STUDIES IN ESSENTIAL OILS

A
THESIS
SUBMITTED TO
THE UNIVERSITY OF POONA
for
THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY

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

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AUGUST 1963.

M. L. Maheshwari
26.8.63.

C O N T E N T S

INTRODUCTION

This thesis embodies results of experiments on the essential oil from agarwood (Aquillaria agallocha Roxb.) carried out by the candidate. It consists essentially of two parts:

(A) Extraction of the oil from the infected wood of Aquillaria agallocha Roxb., known as agarwood belonging to the natural order Thymelaeaceae[1] and characterisation of some of the constituents.

(B) Extraction and composition of oil from the uninfected wood of Aquillaria agallocha Roxb.

Agarwood trees are distributed on the north eastern border districts of India. These trees which were once thought to be rare are now plentiful in China.[2] Agarwood oil is dark brown in colour and balsamic in nature. Agarwood oil occupies a place of prominence among the important essential oils. This rare perfume has been known to the oriental people even during the early years of the Christian era. It is possible that due to the inherent difficulties of getting this expensive oil in quantity, its critical examination did not receive the attention it deserved.

It is generally believed that the oil occurs in plants which have been infected by a fungus growth. Healthy plants do not contain appreciable quantity of the oil.

Agarwood which is odoriferous, consists of irregular patches of dark streaks, highly impregnated with an oleoresin arising due to certain fungus infestation,[3] and is found in the interior of comparatively old and mature trees. On account of the resinous deposition, such patches of wood often become heavier than water and are known as "Agar" or "Agaru".

Very little is known about the composition of the agarwood oil. The examination of this commercially important commodity was undertaken a few years ago at the instance of the Essential Oil Research Committee of the Council of Scientific and Industrial Research, India.

One of our colleagues had isolated and characterised two new selinanic constituents from infected agarwood oil, agarol,[4] an α,β-unsaturated primary alcohol, and an α,β-conjugated secondary ketol, hydroxy agariphilone, possessing the molecular formula, $C_{15}H_{24}O_2$.

Besides these two constituents, the oil from infected variety of wood contains several other oxygenated bodies.

Healthy uninfected agarwood gives the oil in very poor yield (0.08%), which is predominantly rich in hydrocarbons.

The formation of oxygenated bodies in the fungus infected wood is of considerable interest from the point

of view of plant physiology and enzyme chemistry. A similar example is the rotting of sweet potatoes, where the essential oil content increases due to abnormal cell functions through the effect of fungus.[5] Abnormal metabolism of the plant induced by fungus infestation thus seems to be responsible for the formation of oxygenated bodies in agarwood. Such microbiological hydroxylation is also a well-known phenomenon in the terpenes and steroids.[6]

PRESENT INVESTIGATION

Agarwood oil from infected as well as uninfected wood was obtained by low temperature solvent extraction procedure. The dark brown, viscous oil was separated into several fractions by high vacuum distillation and elaborate column chromatography.

The first chapter of the thesis deals with isolation of different constituents of infected agarwood oil. These include, among others the following six sesquiterpenic furanoids of the selinanic group:

(i) Dihydroagarofuran

(ii) β-Agarofuran

(iii) α-Agarofuran

(iv) Nor-ketoagarofuran

(v) 4-Hydroxy dihydroagarofuran

(vi) 3,4-Dihydroxy dihydroagarofuran.

Preliminary investigations on the higher boiling fractions are also incorporated in the same chapter. The polyoxygenated compounds present in higher boiling fractions shall receive our attention at a future date.

The chemistry of the first three new sesquiterpenic furanoids listed above has been discussed in the second chapter. From degradative studies and physical measurements, their structures and absolute configurations have been determined.[7]

The third chapter includes the structures and absolute configurations of the remaining three furanoids.

Their structures and absolute configurations have been determined by inter-conversions into the known compounds[8] dealt with in the second chapter.

The results described in this chapter establish the close relationship between the various naturally occurring derivatives of agarofuran. It is also seen that the β-agarofuran via nor-ketoagarofuran and 4-hydroxy-dihydroagarofuran can be easily converted to α-agarofuran.

Only few furanoid compounds are known in the field of terpenes and specially very few are known in the case of sesquiterpenes. Names of some of the furanoid terpenic compounds have been given here: Diterpenes-marrubiin,[9] vinhaticoic acid,[10] vouacapenic acid,[11] grindelic acid,[12,13]

oxygrindelic acid,[12,13] polyalthic acid,[14] cafestol,[15] kahweol,[16] columbin,[17] clerodin,[18] cascarillin,[19] and campanulin,[20] (triterpene); sesquiterpenes - maalioxide,[21] santanolide ethers,[22] guaioxide,[23] furanoeremophilane,[24] furano-eremophilone,[24] petasalbin,[24] albopetasin,[24] albopelasol,[24] furano-petasin,[24] and daucol.[25]

Besides the work on the oil from infected plant, study of the oil from uninfected plant has also been carried out[26] and the results described in the fourth chapter.

The oil occurring in the uninfected plant is obtained in poor yield (0.08%) and is predominantly rich in hydrocarbons. It has been found to contain elemental sulphur, selinane, two unidentified sesquiterpene hydrocarbons, agarol, a crystalline sesquiterpene alcohol and a crystalline hydroxy ketone.

In addition, fractions containing ketonic and conjugated hydroxy ketonic functions have also been isolated. The occurrence of five isomeric decenes in the oil has been established by gas liquid chromatography.

During this investigation liberal use of ultraviolet and infrared spectrophotometry, nuclear magnetic resonance and rotatory dispersion studies has been made in making various deductions. At all stages, products

according to their specific requirements were subjected to vapour phase chromatography, distillation through batch-strip head and elaborate column chromatography using different grades of neutral alumina.

Any general introduction to the chemistry of terpene compounds has been avoided for the sake of brevity. However, appropriate reference is made to relevant literature in the body of the thesis wherever it was found necessary to illustrate and emphasise a particular point.

REFERENCES

1. K.M.Nadkarni, Indian Materia Medica, Vol.I, p. 120, Popular Book Depot, Bombay (1954).

2. Chiang Hsi-chang, Perf. & Ess.Oil Rec., 49, 11 (1958); Indian Soap Journal, 23, 125 (1958).

3. Tunstall, Progress of Forest Research Report of Assam (1929-30).

4. T.C. Jain and S.C.Bhattacharyya, Tetrahedron Letters, No.9, pp. 13-17 (1959).

5. T.Kubota, Tetrahedron, 4, 68 (1958).

6. (i) P.K.Bhattacharyya, B.R.Prema, B.D.Kulkarni and S.K.Pradhan, Nature, 187, 689 (1960).

   (ii) M.Hayano, A.Saito and R.I.Dorfman, Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto (1957), Pergamon Press, N.Y. p.204(1958).

7. M.L.Maheshwari, T.C.Jain, R.B.Bates and S.C.Bhattacharyya, Tetrahedron, 19, 1079 (1963).

8. M.L.Maheshwari, K.R.Varma and S.C.Bhattacharyya, Tetrahedron - in press.

9. (i) F.Mercier etal., Comp.Rend., 195, 1102 (1932).

   (ii) W.Cocker, B.E.Cross, S.R.Duff and T.F.Holley, Chem. & Ind., 827 (1952).

   (iii) W.Cocker, J.T.Edward and T.F.Holley, Chem. & Ind., 1227 (1953); 772 (1955).

   (iv) W.Klyne, J.Chem.Soc., 3072 (1953).

10. (i) F.E.King, T.J.King, and K.G.Neill, J.Chem.Soc., 1055 (1953).

    (ii) F.E.King, and T.J.King, J.Chem.Soc., 4158(1953).

    (iii) F.E.King and K.G.Neill, J.Chem.Soc., 4752(1952).

11. (i) D.B.Spoelstra, Rec.Trav.Chim., 49, 226 (1930).

    (ii) F.E.King, D.H.Godson and T.J.King, J.Chem.Soc., 1117 (1955).

12. L.Panizzi, L.Mangoni and M.Belardini, Tetrahedron Letters, No.11, pp. 376-81 (1961).

13. T.Bruun, L.M.Jackman and E.Stenhagen, Acta Chem.Scan., 16, 1675 (1962).

14. K.W.Gopinath, T.R.Govindachari, P.C.Parthasarathy, and N.Viswanathan, Helv.Chim.Acta, 44, 1040 (1961).

15. A.Wettstein and K.Miescher, Helv.Chim.Acta, 26, 788 (1943).

16. (i) C.Djerassi, H. Bendas and P.Sen Gupta, J.Org. Chem., 20, 1046 (1955).

(ii) C.Djerassi, M.Cais and L.A.Mitscher, J.Amer.Chem. Soc., 81, 2386 (1959).

(iii) R.A.Finnegan and C.Djerassi, J.Amer.Chem.Soc., 82, 4342 (1960).

(iv) A.Wettstein and K.Miescher, Helv.Chim.Acta, 26, 631 (1943).

17. D.H.R.Barton and D.Elad, J.Chem.Soc., 2085 and 2090 (1956).

18. (i) D.H.R.Barton, H.T.Cheung, A.D.Cross, L.M.Jackman and M.Martin-Smith, J.Chem.Soc., 5061 (1961); Proc. Chem.Soc., 76 (1961).

(ii) G.A.Sim, T.A.Hamor, I.C.Paul and J.M.Robertson, Proc.Chem.Soc., 75 (1961).

19. J.S.Birtwistle, D.E.Case, P.C.Dutta, T.G.Halsall, G.Mathews, H.D.Sabel and V.Thaller, Proc.Chem.Soc., 329 (1962).

20. S.Rangaswami and K.Sambamurthy, Proc.Indian Acad. Sci., 54A, 132-9 (1961).

21. (i) G.Büchi, M.Schach V.Wittenau and Dwain M.White, J.Amer.Chem.Soc., 81, 1968 (1959).

(ii) C.S.Narayanan etal., Tetrahedron - in press.

22. O.Kovács, V.Herout, M.Horák and F.Šorm, Coll.Czech. Chem.Commun., 21, 225 (1956).

23. R.B.Bates and R.C. Slagel, Chem. and Ind., 1715(1962).

24. L.Novotný, V.Herout and F.Šorm, Tetrahedron Letters, No. 20, pp. 697-701 (1961).

25. V.Sykora, L.Novotný, and F.Šorm, Tetrahedron Letters, No. 14, pp. 24-28 (1959).

26. T.C.Jain, M.L.Maheshwari, and S.C.Bhattacharyya, Perf. & Ess.Oil Rec., 53, 294 (1962).

CHAPTER I

ISOLATION AND CHARACTERISATION OF SOME OF THE CONSTITUENTS OF INFECTED AGARWOOD OIL

S U M M A R Y

"Agarwood oil" obtained by low temperature solvent extraction procedure from the infected variety of Aquillaria agallocha Roxb. is an intricate mixture of a large number of components which are difficult to separate. By careful fractional distillation and elaborate column chromatography alongwith the use of VPC analysis, the presence of two sesquiterpenoid hydrocarbons, six new sesquiterpene furanoids of the selinanic group - dihydroagarofuran, β-agarofuran, ∝-agarofuran, nor-ketoagarofuran, 4-hydroxy dihydroagarofuran, and 3,4-dihydroxydihydroagarofuran has been confirmed. The higher boiling fractions containing polyoxygenated bodies have also been characterised.

Infrared and ultraviolet spectral characteristics of all the compounds have been recorded.

The Indian sub-continent because of its variety of climatic conditions in different parts, produces many essential oil bearing plants. Agarwood oil commonly known as 'Agar' or 'Agaru' in the trade obtained from the plant Aquillaria agallocha Roxb. belonging to the natural order Thymelaeceae[1] is one of the important members of this group. Agar trees are distributed on the north-eastern border districts of India. Agarwood oil is prepared locally by hydro distillation[2] of the powdered wood known as 'Dhum'. It is one of the most rare perfumes and was known to the oriental people even during the early years of the Christian era.[2] It finds extensive use as an ingradient of perfumery preparations specially in Arab countries. Good amount of oil as well as wood chips are exported to Arabia, Turkey, Persia etc. at fairly high price. People in these countries use this oil for massage; wood chips are burnt in houses and are mixed with tobacco for flavouring.

It is interesting to note that agar oil occurs in plants which have been infected by a fungus growth. Healthy plants do not contain any appreciable quantity of the oil. Abnormal metabolism of the plant induced by the fungus infestation seems to be responsible for the formation of oxygenated bodies in agarwood.[3-5]

However, these observations do not seem to have been substantiated by any systematic examination of the oil, probably due to the inherent difficulty of getting this expensive oil in quantity. Considering the importance of agarwood oil as a commercial commodity in India, it was felt desirable to investigate the composition of the oil from the infected variety of the wood.

PRESENT INVESTIGATION

During our investigation, the dark brown and viscous agarwood oil was obtained from the infected variety of agarwood by low temperature solvent extraction procedure. Overall yields of the extractive (concrete) was 0.4%. Waxy material was removed from the oil by extracting it with ethanol (absolute) followed by cooling and filtration.

Agar absolute obtained after removal of solvent was distilled under high vacuum using a mercury diffusion pump and separated into (i) lower boiling fraction, and (ii) higher boiling residue.

Agar distillate was then subjected to chromatography on grade II alumina (10 times in two lots). Several fractions obtained were appropriately mixed to form six main cuts on the basis of their physical constants. Results are recorded in Table I.

T A B L E I

Fraction	Eluent	Volume of eluent (l)	Wt.of the fraction (g)	n_D^{22}
A_1	Pet.ether	10	24.0	1.5008
A_2	"	8	678.0	1.5129
A_3	"	16	48.0	1.5181
A_4	Benzene	30	106.0	1.5258
A_5	Ether	40	164.0	n_D^{31} 1.5175
A_6	Ethanol	25	30.0	extremely viscous

At present we are restricting ourselves to the fractions A_1 and A_2. The various components present in the fractions A_3 to A_6 will receive our attention at a future date, though some of the constituents have been isolated in nearly pure form.

First two fractions (A_1 and A_2) were combined together and subjected to fractional distillation (divided into two batches) using an efficient packed column (Fanske glass helices) fitted with a batch strip head and the different fractions collected were mixed together according to their refractive indices, optical rotations and boiling points to constitute eighteen main fractions as recorded in Table II.

T A B L E II

Fraction	Wt.of the fraction (g)	B.P./Pressure (mm)	n_D^{23}	$(\alpha)_D^{28}$
B_1	24.0	101°/0.2	1.4948	-
B_2	31.0	"	1.5081	- 41.3°
B_3	35.0	103°/0.2	1.5079	-
B_4	34.5	106°/0.2	1.5095	- 29.65°
B_5	35.8	"	"	-
B_6	36.5	"	1.5096	- 30.2°
B_7	36.1	"	1.5101	-
B_8	43.0	"	1.5104	- 30.4°
B_9	38.5	"	1.5108	-
B_{10}	36.6	"	"	- 22.16°
B_{11}	34.3	109°/0.2	1.5111	-
B_{12}	34.0	115°/0.2	1.5131	- 21.6°
B_{13}	36.4	120°/0.2	1.5148	- 29.1°
B_{14}	38.0	124°/0.2	1.5162	- 25.2°
B_{15}	37.5	126°/0.2	1.5172	-
B_{16}	39.1	131°/0.2	1.5185	- 27.41°
B_{17}	36.2	140°/0.2	1.5205	-
B_{18}	23.2	145°/0.2	1.5226	-

All the above fractions were chromatographed independently on alumina (grade I, II and III) and separated into about two hundred fractions. On the basis of refractive indices, optical rotations, IR and UV spectra, identical fractions were combined and finally made into six main cuts as described in Table III.

TABLE III

Fraction	Eluent	Wt.of the fraction (g)
C_1	Pet.ether	23.0
C_2	"	13.0
C_3	Pet.ether + Benzene	5.0
C_4	Benzene	2.0
C_5	"	340.0
C_6	Ether	230.0

Fraction C_1

This fraction which was composed of hydrocarbons was chromatographed on grade I alumina. Identical fractions were mixed together and each was fractionally distilled in a spinning band column to furnish two main products A and B in the range of 115° to 155° at 1.5 mm.

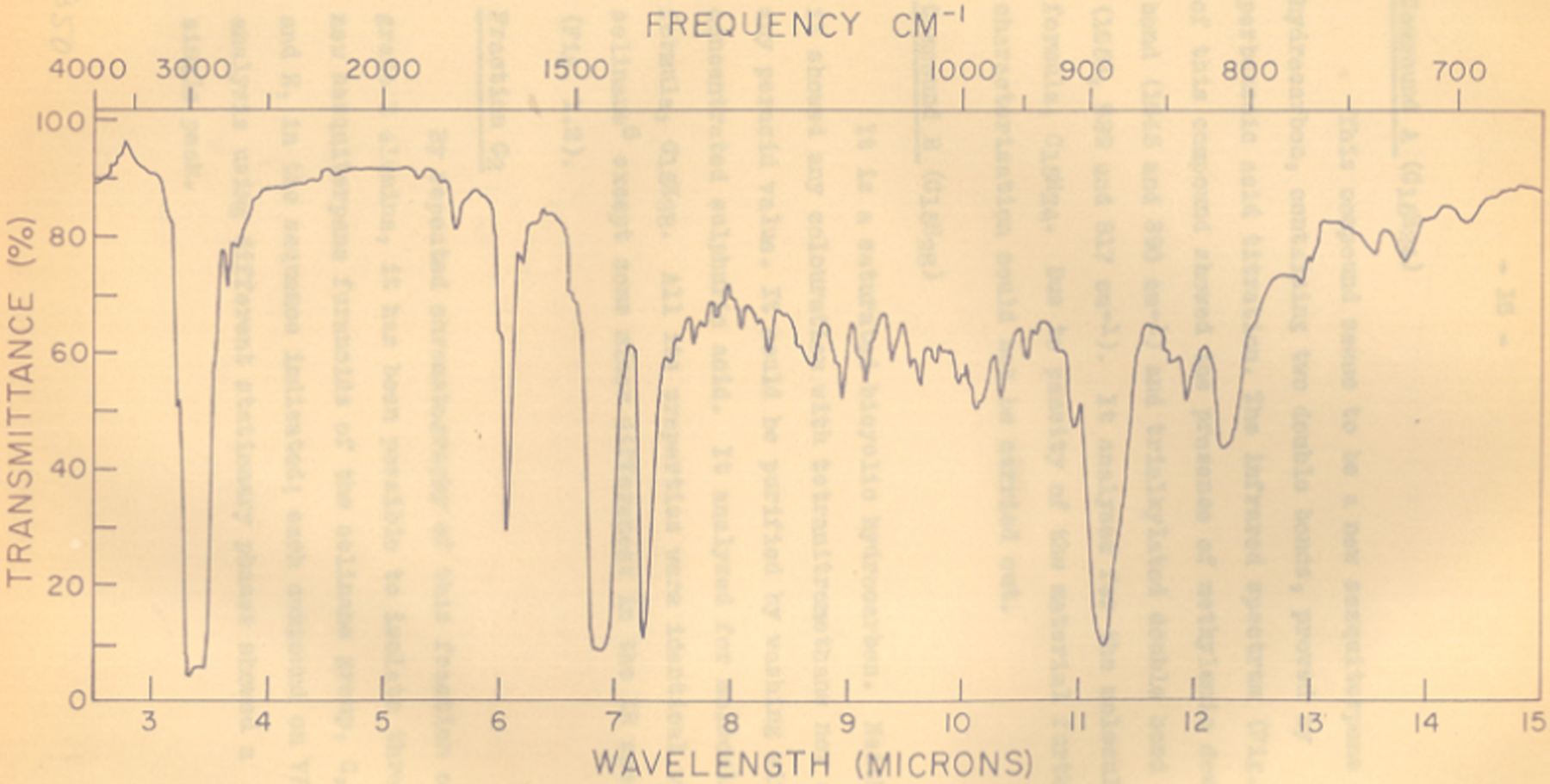


FIG. I.I IR SPECTRUM (IN 0·05 MM. CELL) OF COMPOUND A($C_{15}H_{24}$)

Compound A ($C_{15}H_{24}$)

This compound seems to be a new sesquiterpene hydrocarbon, containing two double bonds, proved by perbenzoic acid titration. The infrared spectrum (Fig.1.1) of this compound showed the presence of methylenic double bond (1645 and 890 cm^{-1}) and trialkylated double bond (1658, 839 and 817 cm^{-1}). It analysed for the molecular formula, $C_{15}H_{24}$. Due to paucity of the material further characterisation could not be carried out.

