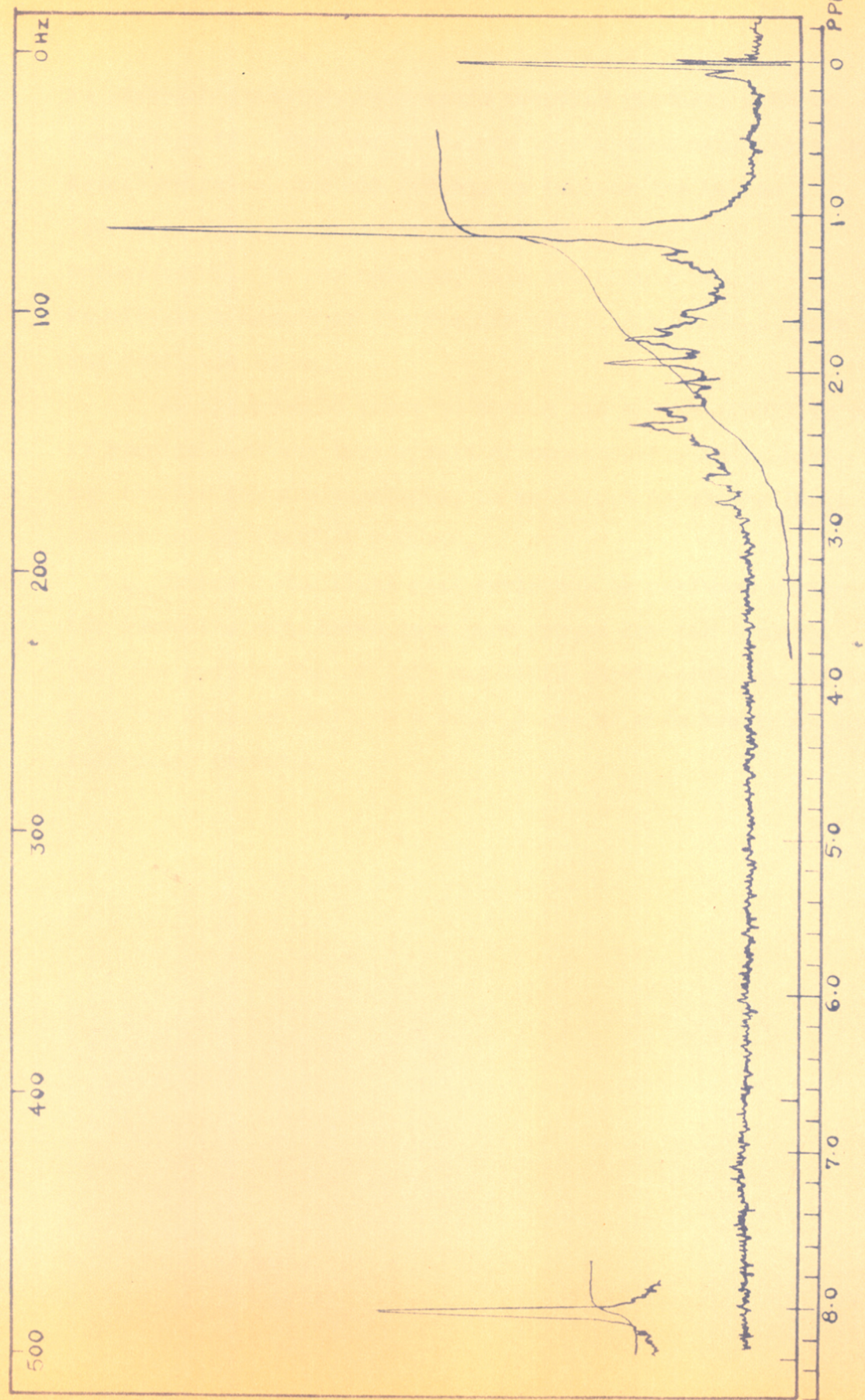


FIG 6 NMR SPECTRUM OF HOMOCARYOPHYLLENIC ACID (IN CCl_4)

in workable quantity. The crude reaction product, without further purification was subjected to alkaline hydrolysis and decarboxylation, but all efforts to isolate the ketone (IR, Fig. 7) via its semicarbazone have so far been unsuccessful. In our opinion, this was mainly due to insufficient amount of the diester (XXIV, $R = \text{COOCH}_3$) which was available to us.