Compound B ($C_{15}H_{28}$)

It is a saturated bicyclic hydrocarbon. Neither it showed any colouration with tetranitromethane nor showed any peracid value. It could be purified by washing with concentrated sulphuric acid. It analysed for molecular formula, $C_{15}H_{28}$. All its properties were identical with selinane[6] except some minor differences in the IR spectrum (Fig. 1.2).

Fraction C_2

By repeated chromatography of this fraction on grade I alumina, it has been possible to isolate three new sesquiterpene furanoids of the selinane group, C, D and E, in the sequence indicated; each compound on VPC analysis using different stationary phases showed a single peak.

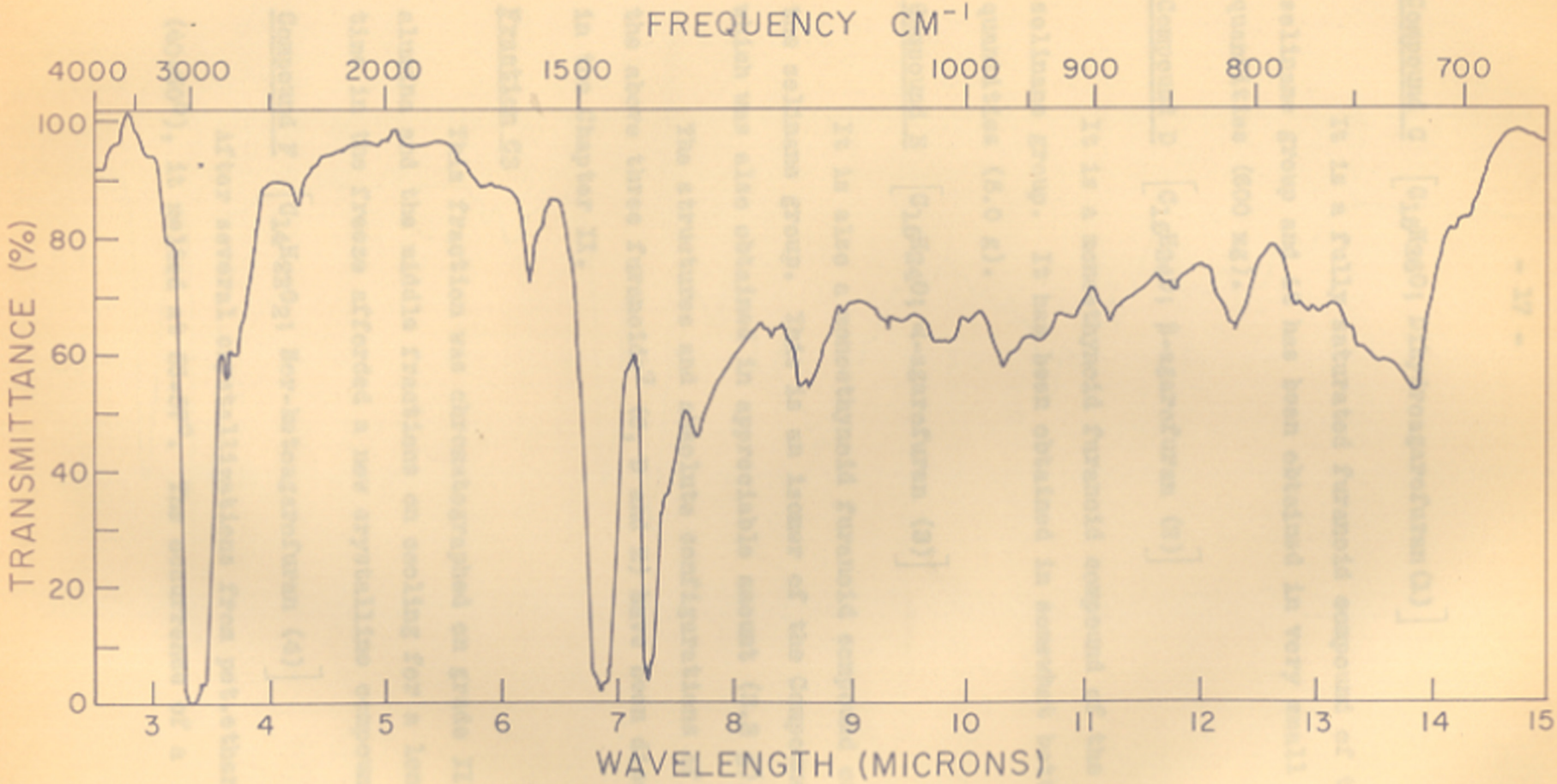


FIG. 1.2 IR SPECTRUM (IN 0·1 MM. CELL) OF COMPOUND B($C_{15}H_{28}$)

Compound C $\left[C_{15}H_{26}O;\ \text{Dihydroagarofuran (1)}\right]$

It is a fully saturated furanoid compound of the selinane group and it has been obtained in very small quantities (600 mg).

Compound D $\left[C_{15}H_{24}O;\ \beta\text{-agarofuran (2)}\right]$

It is a monoethynoid furanoid compound of the selinane group. It has been obtained in somewhat better quantities (5.0 g).

Compound E $\left[C_{15}H_{24}O;\ \alpha\text{-agarofuran (3)}\right]$

It is also a monoethynoid furanoid compound of the selinane group. This is an isomer of the Compound D, which was also obtained in appreciable amount (3.3 g).

The structures and absolute configurations of all the above three furanoids[7] (C, D and E) have been discussed in the Chapter II.

Fraction C3

This fraction was chromatographed on grade II alumina and the middle fractions on cooling for a long time in the freeze afforded a new crystalline compound F.

Compound F $\left[C_{14}H_{22}O_2;\ \text{Nor-ketoagarofuran (4)}\right]$

After several crystallizations from pet.ether (40-60°), it melted at 56-57°. The occurrence of a

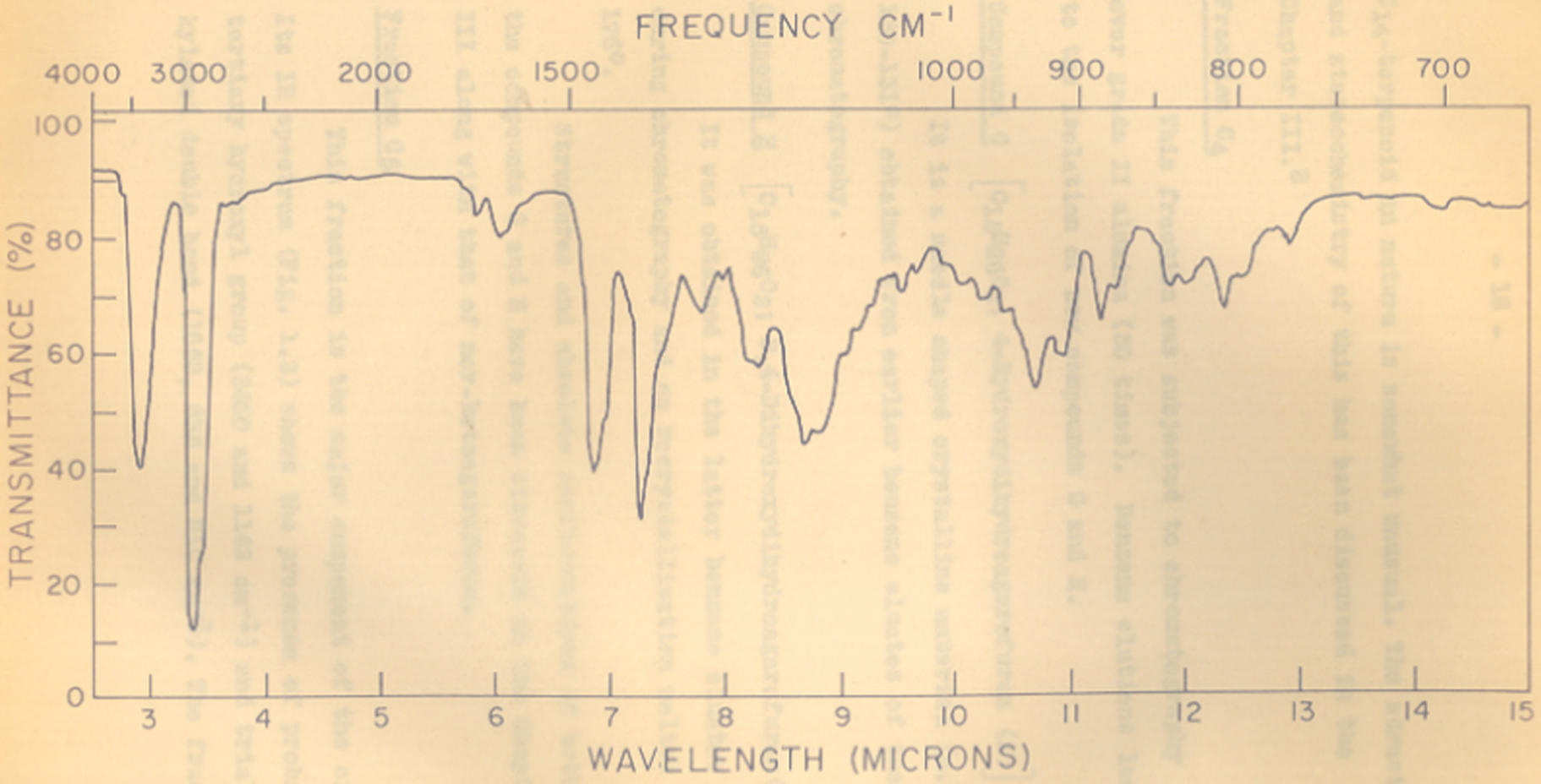


FIG. I.3 IR SPECTRUM (LIQUID FILM) OF FRACTION C_5

C_{14}-terpenoid in nature is somewhat unusual. The structure and stereochemistry of this has been discussed in the Chapter III.[8]

Fraction C_4

This fraction was subjected to chromatography over grade II alumina (50 times). Benzene elutions led to the isolation of new compounds G and H.

Compound G $\left[C_{15}H_{26}O_2;\ 4\text{-Hydroxydihydroagarofuran (5)}\right]$

It is a needle shaped crystalline material (m.p. 130-131°) obtained from earlier benzene eluates of the chromatography.

Compound H $\left[C_{15}H_{26}O_3;\ 3,4\text{-Dihydroxydihydroagarofuran(6)}\right]$

It was obtained in the latter benzene eluates during chromatography and on recrystallization melted at 176°.

Structures and absolute configurations of both the compounds G and H have been discussed in the Chapter III along with that of nor-ketoagarofuran.

Fraction C_5

This fraction is the major component of the oil. Its IR spectrum (Fig. 1.3) shows the presence of probably tertiary hydroxyl group (3460 and 1142 cm^{-1}) and trialkylated double bond (1658, 835 and 810 cm^{-1}). The fraction

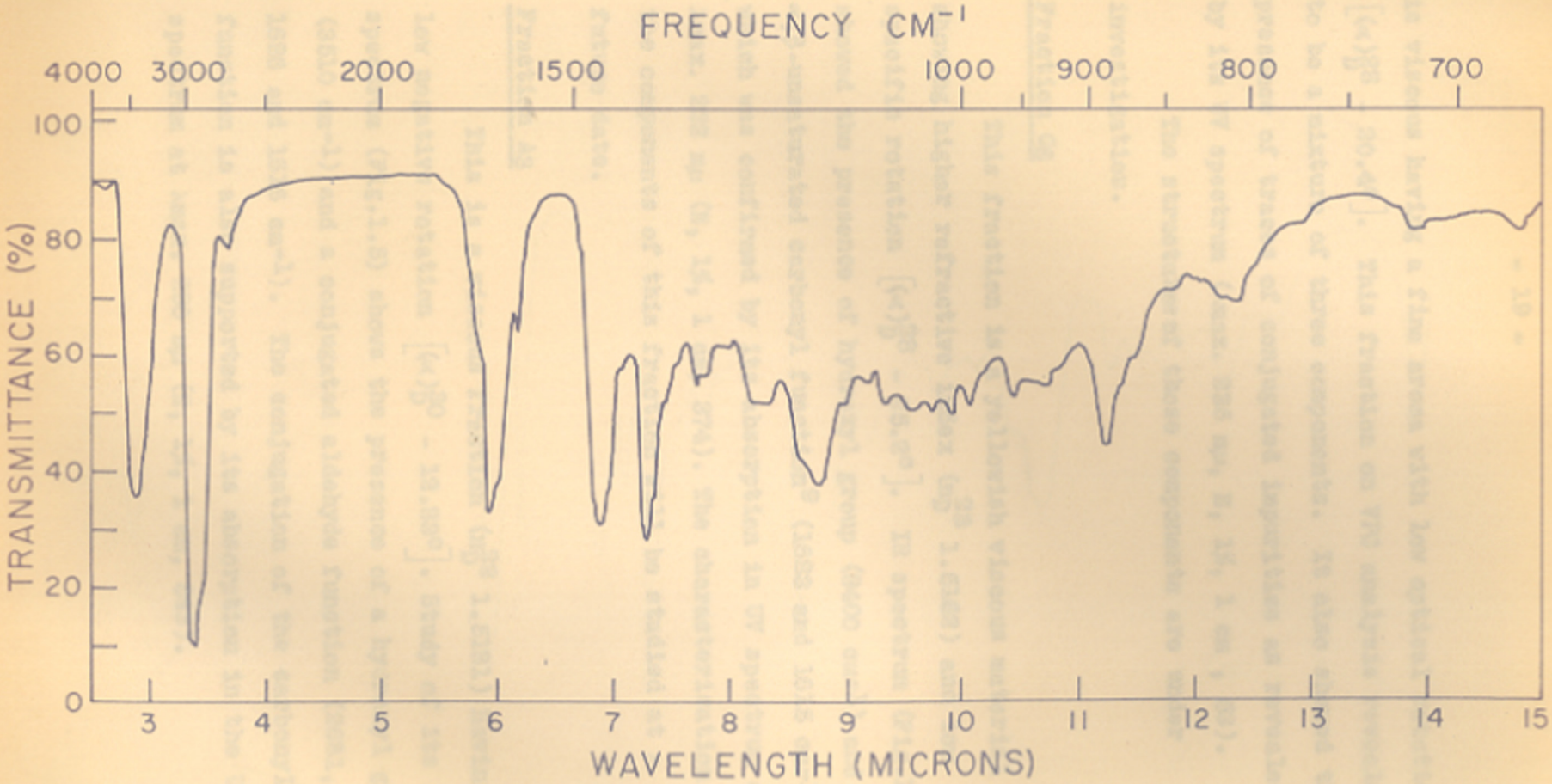


FIG. 1.4 IR SPECTRUM (LIQUID FILM) OF FRACTION C_6.

is viscous having a fine aroma with low optical rotation $\left[(\alpha)_D^{28} - 20.4^o\right]$. This fraction on VPC analysis revealed to be a mixture of three components. It also showed the presence of traces of conjugated impurities as revealed by its UV spectrum (λmax. 235 mμ, E, 1%, 1 cm , 53).

The structures of these components are under investigation.

Fraction C6

This fraction is a yellowish viscous material showing higher refractive index (n_D^{23} 1.5162) and low specific rotation $\left[(\alpha)_D^{28} - 25.2^o\right]$. IR spectrum (Fig.1.4) showed the presence of hydroxyl group (3400 cm^{-1}) and α,β-unsaturated carbonyl function[9] (1683 and 1615 cm^{-1}), which was confirmed by its absorption in UV spectrum at λmax. 232 mμ (E, 1%, 1 cm, 374). The characterisation of the components of this fraction will be studied at a future date.

Fraction A3

This is a viscous fraction (n_D^{22} 1.5181) having low negative rotation $\left[(\alpha)_D^{30} - 12.83^o\right]$. Study of its IR spectrum (Fig.1.5) shows the presence of a hydroxyl group (3510 cm^{-1}) and a conjugated aldehyde function (2681, 1686 and 1616 cm^{-1}). The conjugation of the carbonyl function is also supported by its absorption in the UV spectrum at λmax. 230 mμ (E, 1%, 1 cm, 543).