An alternative route to obtain the ketone (XXVI) is the $\beta\gamma$ pyrolysis of the barium salt of homocaryophyllenic acid. Our studies are continuing in an effort to obtain large quantities of (XXIV, $R = \text{COOCH}_3$).

Although the final goal could not be achieved during the present study, there is a good ground for the feeling that the preparation of (XX) and (XXI) should offer no difficulty, once the diester (XXIV, $R = \text{COOCH}_3$) can be made available in sufficient amounts.

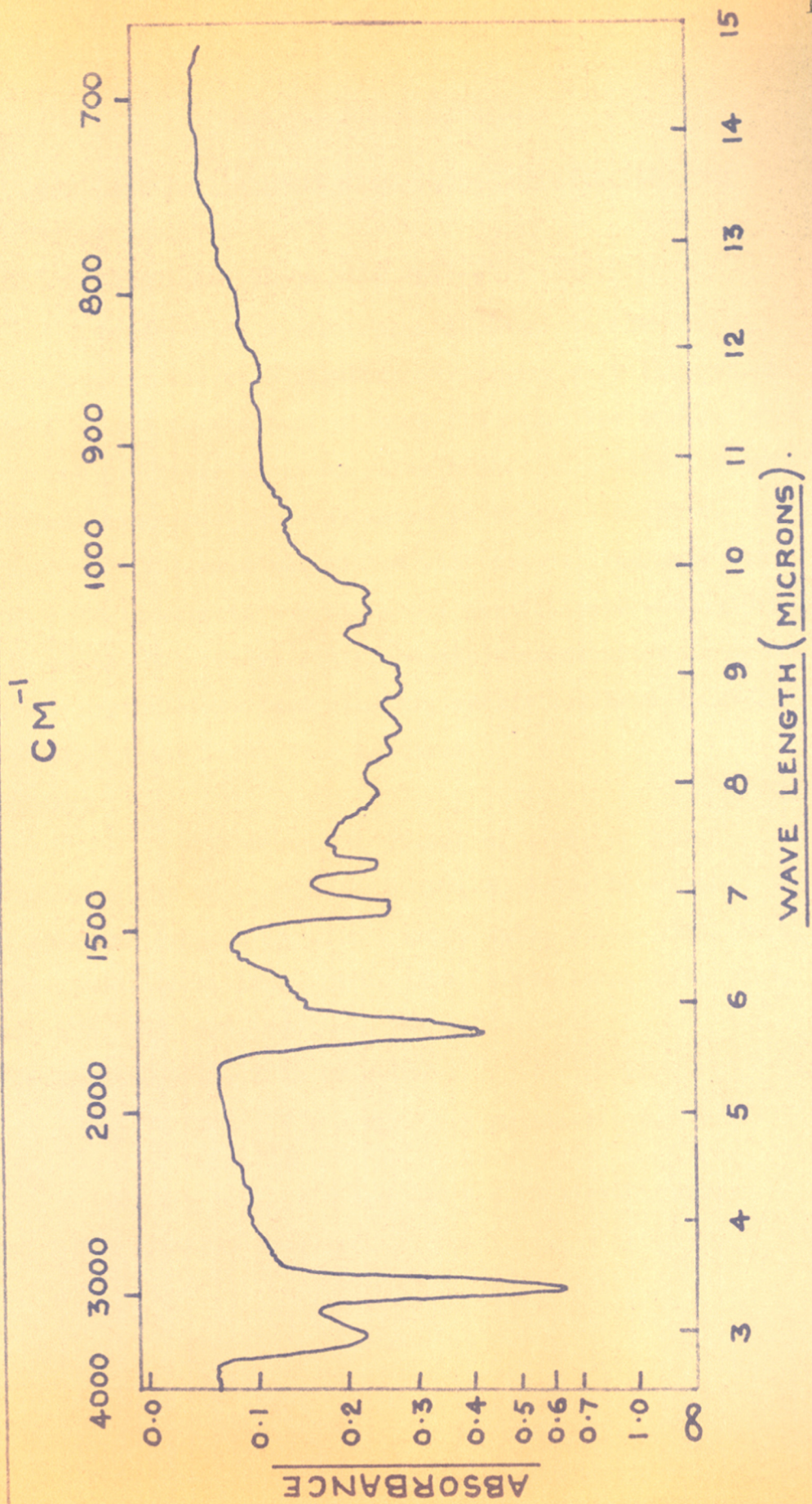


FIG. 7 IR SPECTRUM OF THE REACTION PRODUCT WHICH CONTAINS KETONE (NEAT).

EXPERIMENTAL(A) Substituted quinolines from citral pyruvic acid and aromatic aminesCitryl-naphthocinchoninic acid $C_{23}H_{23}O_2N$ (XII)

A solution of β -naphthylamine (2 g) in ~~abs~~ absolute alcohol was added to a solution of pyruvic acid (1.2 g) and citral (2 g) in absolute alcohol (10 ml). The mixture was refluxed for three hours on water bath. On cooling, citryl-naphthocinchoninic acid separated in crystalline form. The product was filtered and washed with ether. The acid was dissolved in ammonia and filtered; the filtrate was acidified with acetic acid where citryl-naphthocinchoninic acid precipitated. It was crystallised from alcohol, yellow leaflets (0.89 g) m.p. 201° mol.wt. 345 Mass spectrum.

Analysis

Found: C, 79.82; H, 6.90; N, 3.96%

Calculated for $C_{23}H_{23}O_2N$ C, 79.97; H, 6.71; N, 4.06%.

IR spectrum (Fig. 1 in nujol) 3500, 2850, 2400, 2000, 1650, 1600, 1455, 1370, 1340, 1300, 1275, 1180, 1150, 1110, 990, 895, 875, 855, 825, 800, and 765 cm^{-1} .

NMR spectrum (Fig. 2 in CF_3COOH)

δ 1.72 (6H $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{C} = \text{O} \\ \diagdown \\ \text{CH}_3 \end{array}$) δ 2.29 and δ 2.38

(3H, $\text{CH}_3 - \text{C} \begin{array}{c} \text{H} \\ \diagup \\ \text{C} = \text{C} \end{array}$) δ 5.1 and δ 6.25 vinyl protons

and δ 7.5 aromatic hydrogen.

Methyl ester of citrylnaphthocinchoninic acid $C_{24}H_{25}O_2N$

A solution of citrylnaphthocinchoninic acid (0.4 g) in absolute alcohol was added to an ethereal solution of diazomethane until gas evolution ceased. The mixture was stirred for half an hour and washed with aqueous potassium hydroxide solution (5%) and water. The ethereal solution was dried over anhydrous sodium sulphate and removal of solvent gave citrylnaphthocinchoninic acid methyl ester (0.38 g). The ester was purified by distillation b.p. 102° (bath)/2 mm.

Mol. weight 359 mass spectrum.

Analysis

Found: C, 79.67; H, 7.10 N, 3.92

Calculated for $C_{24}H_{25}O_2N$: C, 80.19; H, 7.01; N, 3.90

NMR spectrum

δ 1.72 (6H , $C=C$), δ 2.29 and δ 2.38
 3H, $CH_3 - C \begin{smallmatrix} H \\ \diagup \end{smallmatrix}$ δ 3.88 (3H )

δ 5.1 and 6.25 vinyl proton and δ 7.5 aromatic hydrogen.