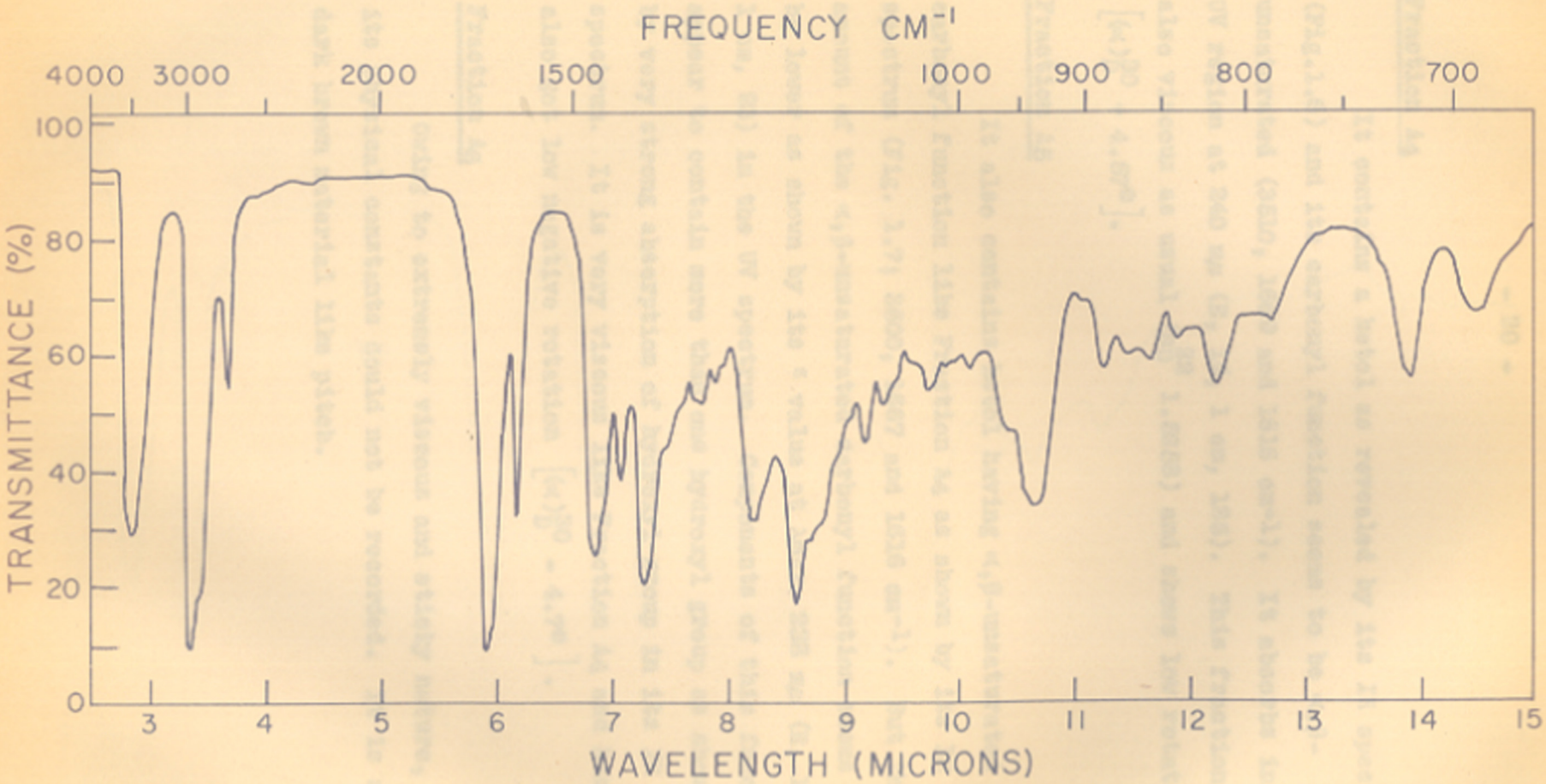


FIG. 1.5 IR SPECTRUM (LIQUID FILM) OF FRACTION A_3.

Fraction A4

It contains a ketol as revealed by its IR spectrum (Fig.1.6) and its carbonyl function seems to be α,β-unsaturated (3510, 1669 and 1616 cm^{-1}). It absorbs in the UV region at 240 mμ (E, 1%, 1 cm, 185). This fraction is also viscous as usual (n_D^{22} 1.5258) and shows low rotation $\left[(\alpha)_D^{30} + 4.57^o\right]$.

Fraction A5

It also contains ketol having α,β-unsaturated carbonyl function like Fraction A4 as shown by its IR spectrum (Fig. 1.7; 3500, 1667 and 1616 cm^{-1}). But the amount of the α,β-unsaturated carbonyl function seems to be lower as shown by its ε value at λmax. 238 mμ (E, 1%, 1 cm, 93) in the UV spectrum. Components of this fraction appear to contain more than one hydroxyl group as shown by very strong absorption of hydroxyl group in its IR spectrum. It is very viscous like Fraction A4 and has also got low negative rotation $\left[(\alpha)_D^{30} - 4.7^o\right]$.

Fraction A6

Owing to extremely viscous and sticky nature, its physical constants could not be recorded. It is a dark brown material like pitch.

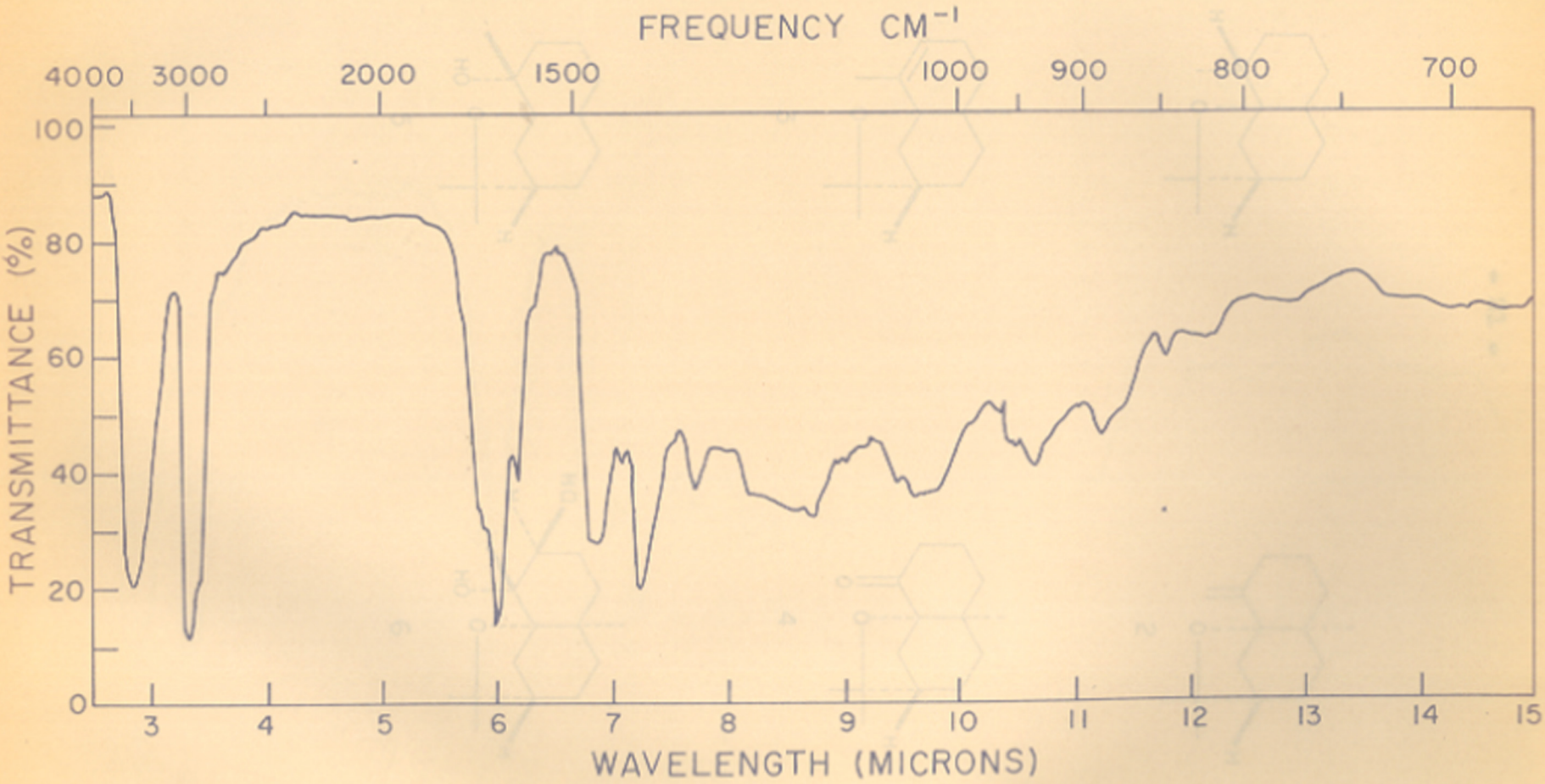
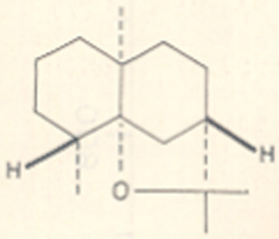


FIG. 1.6 IR SPECTRUM (LIQUID FILM) OF FRACTION A_4.



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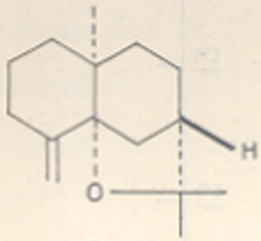

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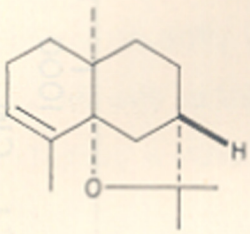

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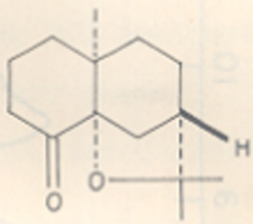

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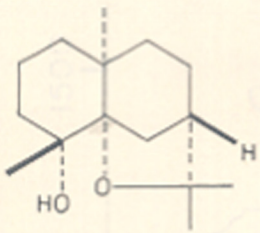

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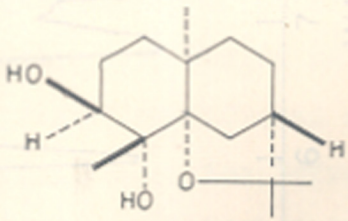

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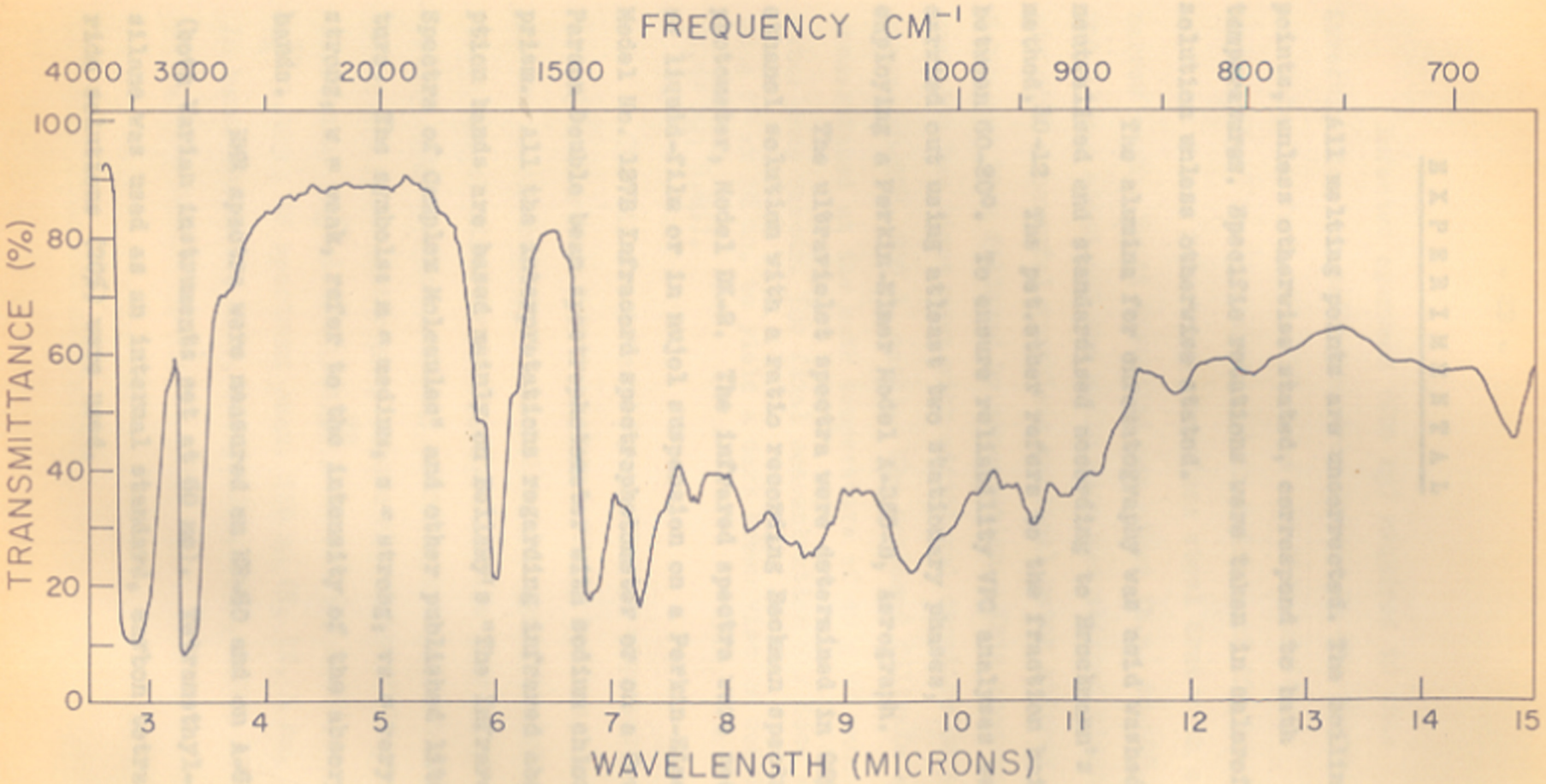


FIG. 1.7 IR SPECTRUM (LIQUID FILM) OF FRACTION A_5

E X P E R I M E N T A L

All melting points are uncorrected. The boiling points, unless otherwise stated, correspond to bath temperatures. Specific rotations were taken in chloroform solution unless otherwise stated.

The alumina for chromatography was acid washed, neutralised and standardised according to Brockmann's method.[10-12] The pet.ether refers to the fraction boiling between 60-80°. To ensure reliability VPC analyses were carried out using atleast two stationary phases, employing a Perkin-Elmer Model A-350-B, Aerograph.

The ultraviolet spectra were determined in 95% ethanol solution with a ratio recording Beckman spectrophotometer, Model DK-2. The infrared spectra were recorded as liquid-film or in nujol suspension on a Perkin-Elmer Model No. 137B Infracord spectrophotometer or on a Grubb-Parson Double beam spectrophotometer with sodium chloride prism. All the interpretations regarding infrared absorption bands are based mainly on Bellamy's "The Infrared Spectra of Complex Molecules" and other published literature. The symbols: m = medium, s = strong, vs = very strong, w = weak, refer to the intensity of the absorption bands.

NMR spectra were measured on HR-60 and on A-60 (both Varian instruments set at 60 mc). Tetramethylsilane was used as an internal standard, carbon tetrachloride solutions (20%) were used.

Infected agarwood (600 kg) required in this investigation was collected from Assam. It was initially cut into the form of small chips and then powdered thoroughly. The powdered wood in batches of 100 kg. each was taken in a large stainless steel drum with a narrow mouth. To it distilled pet.ether (b.p. 40-60°, 200 l) was added and the whole mass was stirred vigorously with a powerful sparkless vertical motor stirrer for 5 hrs and then filtered by percolation. This process was repeated two times more with additional quantity of pet. ether (40-60°, 200 l. each). The combined filtrate was concentrated at the bath temperature of 40 ± 2° under water pump vacuum. The final concentration was done at 30 ± 2° at 10 mm. to yield a dark brown viscous concrete (2.49 kg; 0.4%).

The oil was dewaxed by shaking it vigorously (4 hr) with ethanol (2 l. each) in three batches, cooling in the freeze overnight and filtration. Agar absolute (2.04 kg) obtained by drying the filtrate over anhydrous sodium sulphate and removing solvent on a water bath (40 ± 2°) under water pump vacuum showed the following constants: n_D^{22} 1.5181; $(\alpha)_D^{25}$ - 13.25° (c, 1.25); d_4^{30} 1.0230; UV spectrum: λmax. 235 mμ (E, 1%, 1 cm, 137). IR bands at: 3355, 1689, 1660, 1370, 1217, 1148, 1031, 1009, 936, and 886 cm^{-1}.

Agar absolute was distilled (45-160° at 2.46 X 10^{-3} mm) in vacuum using a mercury diffusion pump. Pot residue (770 g) has been preserved for studying on a future occasion. Agar distillate (1.25 kg., 56.4% yield) had the following properties: n_D^{23} 1.5135; d_4^{30} 1.0060; $(\alpha)_D^{25}$ - 11.94° (c, 2.13); UV spectrum: λmax. 233 mμ (E, 1%, 1 cm , 115); IR bands at: 3380, 1691, 1663, 1615, 1377, 1220, 1155, 935, 885, and 812 cm^{-1}.

Separation of components of the Agar distillate

The components were separated by fractional distillation through a packed column with batch-strip head and by rigorous chromatography. The results are tabulated in Tables I, II and III. For the sake of brevity only the essential details regarding the characteristic components are being discussed here.

Isolation of Compounds A and B

These compounds were contained in the fraction C_1 (Table III, p. 15). This fraction was chromatographed over alumina (grade I, 100 times), eleven fractions were collected (elution by pet.ether). Identical fractions were mixed together and each fraction was fractionally distilled in a high efficiency spinning band column. The course of separation was followed by noting the boiling points and refractive indices. The difference between the boiling points of compounds A and B was reasonable enough to ensure clear cut separation.

Compound A: b.p. 115° at 1.5 mm., n_D^{27} 1.4950, $(\alpha)_D^{30}$ + 12.33°, d_4^{30} 0.94958, yellow colouration with tetranitromethane, end absorption in UV spectrum (ε 223, 3265), IR bands at: 2915, 1658, 1645, 1383, 890, 839, and 817 cm-1 (in 0.05 mm cell). (Found: C, 87.81; H, 12.00. $C_{15}H_{24}$ requires: C, 88.16; H, 11.84%).

Compound B: It is a saturated hydrocarbon and was obtained in slightly larger amount. For further purification it was thoroughly washed with concentrated sulphuric acid, and then with water, dried over anhydrous sodium sulphate and distilled over sodium to give analytically pure sample of the saturated hydrocarbon having the following properties: b.p. 155° at 1.5 mm., n_D^{29} 1.4808, $(\alpha)_D^{28}$ + 0.17°, d_4^{30} 0.8867; M_R 67.08, $C_{15}H_{28}$ $\sqrt{\text{nil}}$ requires 67.07; IR bands at: 1380, 1339, 1299, 1170, 1020, 972, 894, 816, 763, and 722 cm-1 (in 0.1 mm. cell). (Found: C, 86.70; H, 13.26. $C_{15}H_{28}$ requires: C, 86.46; H, 13.54%).

Isolation of compounds C, D and E

Fraction C_2 of Table III (p. 15) was subjected to rigorous chromatography on grade I alumina (100 times). Identical fractions were mixed by observing their refractive indices, optical rotations, IR spectra and VPC to furnish the three major fractions containing the compounds C, D and E in the sequence indicated.

Compound C (Dihydroagarofuran) (1)

The first major fraction of the above chromatography contained traces of compound D (β-agarofuran) in addition to the major component - dihydroagarofuran, which could not be separated even by using very high ratio of grade I alumina for chromatography.

For further purification, this whole fraction(1 g) was subjected to ozonolysis in chloroform (25 ml) for 1 hr. The neutral ozonisation product (850 mg) after decomposition was chromatographed on grade I alumina (100 times) to furnish pure dihydroagarofuran in pet.ether eluate and carbonyl compound in the ether eluate. This carbonyl compound could be utilised during the degradative studies of β-agarofuran (p. 34).

Dihydroagarofuran was further purified by vacuum distillation to afford VPC pure specimen (600 mg) having the following properties: b.p. 135° at 8 mm., n_D^{29} 1.4912, $(\alpha)_D^{30}$ - 77.01° (c, 4.4); no colouration with tetranitromethane, IR bands at: 1379, 1359, 1295, 1229, 1155, 1144, 1114, 1089, 1062, 1015, 998, 980, 952, 887, and 869 cm^{-1}. (Found: C, 81.40; H, 12.0. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%).

Compound D (β-agarofuran) (2)

The corresponding chromatographic fractions on rechromatography and vacuo distillation furnished the

VPC pure specimen of β-agarofuran (5 g) showing the following constants: b.p. 130° at 8 mm., n_D^{28} 1.4973, d_4^{30} 0.9646, $(\alpha)_D^{30}$ - 127.1° (c, 8.3); yellow colouration with tetranitromethane, peracid value 1.01; IR bands at: 1639, 1374, 1362, 1299, 1235, 1152, 1140, 1121, 1090, 1075, 1015, 898, 887, and 870 cm^{-1} (Found: C, 81.30; H, 11.10. $C_{15}H_{24}O$ requires: C, 81.70; H, 10.98%).

Compound E (α-agarofuran) (3)

The appropriate chromatographic fractions on rechromatography and vacuum distillation afforded VPC pure sample of α-agarofuran (3.3 g). Its physical properties were as follows: b.p. 134° at 6 mm., n_D^{30} 1.5061, $(\alpha)_D^{30}$ + 37.09° (c, 6.12); yellow colouration with tetranitromethane, peracid value 0.9; IR bands at: 1653 (w), 1389, 1370, 1326, 1307, 1285, 1250, 1239, 1202, 1164, 1155, 1136, 1099, 1080, 1046, 1012, 965, 950, 935, 887, 856, 838 (s), 825, 805, 770, and 703 cm^{-1} (Found: C, 81.64; H, 10.79. $C_{15}H_{24}O$ requires: C, 81.70; H, 10.98%).

Isolation of compound F (Nor-ketoagarofuran) (4)

Fraction C_3 of Table III (p. 15) was chromatographed on grade II alumina (500 g), middle fractions obtained by pet.ether-benzene (1:1) elution were cooled in the freeze for several weeks to afford the crystalline nor-ketoagarofuran.

It was further purified by several crystallizations from pet.ether (40-60°) to give pure compound (3.0 g)

which melted at 56-57° having the following properties: $(\alpha)_D^{30}$ - 118.86° (c, 3.88); IR bands at: 1712, 1418, 1379, 1361, 1295, 1229, 1149, 1110, 1067, 1013, and 888 cm^{-1}. (Found: C, 75.71; H, 10.15. $C_{14}H_{22}O_2$ requires: C, 75.63; H, 9.97%). Semicarbazone prepared by acetate procedure after repeated crystallization melted at 216°; IR bands at: 3580, 3247, 1712, 1597, 1383, 1364, 1274, 1232, 1155, 1120, 1103, 1087, 1075, 1039, 1018, 974, 956, 891, 873, 834, 809, 768, and 700 cm^{-1} (Found: C, 64.16; H, 8.75; N, 15.1. $C_{15}H_{25}O_2N_3$ requires: C, 64.48; H, 9.02; N, 15.04%).

Isolation of Compounds G and H

The fraction C_4 of Table III (p. 15) was subjected to rigorous chromatography on grade II alumina (100 g). Benzene elution could resolve it into two distinct compounds G and H.

Compound G (4-hydroxydihydroagarofuran) (5)

It was obtained as a needle-shaped crystalline material from its chromatographic fraction (earlier benzene eluates, 400 ml) and further purified by recrystallizations from pet.ether to afford the material, which melted at 130-131° and showed the following constants: $(\alpha)_D^{30}$ - 75.7° (c, 1.29); no colouration with tetranitromethane; IR bands at: 3460, 1374, 1359, 1323, 1290, 1242, 1229, 1195, 1148, 1136, 1114, 1099, 1082, 1054, 1028, 1011, 1001, 985, 966, 954, 939, 929, 910, 885, 875, 852, 831, 810, 789,

768, and 700 cm^{-1}. (Found: C, 75.82; H, 10.98. $C_{15}H_{26}O_2$ requires: C, 75.58; H, 11.0%).

Compound H (3,4-dihydroxydihydroagarofuran (6)

This was obtained in the latter benzene eluates (1 l) during chromatography and was purified by recrystallizations from ethyl acetate and drying under vacuum at 90°; m.p. 176°, $(\alpha)_D^{30}$ - 40.98° (c, 0.29), no colouration with tetranitromethane; IR bands at: 3520, 1431, 1397, 1389, 1316, 1295, 1235, 1140, 1106, 1089, 1075, 1038, 1015, 1003, 983, 962, 945, 933, 909, 881, 836, 828, and 811 cm^{-1}. (Found: C, 70.53; H, 10.42. $C_{15}H_{26}O_3$ requires: C, 70.83; H, 10.30%).

REFERENCES

1. K.M.Nadkarni, Indian Materia Medica, Vol.I, p. 120, Popular Book Depot, Bombay (1954).

2. Sadgopal and B.S.Varma, Soap, Perf. & Cosmetics, 25, 169 (1952).

3. Tunstall, Progress of Forest Research: Report of Assam (1929-30).

4. S.R.Bose, Science and Culture, No.2, 4 (1938).

5. Forest Research in India, Part II, p. 14 (1953-54).

6. (i) J.L.Simonsen and D.H.R.Barton, The Terpenes, Vol. III, Cambridge University Press, p. 33 (1952).

   (ii) J. Plíva, V.Herout, B.Schneider and F.Šorm, Coll. Czech.Chem.Commun., 18, 500 (1953).

   (iii) O.Motl, V.Herout and F.Šorm, Coll.Czech.Chem. Comm., 23, 1293 (1958).

7. M.L.Maheshwari, T.C.Jain, R.B.Bates and S.C.Bhattacharyya, Tetrahedron, 19, 1079 (1963).

8. M.L.Maheshwari, K.R.Varma, and S.C.Bhattacharyya, Tetrahedron - in press.

9. R.N.Jones and C.Sandorfy "Chemical Applications of Spectroscopy in Technique of Organic Chemistry", (Ed. A. Weissberger), Vol. IX, pp. 447, Interscience Publishers Inc., N.Y. (1959).

10. Cf. foot note[29] in C.Djerassi and W.Rittel, J.Amer. Chem.Soc., 79, 3528 (1957).

11. E.Lederer and M.Lederer "Chromatography" p. 26, Elsevier Publishing Co., N.Y. (1957).

12. H.Brockmann and H.Schodder, Ber., 74, 73 (1941).

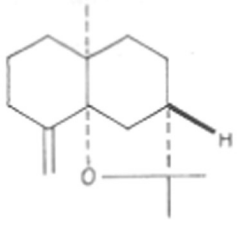
CHAPTER II

STRUCTURE AND ABSOLUTE CONFIGURATION OF α-AGAROFURAN, β-AGAROFURAN & DIHYDROAGAROFURAN

S U M M A R Y

In Chapter I (p. 16) isolation of the sesquiterpenic furanoids of 'infected agarwood oil' has been described. Results concerning the elucidation of the structures of three constituents are presented in this chapter.

By degradative studies and physical measurements the structures and absolute configurations of these compounds have been determined and they have been named as β-agarofuran (I), ∝-agarofuran (II) and dihydroagarofuran (III) respectively.



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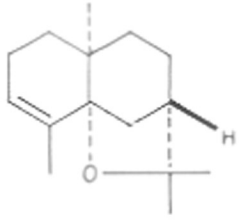

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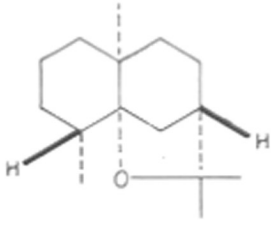

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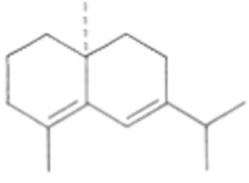
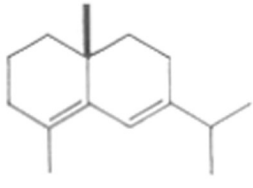


IV



V

During the course of investigation, a diene (IV) was obtained, which established the stereochemistry of angular methyl group (α-orientation). For the sake of comparison, the antipodal of diene (IV) was also prepared from β-eudesmol and following names have been assigned to them according to their signs of specific rotations:

(-)-δ-selinene(IV) and (+)-δ-selinene (V).

The stereochemistry of other asymmetric carbon atoms has been determined by application of rotatory dispersion, model and NMR studies.

Isolation of three new furanoid sesquiterpenes of the selinanic group in the pure (VPC) form in small quantities by elaborate column chromatography of the lower boiling fractions of agarwood oil has already been described earlier (vide Chapter I, p. 16). These are:-

(i) Fully saturated dihydroagarofuran, $C_{15}H_{26}O$;

(ii) β-Agarofuran, $C_{15}H_{24}O$, containing one double bond; and

(iii) α-Agarofuran, $C_{15}H_{24}O$, also containing one double bond.

Names given to these compounds indicate their relationship with the word 'agar' for Aquillaria agallocha Roxb. The results of our investigation on the structure and stereochemistry of these compounds[1] are represented in this chapter.

β-Agarofuran

It was obtained in comparatively larger quantities and correctly analysed for $C_{15}H_{24}O$.

Determination of the carbon skeleton

The gross structural feature of β-agarofuran was detected by dehydrogenation with selenium in an atmosphere of nitrogen to afford eudalene (1), which was characterised by its picrate as well as by spectral studies. Formation of eudalene accounts for its fourteen carbon atoms.

Assuming that β-agarofuran is a true isoprenoid,[2,3] 15th carbon atom will be in the form of an angular methyl group at C_{10} and consequently its basic carbon skeleton should be represented by (2). The numbering of the carbon skeleton is in accordance with the principle formulated by Barton.[4]

Nature of unsaturation

Agarofuran gives yellow colouration with tetranitromethane. It contains one double bond as shown by peracid titration and confirmed by its catalytic hydrogenation to give dihydro-β-agarofuran.

The double bond in β-agarofuran was proved to be methylenic ($>C=CH_2$) from its IR spectrum (Fig. 2.1, absorptions at 1639 and 898 cm^{-1}), NMR spectrum(Fig. 2.4a), and further confirmed by formation of formaldehyde on ozonisation. Haloform test was negative with both the trap solution as well as the neutral non-volatile product of ozonolysis.

Position of Unsaturation

Neutral non-volatile product of ozonolysis is a crystalline keto-oxide, $C_{14}H_{22}O_2$, m.p. 56-57°* in

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* The keto-oxide though analytically pure was initially obtained as a liquid but subsequently crystallized to show the m.p. recorded above (refer p. 70 Chapter III).

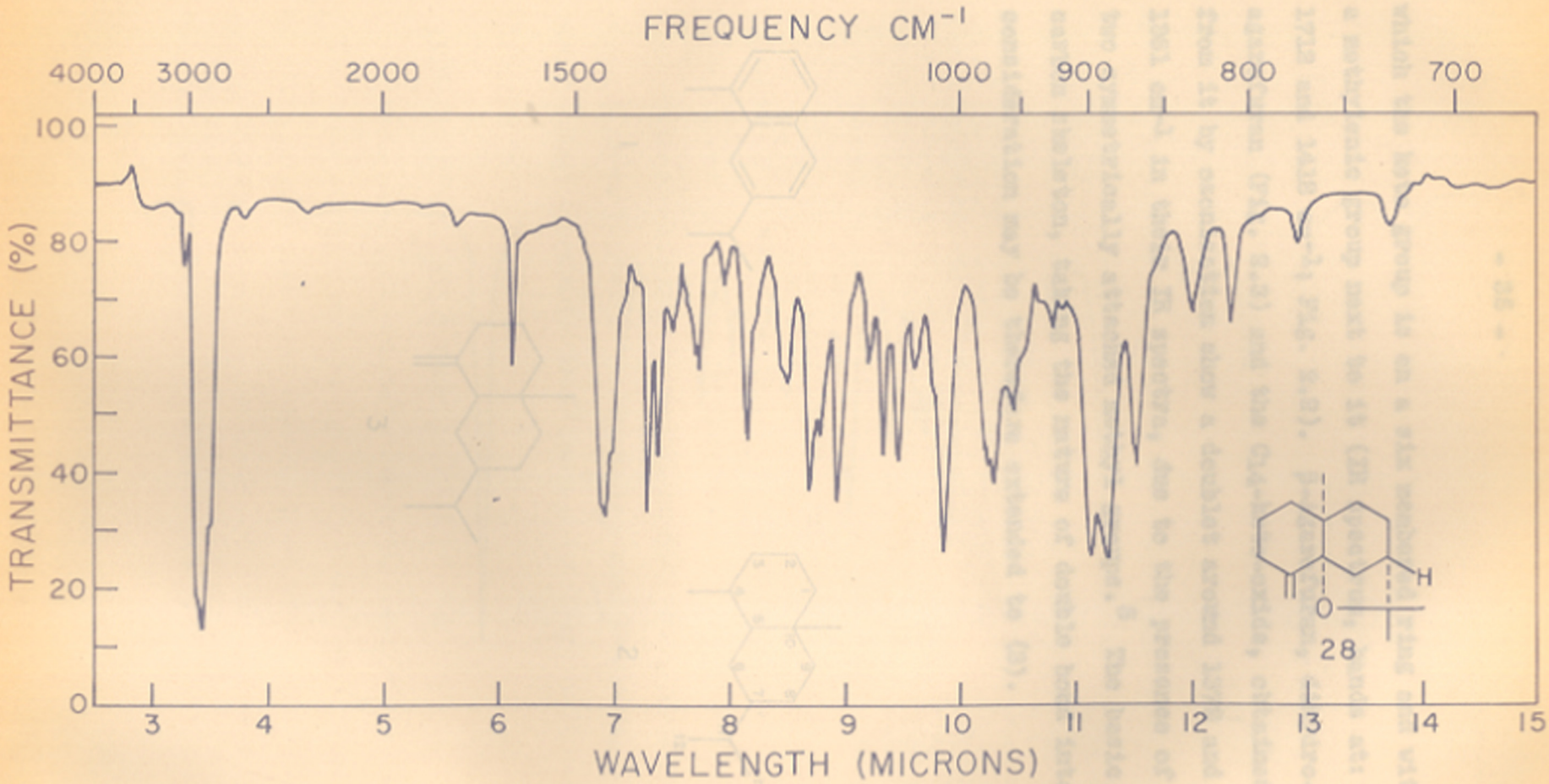
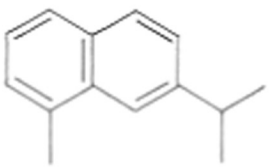


FIG. 2.1 IR SPECTRUM (LIQUID FILM) OF β-AGAROFURAN (28)

which the keto group is on a six membered ring and with a methylenic group next to it (IR spectrum, bands at: 1712 and 1418 cm^{-1}; Fig. 2.2). β-Agarofuran, dihydro-β-agarofuran (Fig. 2.3) and the C_{14}-keto-oxide, obtained from it by ozonisation show a doublet around 1379 and 1361 cm^{-1} in their IR spectra, due to the presence of two symmetrically attached methyl groups.[5] The basic carbon skeleton, taking the nature of double bond into consideration may be therefore extended to (3).