7-Methoxycitryl-cinchoninic acid $C_{20}H_{23}O_3N$

A solution of m-anisidine (1 g) in absolute alcohol was added to a solution of pyruvic acid (1.2 g) and citral (1 g) in absolute alcohol (10 ml). The mixture was refluxed for three hours on water bath. On cooling 7 methoxy citryl-cinchoninic acid separated in crystalline form. The acid was filtered and washed with ether and was purified by dissolving in ammonia and precipitating from the filtrate by acetic acid.

The product was crystallised from alcohol yellow crystals (0.6 g) m.p. 161°.

Analysis

Found: C, 73.79, H, 7.30, N, 4.18%

Calculated for $C_{20}H_{25}O_3N$ C, 73.82, H, 7.12, N, 4.30%

IR spectrum (Fig. 3 in nujol) 2800, 2300, 2110, 1710, 1660, 1600, 1560, 1540, 1460, 1360, 1240, 1190, 1170, 1130, 1010, 890, 835 and 800 cm^{-1} .

NMR spectrum (in CF_3COOH)

δ 1.67 (6H, $\begin{array}{c} CH_3 \\ | \\ C=C \\ | \\ CH_3 \end{array}$), δ 2.27 (3H $CH_3-C\equiv C-H$)

δ 5.1 vinyl protons and δ 7.5 aromatic hydrogen.

Methyl ester of 7-methoxy citryl cinchoninic acid $C_{21}H_{25}O_3N$

A solution of 7-methoxy citryl cinchoninic acid (0.2 g) in absolute alcohol was added to an ethereal solution of diazomethane until gas evolution ceased. The mixture was stirred for half an hour and ethereal solution was washed with aqueous potassium hydroxide (5%) and water. The ethereal solution was dried over anhydrous sodium sulphate and removal of solvent afforded 7-methoxy citryl cinchoninic methyl ester which was purified by distillation, b.p. 110°(bath)/2 mm.

Analysis

Found: C, 74.27; H, 7.49; N, 4.06%

Calculated for $C_{21}H_{25}O_3N$ C, 74.31; H, 7.42; N, 4.13%

IR spectrum 2800, 2300, 2110, ~~1710~~ 1750, 1660, 1600, ~~1560~~ 1540, 1460, 1360, 1240, 1190, 1170, 1130, 1010, 890, 835 and 800 cm^{-1} .

NMR spectrum (Fig. 4)

δ 1.67 (6H, $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{C} = \text{C} \\ \diagdown \\ \text{CH}_3 \end{array}$) δ 2.27 (3H $\text{CH}_3 - \text{C} = \text{H}$)

δ 5.1 and δ 6.25 vinyl hydrogens δ 7.5

EXPERIMENTAL(B) Attempted preparation of 2-methylene 6,6-dimethyl,
bicyclo (3.2.0) HeptaneCaryophyllene $C_{15}H_{24}$

Caryophyllene obtained from the laboratory stock was purified by chromatography and distillation b.p. $108-10^{\circ}/6$ mm
 $(\alpha)_D^{30} - 10 n_D^{30} 1.4970$.

Homocaryophyllenic acid $C_{10}H_{16}O_4$ (a) Ozonolysis of caryophyllene $C_{15}H_{24}$

Calculated amount of ozone (2 moles) was passed in a solution of caryophyllene (10 g) in ethyl acetate (50 ml), cooled to 0° . The ozonide was diluted with ethylacetate and warmed on water bath. Ethylacetate was removed under reduced pressure and residue was refluxed with distilled water for four hours to decompose the ozonide. The product was extracted with ether (4 x 50 ml) and the combined ether extract was extracted with sodium carbonate solution (10% and the aqueous extract was neutralised with dilute hydrochloric acid and extracted with ether (4 x 50 ml). The ether layer was washed with water and dried over anhydrous sodium sulphate. On evaporation of the solvent a mixture of mono ($C_{11}H_{18}O_3$) and diketo ($C_{14}H_{22}O_4$) to caryophyllenic acid was obtained.