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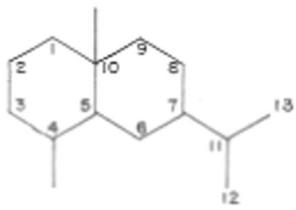

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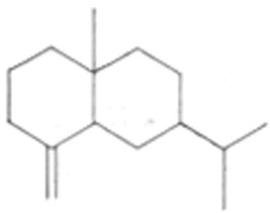

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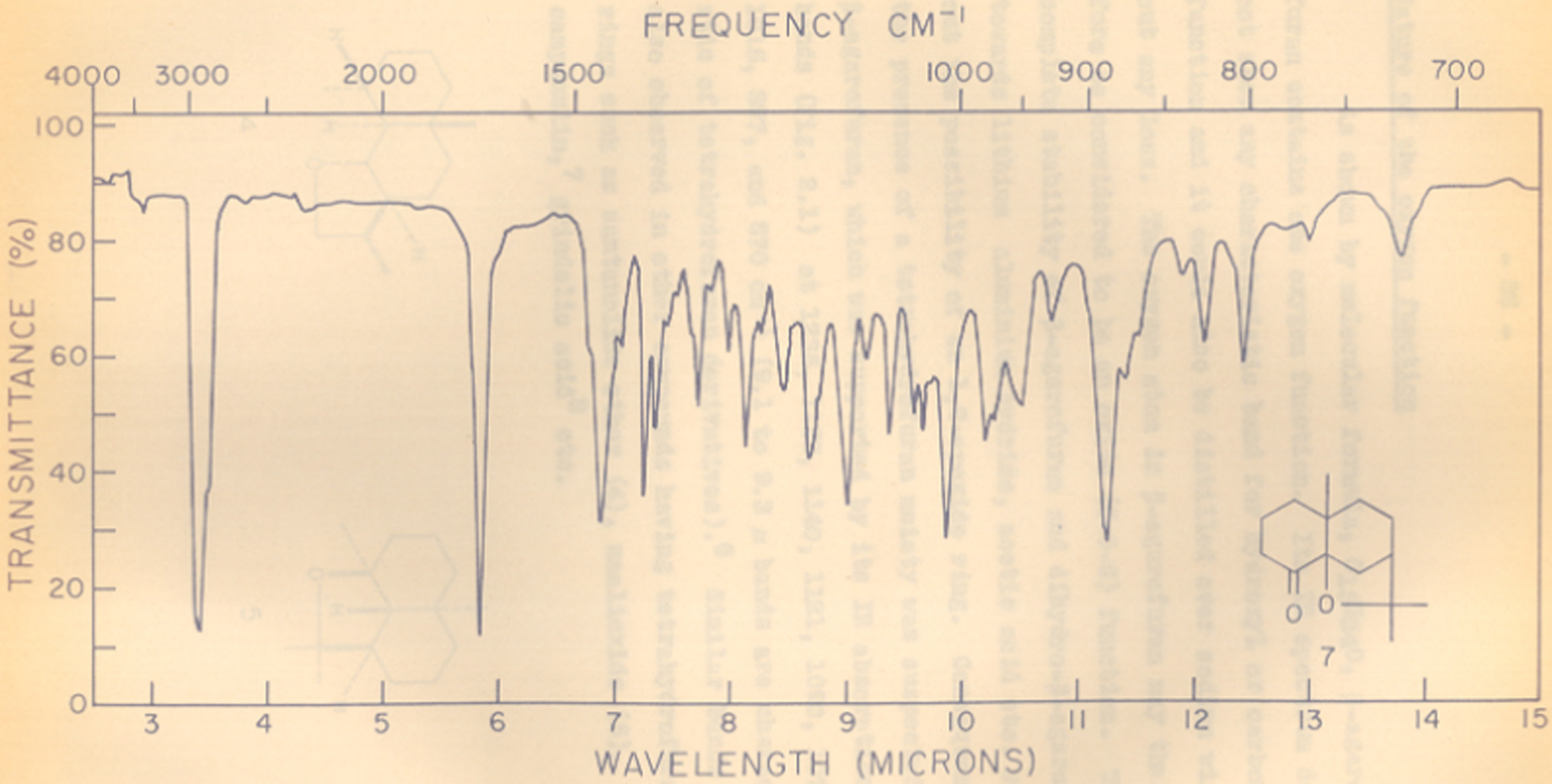
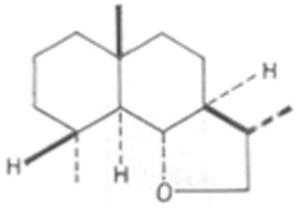


FIG. 2.2 IR SPECTRUM (LIQUID FILM) OF C_{14}-KETO-OXIDE (7)

Nature of the oxygen function

As shown by molecular formula, $C_{15}H_{24}O$, β-agarofuran contains one oxygen function. Its IR spectrum does not show any characteristic band for hydroxyl or carbonyl function and it could also be distilled over sodium without any loss. The oxygen atom in β-agarofuran may therefore be considered to be an oxide (C-O-C) function. The complete stability of β-agarofuran and dihydro-β-agarofuran towards lithium aluminium hydride, acetic acid etc. rules out the possibility of an 1,2-epoxide ring. Consequently, the presence of a tetrahydrofuran moiety was suspected in β-agarofuran, which was supported by its IR absorption bands (Fig. 2.1) at 1235, 1152, 1140, 1121, 1090, 1075, 1015, 887, and 870 cm^{-1} (9.1 to 9.3 μ bands are characteristic of tetrahydrofuran derivatives).[6] Similar bands are also observed in other compounds having tetrahydrofuran rings such as santanolide ether (4), maalioxide (5), campanulin,[7] grindelic acid[8] etc.



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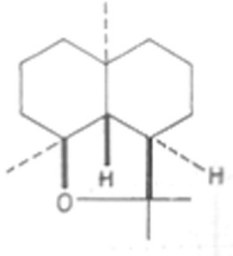

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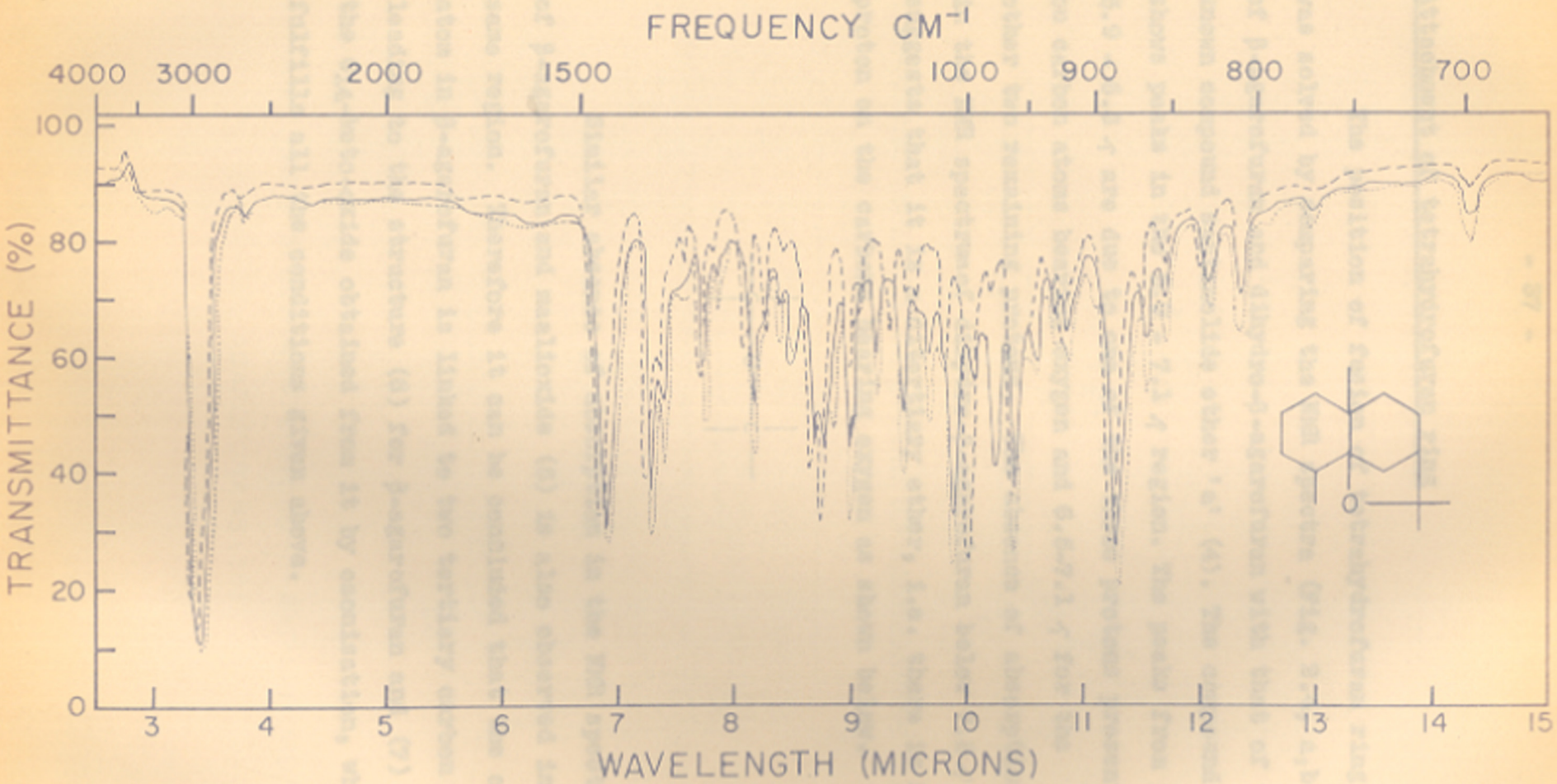
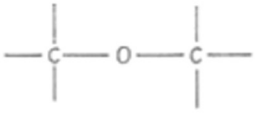


FIG. 2.3 IR SPECTRA (LIQUID FILMS) OF :

——— DIHYDRO-β-AGAROFURAN. – – – – DIHYDRO-α-AGAROFURAN. ····· DIHYDRO AGAROFURAN (NATURAL).

Attachment of tetrahydrofuran ring

The position of fusion of tetrahydrofuran ring was solved by comparing the NMR spectra (Fig. 2.4, a,b) of β-agarofuran and dihydro-β-agarofuran with that of the known compound santanolide ether 'a' (4). The compound (4), shows peaks in the 5.9 - 7.1 τ region. The peaks from 5.9 - 6.3 τ are due to one of the three protons present on carbon atoms bearing oxygen and 6.5-7.1 τ for the other two remaining protons. The absence of absorption in the NMR spectrumof dihydro-β-agarofuran below 7.5τ suggests that it is a ditertiary ether, i.e. there is no proton on the carbons bearing oxygen as shown below.



Similar absence of absorption in the NMR spectra of β-agarofuran and maalioxide (5) is also observed in the same region. Therefore it can be concluded that the oxygen atom in β-agarofuran is linked to two tertiary carbon atoms leading to the structure (6) for β-agarofuran and (7) for the C_{14}-keto-oxide obtained from it by ozonisation, which fulfills all the conditions given above.

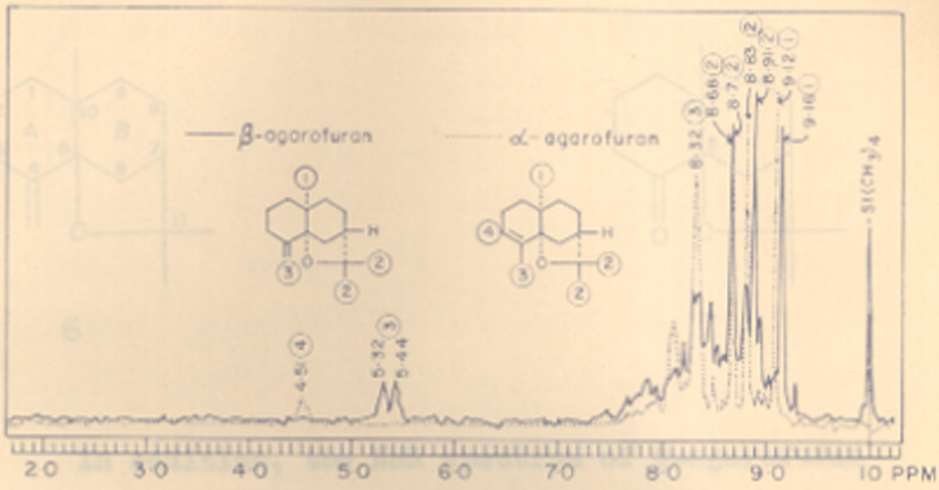


FIG. 2.4.a NMR SPECTRA OF β-AND α-AGAROFURANS (28, 29)

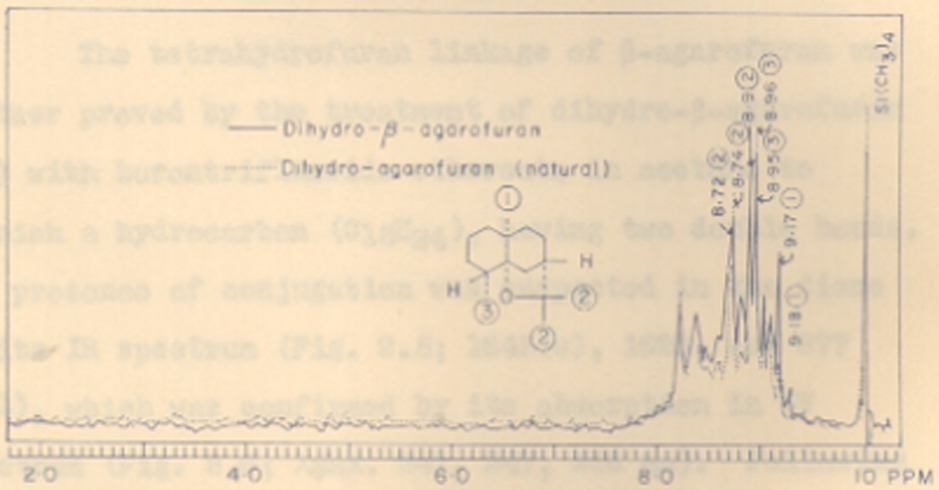
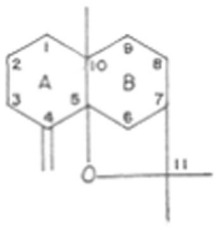
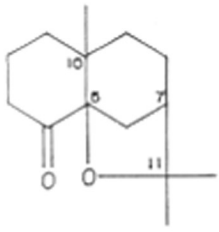


FIG. 2.4.b NMR SPECTRA OF DIHYDRO-β-AND DIHYDRO (NATURAL) AGAROFURANS (38)



6



7

In addition, the NMR spectrum of β-agarofuran (Fig. 2.4a) shows absorption at 5.32 and 5.44 τ which is in accordance with the structure (6) containing two vinyl protons.

The tetrahydrofuran linkage of β-agarofuran was further proved by the treatment of dihydro-β-agarofuran (20) with borontrifluoride ethereate in acetone to furnish a hydrocarbon ($C_{15}H_{24}$), having two double bonds. The presence of conjugation was suspected in the diene by its IR spectrum (Fig. 2.5; 1645(w), 1620, and 877 cm^{-1}), which was confirmed by its absorption in UV spectrum (Fig. 2.6; λ_{max}. 241, 247, 256 mμ). Following three structures (8, 9, 10) are possible for the diene obtained by the treatment of dihydro-β-agarofuran with borontrifluoride ethereate on the basis of the structure (6) of β-agarofuran.

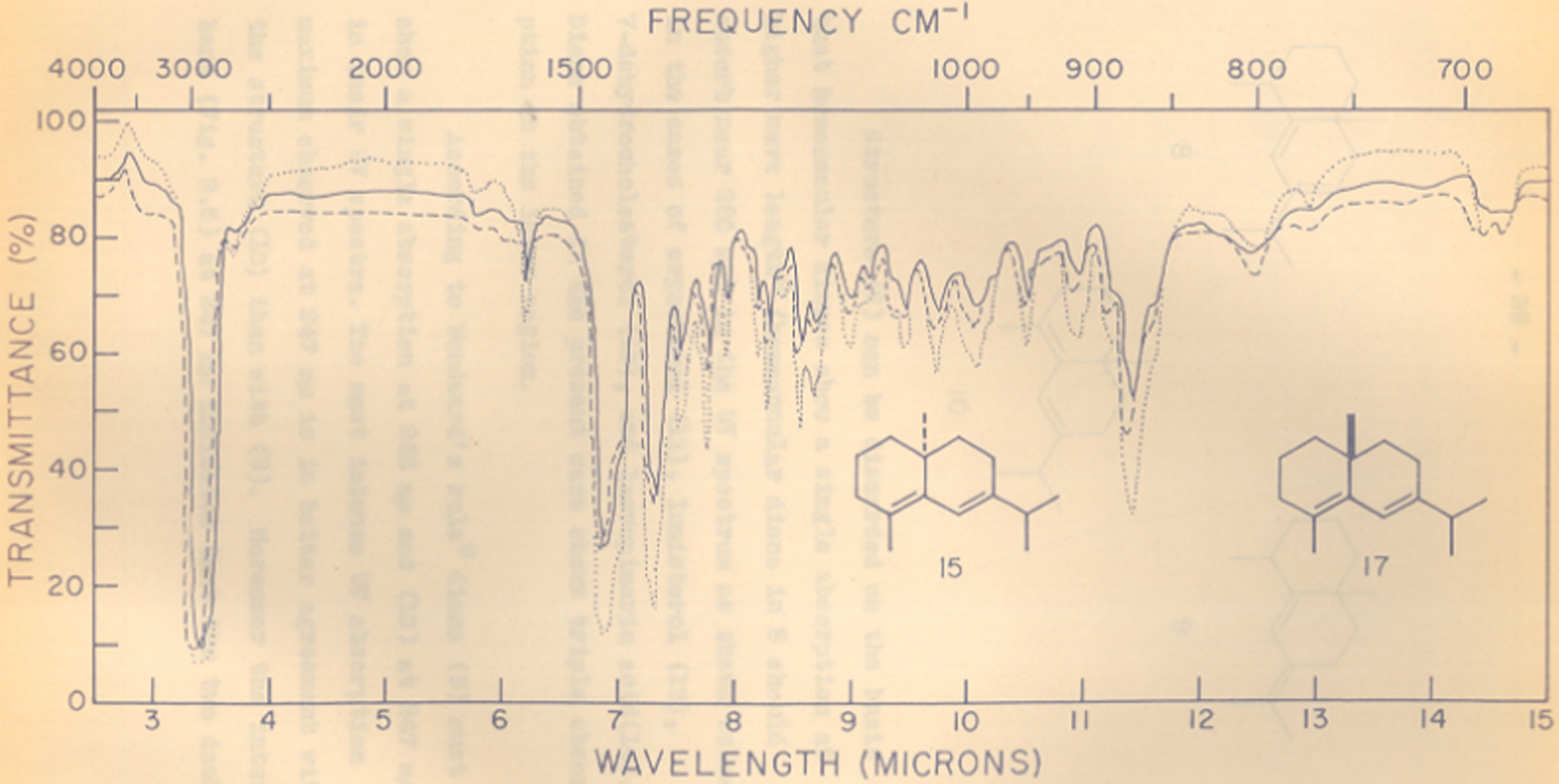
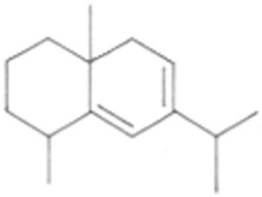
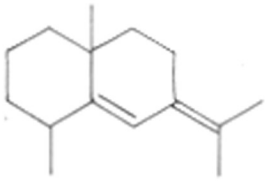


FIG. 2.5 IR SPECTRA (LIQUID FILMS) OF DIENES:

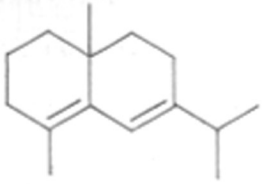
---- DIENE (15) FROM DIHYDRO-β-AGAROFURAN. ····· DIENE (15) FROM MAALIOL. —— DIENE (17) FROM β-EUDESMOL



8



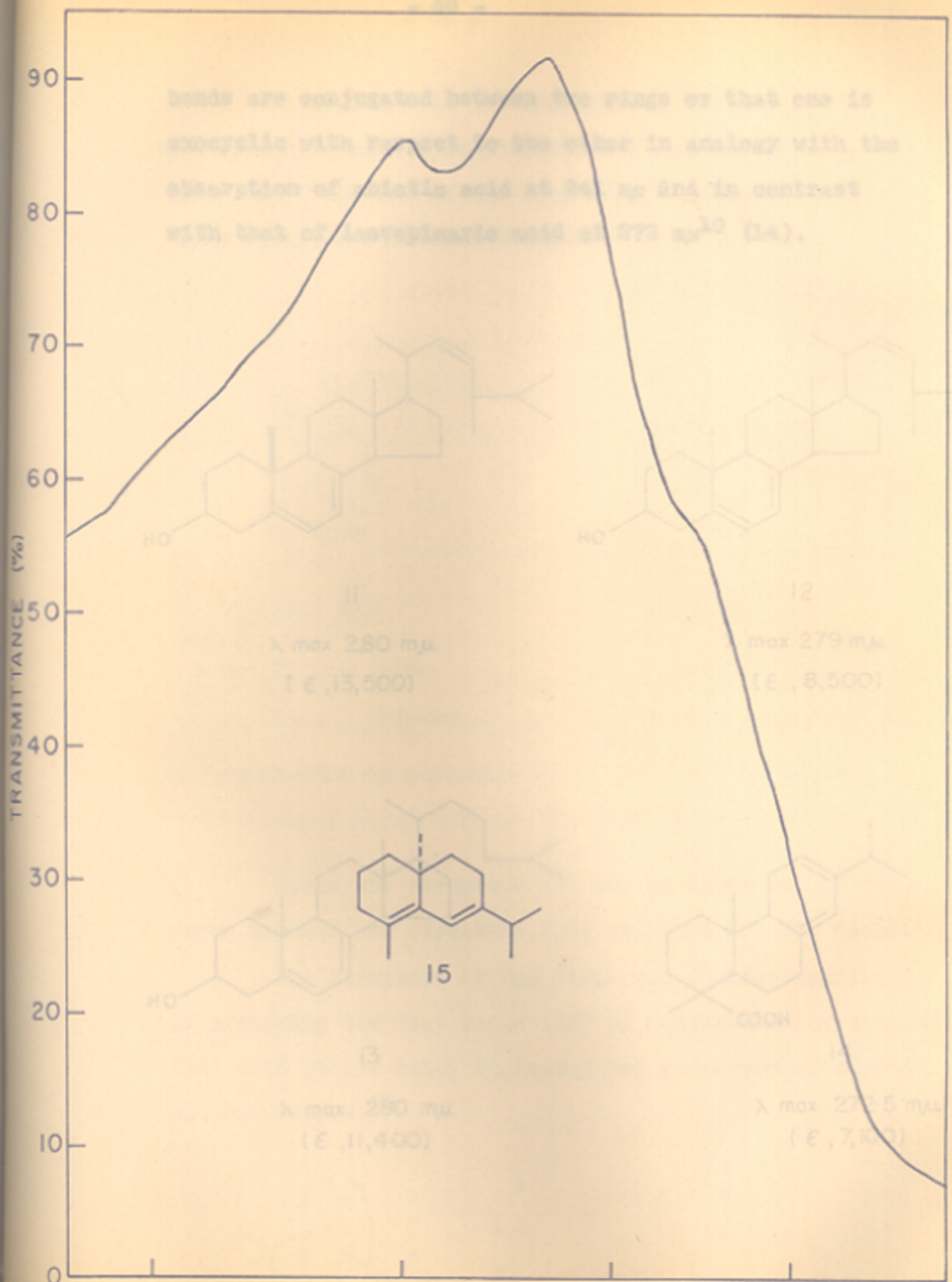
9



10

Structure (8) can be discarded on the basis that homoannular dienes show a single absorption at higher wave length[9] (homoannular diene in 8 should absorb near 280 mμ) in the UV spectrum as shown below in the cases of ergosterol (11), lumisterol (12), 7-dehydrocholesterol (13), and laevopimaric acid(14). Diene obtained in the present case shows triple absorption in the lower region.

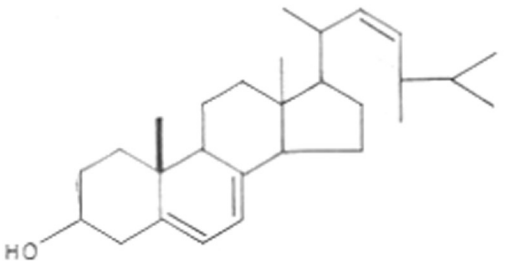
According to Woodward's rule[9] diene (9) must show a single absorption at 252 mμ and (10) at 247 mμ in their UV spectra. The most intense UV absorption maximum observed at 247 mμ is in better agreement with the structure (10) than with (9). Moreower the intense band (Fig. 2.6) at 247 mμ indicated that the two double



15

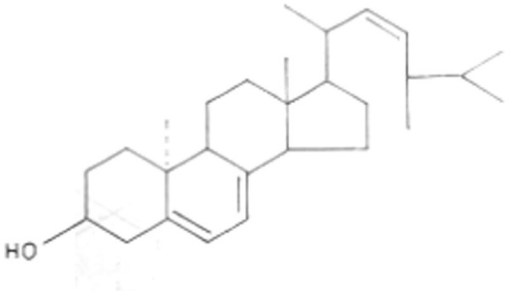
FIG. 2.6 UV SPECTRUM OF (−)-δ- SELINENE (15)

bonds are conjugated between two rings or that one is exocyclic with respect to the other in analogy with the absorption of abietic acid at 241 mμ and in contrast with that of laevopimaric acid at 272 mμ[10] (14).



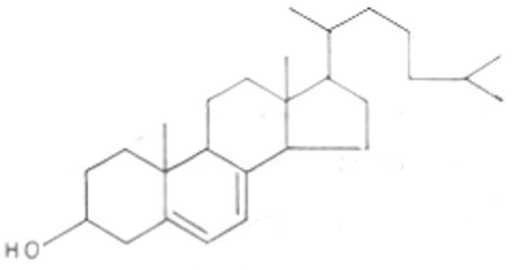
11

λ max 280 mμ
(ε, 13,500)



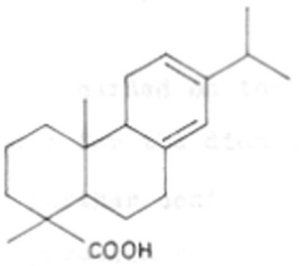
12

λ max 279 mμ
(ε, 8,500)



13

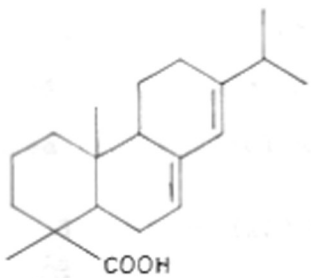
λ max 280 mμ
(ε, 11,400)



14

λ max 272·5 mμ
(ε, 7,100)

In addition to the above fact, the triple absorption is also characteristic of the dienes of the type (10)[11] and not of the type (9),[10] as it will be clear from the following examples.

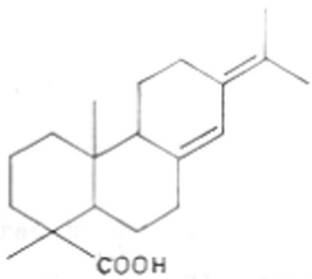


Abietic Acid

λ max 250 mμ (weak)

" 241 " (intense)

"232-233 mμ (medium)



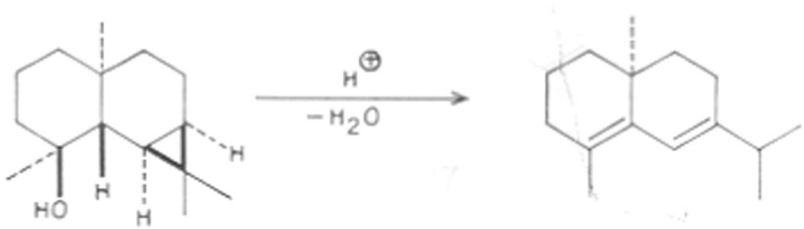
Neo-abietic Acid

λ max 250 mμ

No triple absorption

Hence the structure (9) can be discarded on the above grounds and structure (10) accepted for the diene.

The structure of the diene was further confirmed by preparing the same diene (15) by dehydration of maaliol (16) with formic acid, following the procedure of Büchi and co-workers.[12]

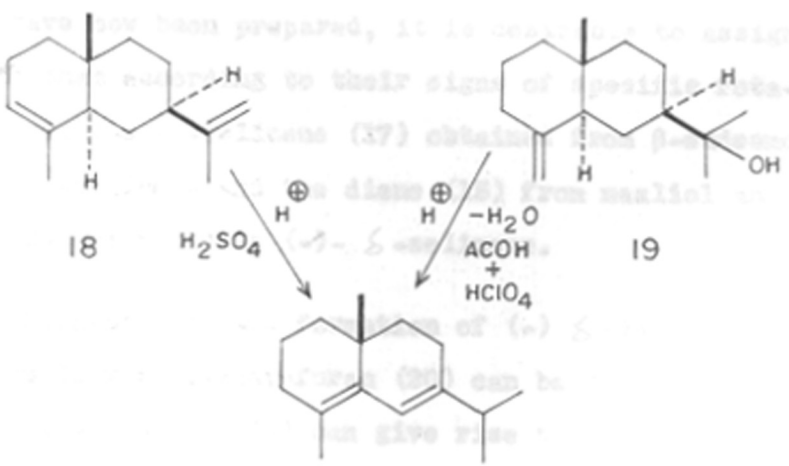


16 15

On comparison, both the dienes (10, 15) were found identical in every respect. Their IR spectra (Fig.2.5) were completely superimposable and both showed the same characteristic triple absorption in UV spectra.

Hence it can be concluded that diene (10) obtained from dihydro-β-agarofuran and diene (15) prepared from maaliol are same.

The diene (15) was considered by Büchi and co-workers to be an enantiomer of δ-selinene (17) prepared by isomerisation of α-selinene (18) with sulphuric acid by Ruzicka.[13] But Buchi and co-workers did not compare the diene (15) and δ-selinene (17) due to non-availability of the latter. Therefore for the purpose of comparison we prepared δ-selinene (17) by dehydration of β-eudesmol (19) with acetic acid and perchloric acid.[14]



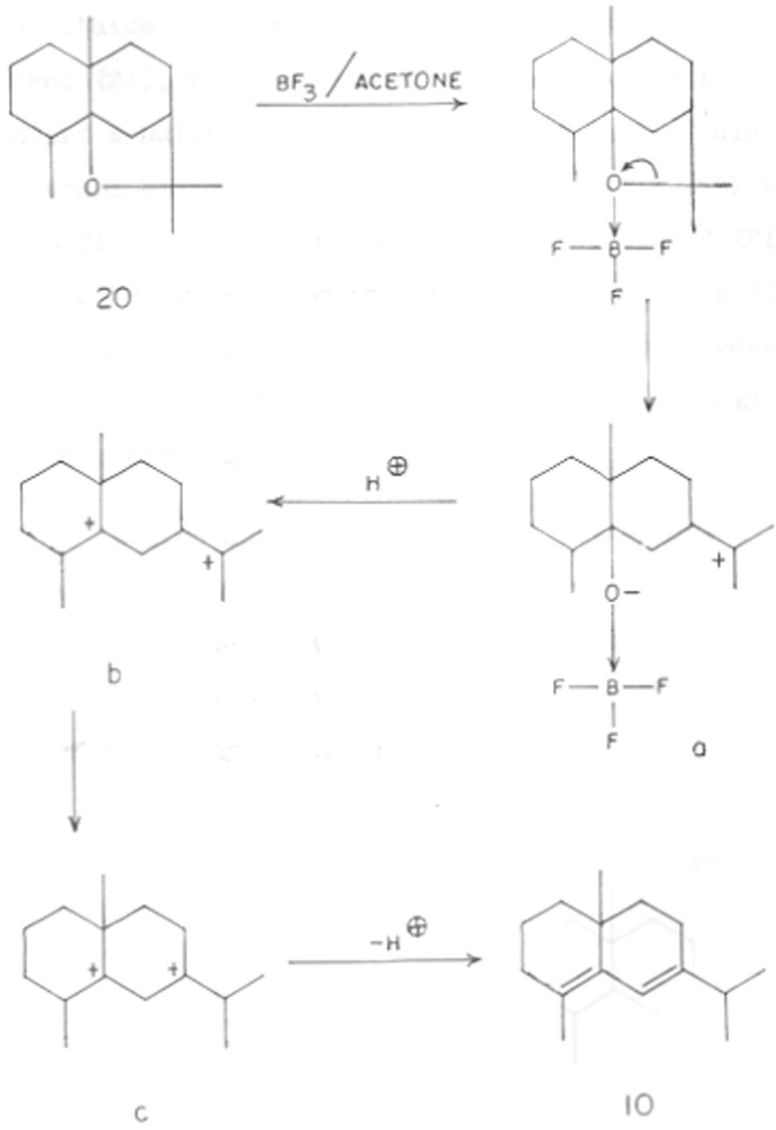
The IR spectrum of δ-selinene (17) was completely superimposable (Fig. 2.5) with the IR spectra of diene (15) and the diene obtained from dihydro-β-agarofuran. UV spectra of all the three conjugated dienes showed the same triple absorption (256, 247, and 241 mμ). The optical rotation of δ-selinene(17) is of very high order $\left[(\alpha)_D^{30} + 265.5^\circ, c, 6.5\right]$. All other physical constants of the three dienes are in agreement.

By observing the constants and specially the signs and magnitudes of rotations it can be concluded that the dienes from dihydro-β-agarofuran $\left[(\alpha)_D^{30} - 188^\circ, c, 2.18\right]$ and maaliol $\left[(\alpha)_D^{30} - 191^\circ, c, 2.85\right]$ are the same and enantiomeric with δ-selinene (17) obtained from β-eudesmol.

In view of the fact that both the enantiomeric dienes have now been prepared, it is desirable to assign names to them according to their signs of specific rotations, i.e. the δ-selinene (17) obtained from β-eudesmol as (+)-δ-selinene and the diene (15) from maaliol and dihydro-β-agarofuran is (-)-δ-selinene.

Mechanism of the formation of (-) δ-selinene (10) from dihydro-β-agarofuran (20) can be given as follows: intermediate (a) can give rise to (b) which

isomerises to more stable intermediate (c) which in its turn furnishes the diene (10) on deprotonation.

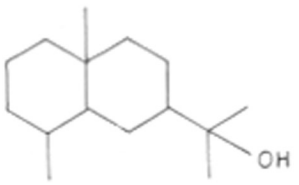


20 a b c 10

Confirmation of the attachment of tetrahydrofuran ring at C_{11} in β-agarofuran

The attachment of the tetrahydrofuran ring at C_{11} was confirmed by reductive cleavage of β-agarofuran with lithium in anhydrous ethylenediamine[15] to give the alcohol (21), the benzoate of which on pyrolysis under standard conditions as employed in the case of dihydro-β-eudesmol benzoate, furnished a hydrocarbon (22) which was sufficient for examination of IR spectrum[16] (Fig.2.7; 1634 and 887 cm^{-1}). The formation of hydrocarbon (22) proved the location of the hydroxyl group in alcohol(21) and consequently that of the attachment of the oxide ring in β-agarofuran at C_{11}.

It also excluded the possibility of the oxide ring occurring as in the eremophilane type of compound (23), which would form on reductive cleavage with lithium either (24) or (25), both of which are incapable of giving on pyrolysis a hydrocarbon having methylenic double bond.



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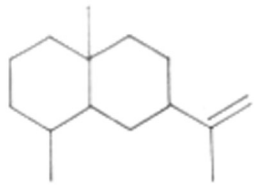


22

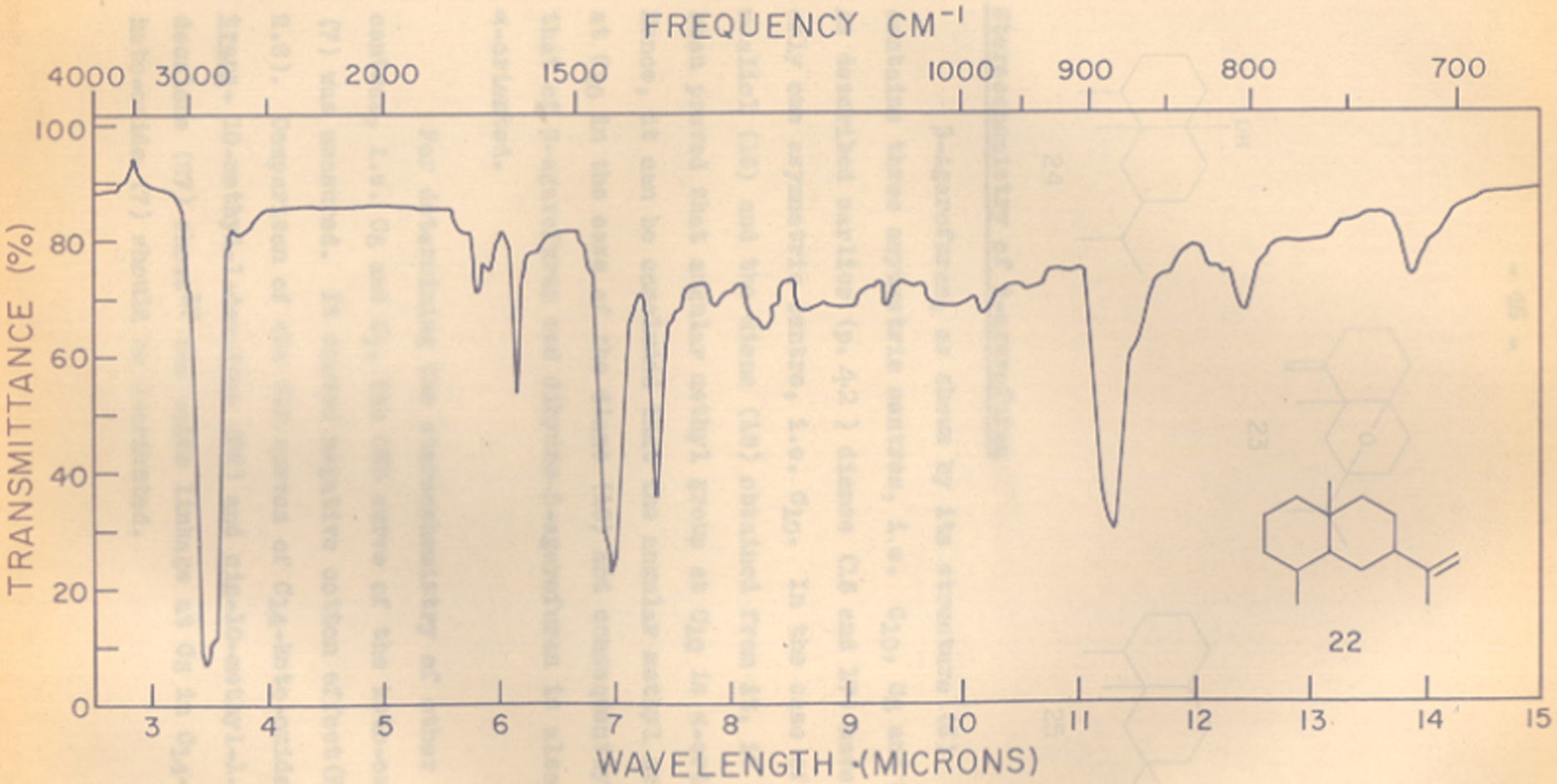
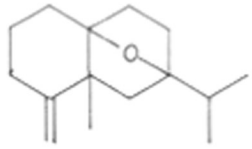
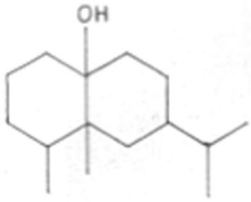


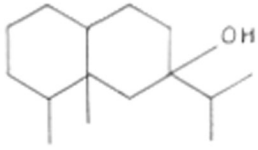
FIG. 2.7 IR SPECTRUM (LIQUID FILM) OF HYDROCARBON (22)



23



24



25

Stereochemistry of β-agarofuran

β-Agarofuran, as shown by its structure (6) contains three asymmetric centres, i.e. C_{10}, C_5 and C_7. As described earlier (p. 42) dienes (15 and 17) have only one asymmetric centre, i.e. C_{10}. In the case of maaliol (16) and the diene (15) obtained from it, it has been proved that angular methyl group at C_{10} is ∝-oriented. Hence, it can be concluded that the angular methyl group at C_{10} in the case of the diene (10) and consequently in that of β-agarofuran and dihydro-β-agarofuran is also ∝-oriented.