NMR spectrum

δ 1.0 (6H, $\begin{smallmatrix} CH_3 \\ \diagup \\ C \\ \diagdown \\ CH_3 \end{smallmatrix}$), δ 1.99 (3H, $CH_3 - \overset{O}{\overset{||}{C}} -$)
 δ 10.38 (1H, COOH).

(b) Oxidation of mono and diketo acidPreparation of standard sodium hypobromite solution

Sodium hydroxide (88 g 2.2 mole) dissolved in distilled water (364 ml) and bromine (28 ml) was added with stirring at 0° when 1.3 M sodium hypobromite solution was obtained.

The mixture of mono and diketo caryophylllic acid (5 g) in ether was added to a stirred solution of sodium hypobromite (50 ml) at 0°. After two hours rapid stirring, the excess of sodium hypobromite was destroyed by slow addition of sodium bisulfite solution (10%). The reaction product was extracted with ether to remove unreacted ketone and the aqueous layer was acidified with conc. HCl and extracted with ether (5 x 50 ml). The combined ether extract was washed with water till free from acid and dried over anhydrous sodium sulphate. Evaporation of solvent yielded homocaryophyllenic acid (1.278 g) which was purified by distillation b.p. 112°(bath)/6 mm.

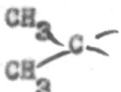
Analysis

Found: C, 60.13; H, 7.97%

Calculated for $C_{10}H_{16}O_4$ C, 59.98; H, 8.05%.

IR spectrum (Fig. # 5) 2950, 1710, 1600, 1460, 1360, and 1160 cm^{-1} .

NMR spectrum (Fig. 6)

δ 1.00 (6H ) and

δ 10.83 (2H, 2 COOH)

Manilide m.p. 182°.

Analysis

Found: C, 75.20; H, 7.44; N, 7.5%

Calculated for $C_{22}H_{26}O_2N_2$ C 75.40; H, 7.48; N, 7.99%.

Dimethyl ester of homocaryophyllenic acid $C_{12}H_{20}O_4$

Homocaryophyllenic acid (1.2 g) in ether was added to an ethereal solution of diazomethane, cooled to 0° . The ether solution was stirred for one hour and washed with aqueous potassium hydroxide (10%) and water and dried over anhydrous sodium sulphate. Evaporation of solvent gave dimethyl ester of caryophyllenic acid (1.1 g) which was purified by chromatography and distillation b.p. 107° (bath)/6 mm.

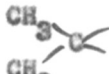
Analysis

Found: C, 63.10; H, 8.76%

Calculated for $C_{12}H_{20}O_4$: C, 63.13; H, 8.83%

IR spectrum 2950, 1750, 1600, 1460, 1360 and 1160 cm^{-1} .

NMR spectrum

δ 1.10 and δ 1.07 (6H ) and
 δ 3.63 (6H, 2 $CH_3O-C=O$)

Cyclopentanone C_5H_8O (via Dieckmann's reaction)

Diethyl adipate (.8 g) was added to sodium (.2 g) suspended in xylene (50 ml) at $115-120^\circ$ under vigorous stirring. After refluxing for four hours the reaction mixture was cooled and carefully treated with acetic acid (10%). The xylene layer was washed with water and dried over anhydrous sodium sulphate. After removal of xylene the β -keto ester was hydrolysed with alkali. The product after working up gave cyclopentanone (.27 g) b.p. 132° semicarbazone/m.p. 200° .

Attempted cyclisation of dimethyl ester of homocaryophyllenic acid

Dimethyl ester of homocaryophyllenic acid (.5 g) in xylene was added to sodium (.18 g) suspended in xylene (40 mg) at 115-120° under vigorous stirring. After refluxing for four hours, the reaction mixture was cooled and carefully treated with acetic acid(10%). The xylene layer was washed with water and dried over anhydrous sodium sulphate. After removal of the crude material (.3) was subjected to alkaline hydrolysis for decarboxylation.

The expected ketone (IR, Fig. 7) did not form any semi-carbazone and could not be isolated in pure form.

IR spectrum (Fig. 7) 2950, 1740, 1460 and 1370 cm^{-1} .

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