For determining the stereochemistry of other two centres, i.e. C_5 and C_7, the ORD curve of the keto-oxide (7) was measured. It showed negative cotton effect (Fig. 2.8). Comparison of the ORD curves of C_{14}-keto-oxide (7), trans- 10-methyl-1-decalone (26) and cis-10-methyl-1-decalone (27) shows[17] the oxide linkage at C_5 in C_{14}-keto-oxide (7) should be ∝-oriented.

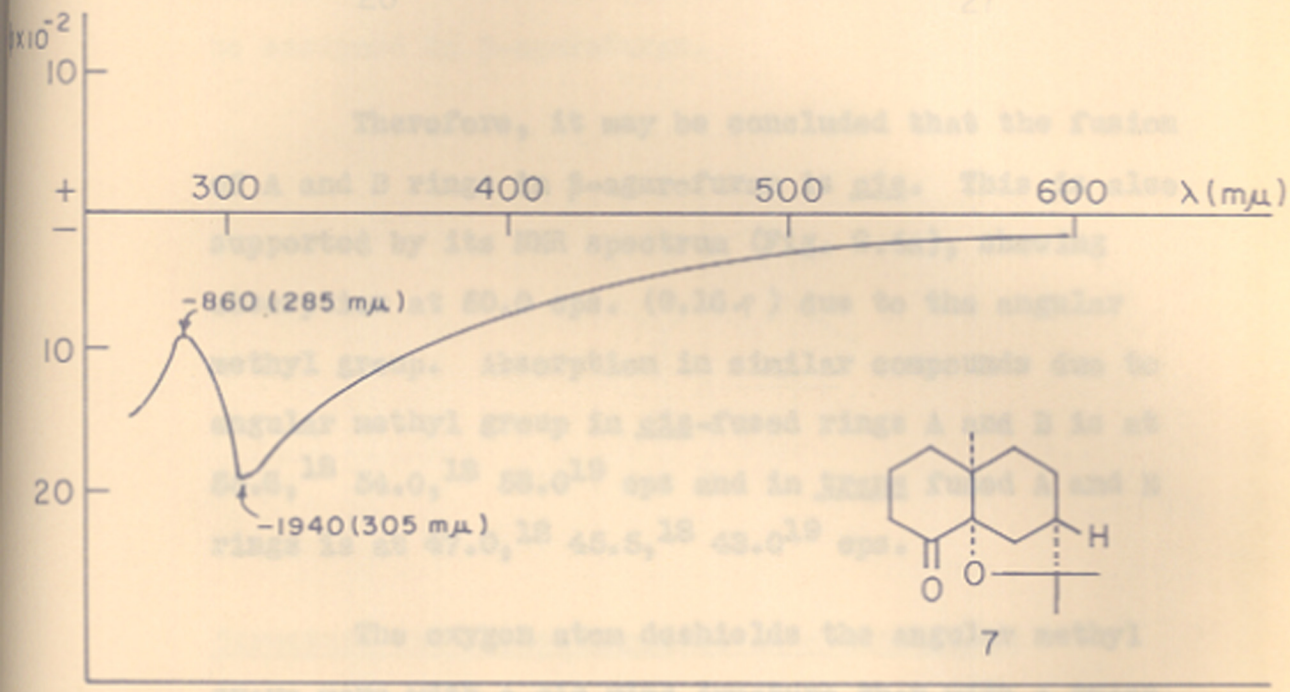
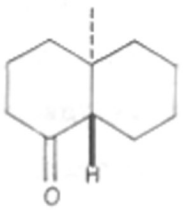
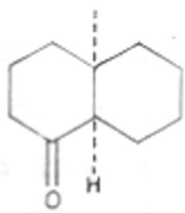


FIG. 2.8 ORD CURVE OF C_{14}-KETO-OXIDE (7)

[NOR-KETOAGAROFURAN (4) REFER TO CHAPTER III, P. 71.]



26

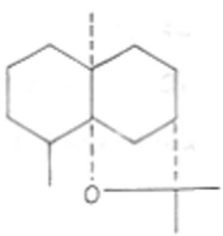


27

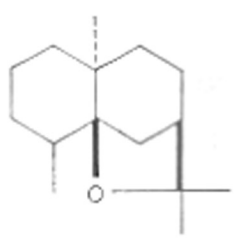
Therefore, it may be concluded that the fusion of A and B rings in β-agarofuran is <u>cis</u>. This is also supported by its NMR spectrum (Fig. 2.4a), showing absorption at 50.0 cps. (9.16 τ) due to the angular methyl group. Absorption in similar compounds due to angular methyl group in <u>cis</u>-fused rings A and B is at 55.6,[18] 54.0,[18] 58.0[19] cps and in <u>trans</u> fused A and B rings is at 47.0,[18] 45.5,[18] 43.0[19] cps.

The oxygen atom deshields the angular methyl group more with a <u>cis</u> ring juncture than with a <u>trans</u>. The average distance between protons of the methyl group and the oxygen nucleus, as measured from Dreiding models, are in parenthesis.

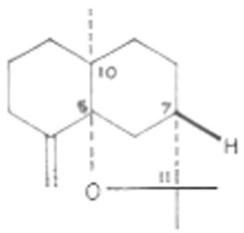
(2·8—3·2 Å)



(4·0 Å)



By model studies it has been observed that the tetrahydrofuran ring can be fused at C_5 and C_7 only when both the linkages (C_5-O and C_7-C_{11}) are axially oriented. Consequently, since the A and B rings have cis-fusion and the angular methyl group and the oxide linkage (C_5 - O) are ∝-oriented, the C7-C11 bond also automatically becomes ∝-oriented. Hence the stereochemical structure (28) can be assigned to β-agarofuran.



28

Structure and stereochemistry of ∝-agarofuran

∝-Agarofuran is the double bond isomer of β-agarofuran and therefore we shall discuss it only in brief.

∝-Agarofuran analysed for the molecular formula, $C_{15}H_{24}O$ and from the experimental evidences recorded herein, can be represented by structure (29). Its IR spectrum (Fig. 2.9) pattern was essentially similar to that of β-agarofuran, specially in the finger print region

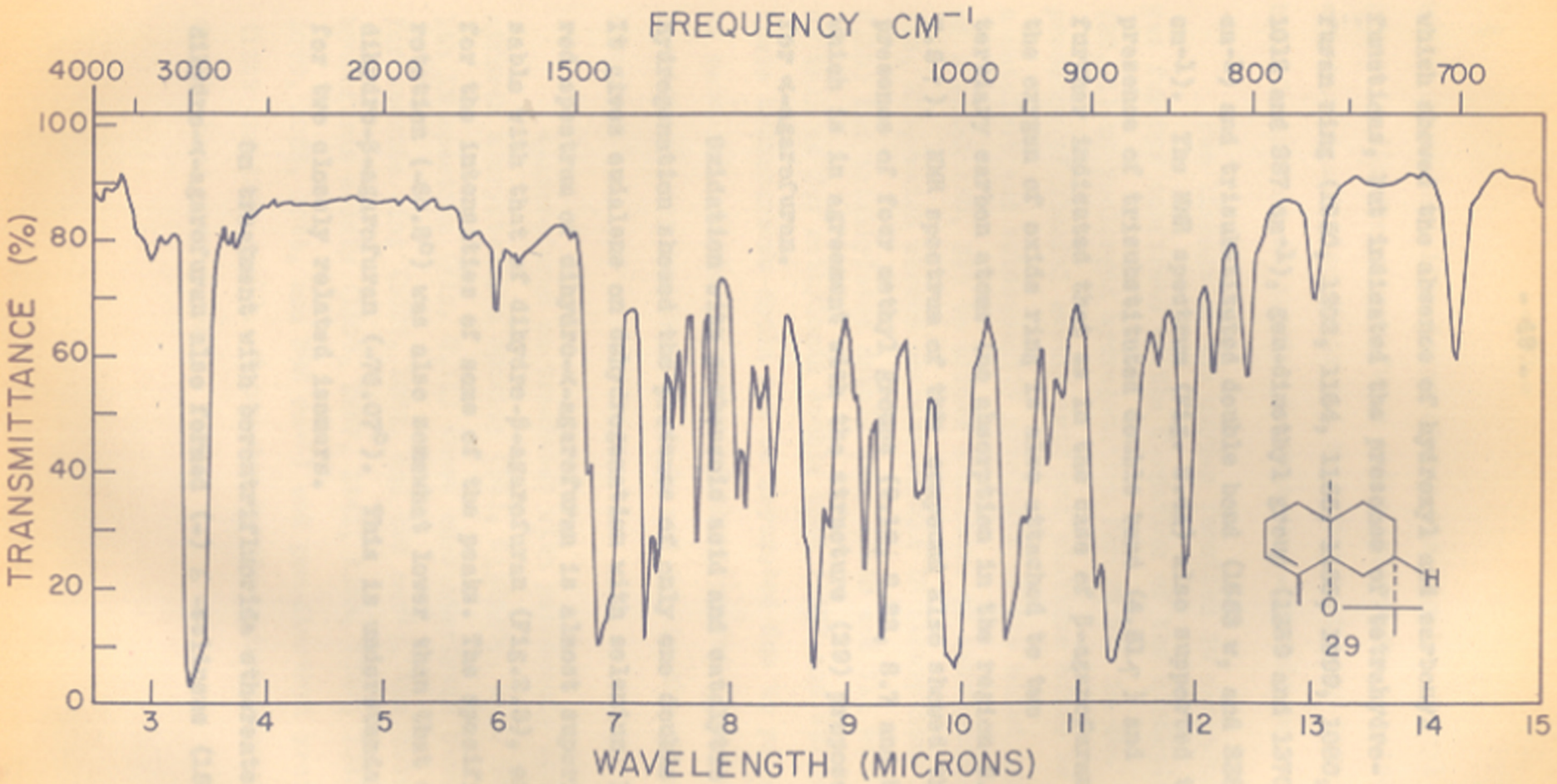


FIG. 2.9 IR SPECTRUM (LIQUID FILM) OF α-AGAROFURAN (29)

which showed the absence of hydroxyl and carbonyl functions, but indicated the presence of tetrahydrofuran ring (1250, 1202, 1164, 1155, 1136, 1099, 1080, 1012 and 887 cm^{-1}), gem-dimethyl group (1389 and 1370 cm^{-1}) and trisubstituted double bond (1653 w, and 838 s cm^{-1}). The NMR spectrum (Fig. 2.4a) also supported the presence of trisubstituted double bond (4.51τ) and further indicated that as in the case of β-agarofuran the oxygen of oxide ring is also attached to two tertiary carbon atoms (no absorption in the region 5.9 to 7.5τ). NMR spectrum of this compound also showed the presence of four methyl groups (9.12, 8.83, 8.7 and 8.32τ) which is in agreement with the structure (29) proposed for α-agarofuran.

Oxidation with perbenzoic acid and catalytic hydrogenation showed the presence of only one double bond. It gives eudalene on dehydrogenation with selenium. Infrared spectrum of dihydro-α-agarofuran is almost superimposable with that of dihydro-β-agarofuran (Fig.2.3), except for the intensities of some of the peaks. The specific rotation (-60.8°) was also somewhat lower than that of dihydro-β-agarofuran (-76.07°). This is understandable for two closely related isomers.

On treatment with borontrifluoride ethereate, dihydro-α-agarofuran also formed (-) δ -selinene (15)

which shows that like β-agarofuran the angular methyl group in dihydro-∝-agarofuran and ∝-agarofuran is also ∝-oriented and also indicated that the fusion of tetrahydrofuran ring is similar to that in β-agarofuran.

The linkage of the oxygen function in ∝-agarofuran was further confirmed by the formation of the alcohols (30,31) by prolonged hydrogenation for 72 hrs in the presence of glacial acetic acid and platinum oxide as a catalyst and subsequently pyrolysis of the mixed benzoate of alcohol (30, 31) to afford a mixture of hydrocarbons (32, 33), which showed characteristic IR absorptions (Fig. 2.10) for trialkylated double bond (1653 and 811 cm^{-1}) as well as for methylenic double bond (1637 and 891 cm^{-1}). These hydrocarbons could not be separated due to paucity of material but the formation of these proved that tetrahydrofuran ring is attached at C_5 and C_{11} in the parent compound.

∝-Agarofuran on treatment with perbenzoic acid affords a crystalline epoxide (34), $C_{15}H_{24}O_2$, m.p. 88°. Its IR spectrum (Fig. 2.11) shows characteristic bands for epoxide ring (1089, 1024, and 882 cm^{-1}), the tetrahydrofuran moiety (1309, 1287, 1241, 1160, 1144, 1121, 1089, 1058, 1024, and 898 cm^{-1}) and gem-dimethyl group (1389 and 1370 cm^{-1}). Bands due to the trisubstituted double bond were absent.

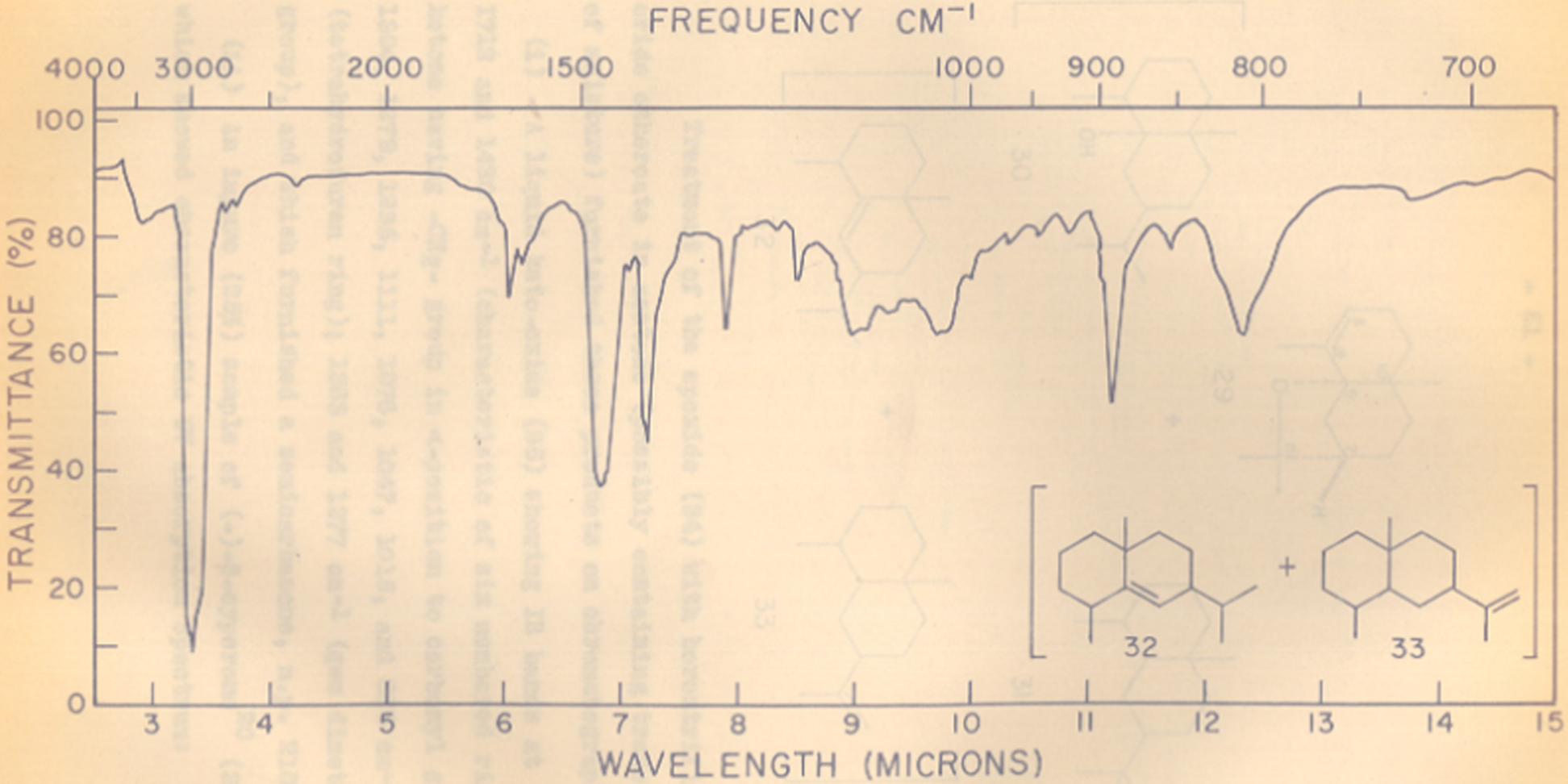
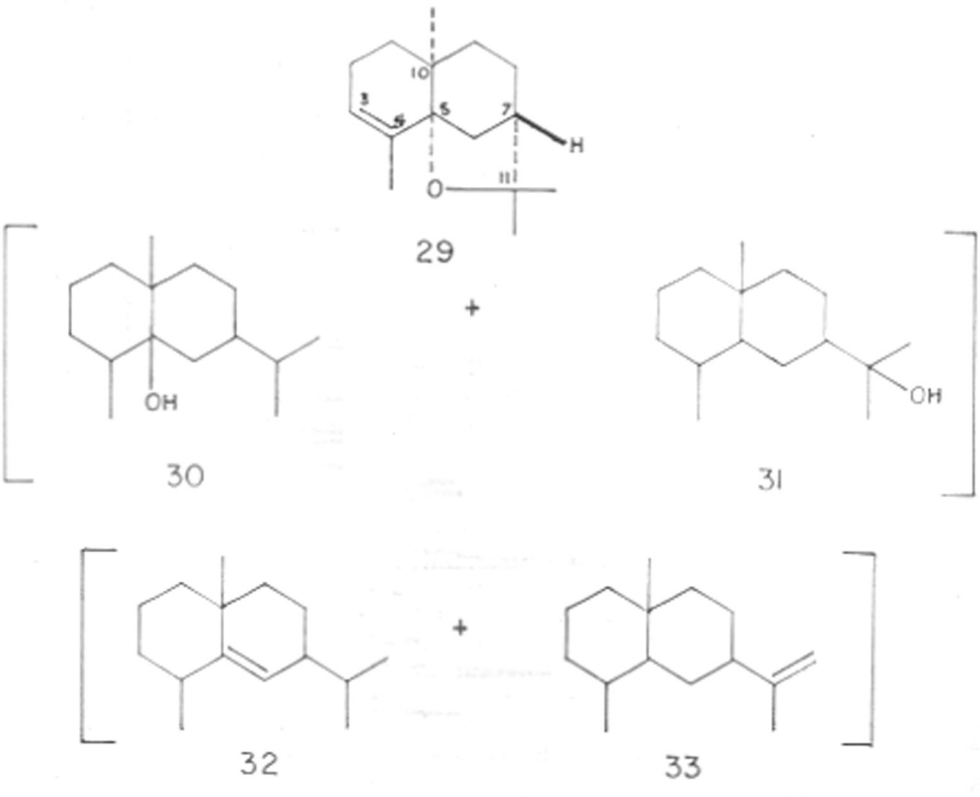


FIG. 2.10 IR SPECTRUM (LIQUID FILM) OF HYDROCARBONS (32,33)



29 + 30 + 31

32 + 33

Treatment of the epoxide (34) with borontrifluoride ethereate in acetone (possibly containing traces of moisture) furnished three products on chromatography.

(i) A liquid keto-oxide (35) showing IR bands at 1712 and 1420 cm^{-1} (characteristic of six membered ring ketone having $-CH_2-$ group in α-position to carbonyl group), 1304, 1279, 1235, 1111, 1076, 1047, 1015, and 889 cm^{-1}, (tetrahydrofuran ring); 1383 and 1377 cm^{-1} (gem dimethyl group), and which furnished a semicarbazone, m.p. 210°;

(ii) An impure (25%) sample of (-)-β-cyperone[20] (36), which showed characteristic UV absorption spectrum:

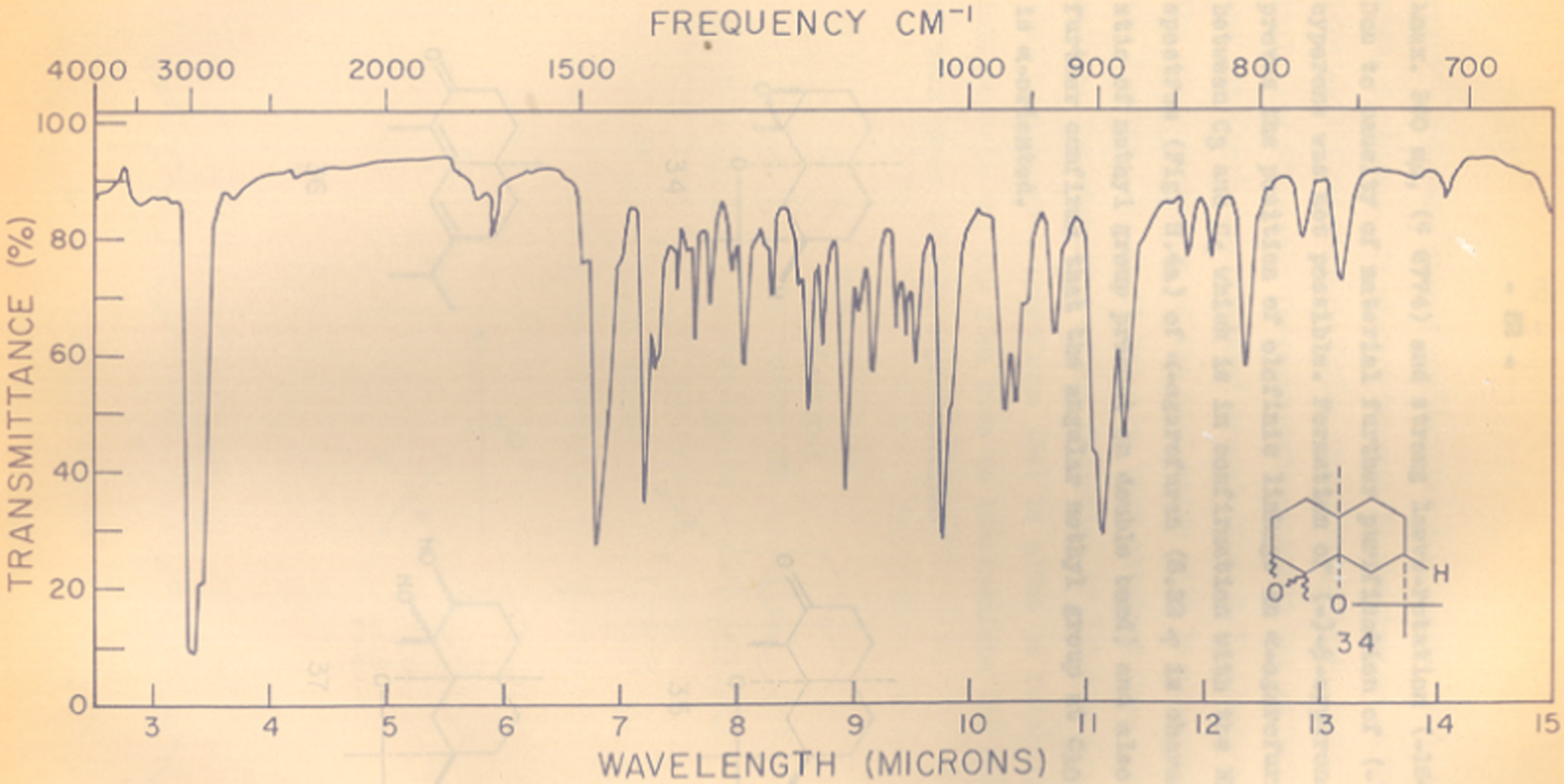
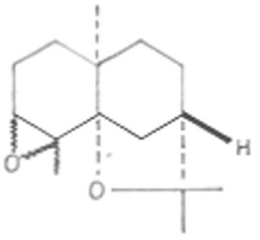


FIG. 2.11 IR SPECTRUM (IN NUJOL) OF EPOXY-α-AGAROFURAN (34)

λmax. 300 mμ, (ε 6774) and strong laevo-rotation (-164.2°). Due to paucity of material further purification of (-)-β-cyperone was not possible. Formation of (-)-β-cyperone proves the position of olefinic linkage in ∝-agarofuran between C_3 and C_4 which is in confirmation with the NMR spectrum (Fig. 2.4a) of ∝-agarofuran (8.32 τ is characteristic of methyl group present on double bond) and also further confirms that the angular methyl group at C_{10} is ∝-oriented.



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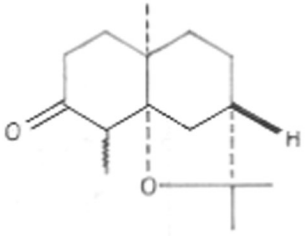

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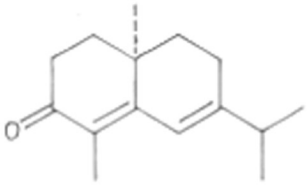

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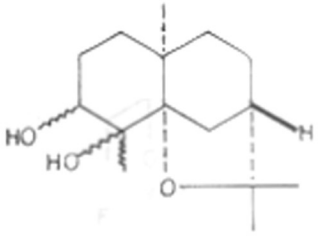
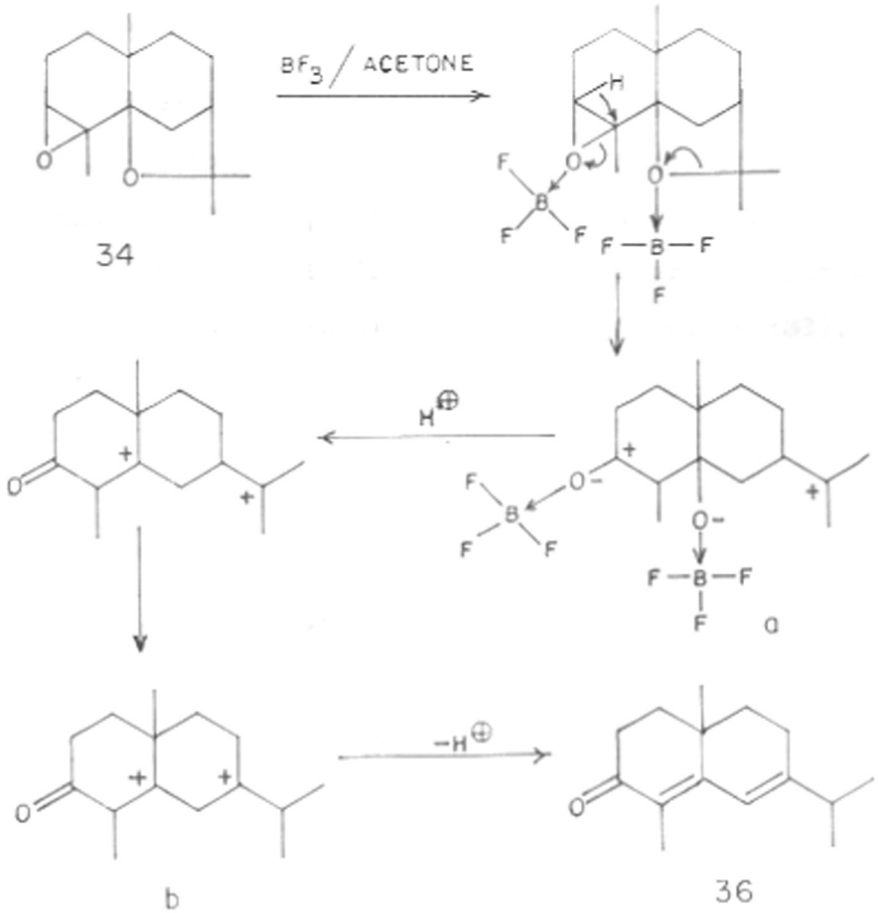


37

(iii) A needle shaped crystalline diol-oxide (37), m.p. 176°, $C_{15}H_{26}O_3$. It showed intense IR absorption at 3520, 1140 cm^{-1} (-OH groups), 1316, 1295, 1235, 1106, 1089, 1015, and 881 cm^{-1} (tetrahydrofuran ring) and at 1397 and 1389 cm^{-1} (gem-dimethyl group) (Fig. 3.5, Chapter III). The stereochemistry of the diol-oxide will be discussed in the Chapter III, as it has also been obtained from the nature. The mechanism of the formation of (-)-β-cyperone (36) from epoxy-α-agarofuran (34) is given as follows, intermediate (a) can give rise to intermediate (b) which in turn gives (36) on deprotonation:



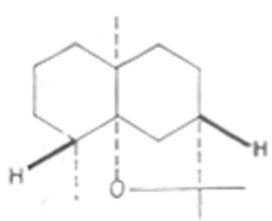
The non-volatile product of ozonisation of ∝-agarofuran showed the presence of an aldehyde function and a methyl ketone group (iodoform test).

All the evidences support the structure (29) for ∝-agarofuran.

Dihydroagarofuran

Along with ∝- and β- agarofurans we also obtained pure dihydroagarofuran from infected agarwood oil, all the properties of which were identical with those of dihydro-β-agarofuran. Their IR spectra (Fig. 2.3) were also completely superimposable. Their NMR spectra (Fig. 2.4b) were also identical.

Assuming that during the formation of dihydro-β-agarofuran the hydrogen attack takes place from the less hindered β-face,[21] the stereochemistry of dihydro-β-agarofuran (which is identical with natural dihydro-agarofuran) can be represented by the structure (38).



E X P E R I M E N T A L

Isolation of dihydroagarofuran (38), β-agarofuran (28) and α-agarofuran (29)

For isolation of these compounds, please refer to Chapter I, p. 25 of the thesis.

Dehydrogenation of β-agarofuran (28) with selenium

β-Agarofuran (202 mg), mixed with Se (202 mg) was heated in an atmosphere of nitrogen at 300° for 10 hrs. The product after extracting with pet.ether was passed through a column of alumina (grade I, 20 g). The eluate (165 mg) after working up in the customary manner, afforded eudalene (1) (150 mg), UV spectrum: λmax. 227.5, 279 mμ (log ε 4.5658, 3.3730); picrate m.p. 94°, undepressed on admixture with an authentic specimen (Found: C, 57.81; H, 4.98; N, 10.1. Calculated for $C_{20}H_{19}O_7N_3$ C, 58.11; H, 4.63; N, 10.17%).

Preparation of dihydro-β-agarofuran

β-Agarofuran (304 mg) dissolved in glacial acetic acid (20 ml) was stirred in an atmosphere of hydrogen with pre-reduced Adams PtO_2 catalyst (50 mg). The equivalent of 0.98 mole of hydrogen was absorbed in 30 minutes. The catalyst was filtered and solvent was removed in vacuum to furnish dihydro-β-agarofuran (270 mg) which on chromatography over 100 fold alumina (grade I, 2.7 g) followed

by vacuum distillation gave the analytical specimen, b.p. 135° at 8 mm., n_D^{29} 1.4910, $(\alpha)_D^{30}$ - 76.07° (c, 8.29); no colouration with tetranitromethane; IR bands at: 1379, 1359, 1155, 1144, 1114, 1089, 1062, 1015, 998, 980, 952, 887 and 869 cm^{-1}, no band at 1639 and 898 cm^{-1} (Found: C, 81.32; H, 12.05. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%).

Ozonolysis of β-agarofuran

β-Agarofuran (266 mg) in chloroform (50 ml) was ozonised for 2 hrs at 0°. The solvent was distilled off and the residue steam distilled for a short time and the distillate led into dimedone solution. The steam distillate on standing in the freeze overnight gave formaldimedone (176 mg, equivalent to 0.5 mole of $>C=CH_2$ grouping), m.p. 187°, undepressed on admixture with an authentic specimen. No acetone was detected.

The non-volatile portion was taken up in ether and worked up in the customary manner to yield the neutral product (180 mg). This was purified by chromatography over alumina (grade II, 10 g), eluted with benzene (50 ml) followed by distillation to give keto-oxide (7), (122 mg), b.p. 145° at 4 mm., n_D^{28} 1.4946, $(\alpha)_D^{30}$ - 69.2° (c, 6.9), IR bands at: 1712, 1418, 1379, 1361, 1295, 1229, 1149, 1140, 1110, 1092, 1067, 1013, 888, and 875 cm^{-1}; negative haloform reaction (Found: C, 75.98; H, 10.40. $C_{14}H_{22}O_2$ requires: C, 75.63; H, 9.97%). Semicarbazone, m.p. 195°.

This C_{14}-keto-oxide was subsequently obtained in crystalline form and melted at 56-57° (for details see p. 77).

Treatment of dihydro-β-agarofuran (38) with boron-trifluoride ethereate in acetone

Freshly distilled borontrifluoride ethereate (1 ml) was added to a solution of dihydro-β-agarofuran (500 mg) in dry acetone (20 ml) and kept at room temperature for 48 hrs. Addition of ice cold water (10 ml) and working up in the customary manner furnished a hydrocarbon (430 mg) which was further purified by chromatography over alumina (grade I, 43 g; elution by 50 ml. of pet.ether) followed by distillation to afford diene (15) (360 mg), b.p. 120° at 8 mm., n_D^{31} 1.5170, $(\alpha)_D^{30}$ - 188° (c, 2.18), UV spectrum: λmax. 241, 247, 256 mμ (ε 28140, 28540, 16700); IR bands at: 2900, 1645 (w), 1620, 1389, 1379, 1355, 1299, 1270, 1215, 1174, 1066, 1034, 995, 953, 877, and 804 cm-1 (Found: C, 87.86; H, 11.9. $C_{15}H_{24}$ requires: C, 88.16; H, 11.84%).

Dehydration of maaliol (16) to the diene (15)

Maaliol (1 g) was dissolved in anhydrous formic acid (10 ml) and the mixture heated on the steam bath for 5 hrs. The material was then poured into ice water and extracted with pet.ether. The organic layer was washed repeatedly with aqueous sodium bicarbonate solution and

water and after drying was passed through a column of alumina (grade I, 20 g), and eluted with pet.ether. After the solvent had been removed by evaporation, the residue was distilled to furnish the diene (15) (508 mg), b.p. 118-120° at 8 mm., n_D^{31} 1.5169, $(\alpha)_D^{30}$ - 191° (c, 2.85), UV spectrum: λmax. 241, 247, 256 mμ (ε 18870, 19900, 13000), IR bands at: 2900, 1645(w), 1620, 1385, 1375, 1295, 1270, 1215, 1175, 1065, 1030, 995, 876, and 805 cm-1 (Found: C, 87.80; H, 12.0. Calculated for $C_{15}H_{24}$ C, 88.16; H, 11.84%).

Dehydration of β-eudesmol (19) to the diene (17)

To a solution of β-eudesmol (2.5 g) in glacial acetic acid (25 ml), perchloric acid (1 ml, 60%) was added. The reaction mixture was allowed to stand at room temperature (25-27°) for 72 hrs. with continuous stirring. The reaction product was diluted with a large excess of water and extracted with ether. The ethereal extract was washed with aqueous sodium bicarbonate, then with water and dried over anhydrous Na_2SO_4. The solvent was removed and the residue (2.29 g) was taken up for further purification by chromatography over alumina (grade I, 100 g), followed by distillation to furnish analytically pure diene (17) (1.9 g), b.p. 105-110° at 1 mm., n_D^{31} 1.5172, $(\alpha)_D^{30}$ + 265.5° (c, 6.5); UV spectrum: λmax. 240,247,256 mμ (ε 31110, 32045, 18615), IR bands at: 2900, 1645(w), 1618, 1385,

1372, 1290, 1266, 1222, 1209, 1171, 1156, 1112, 1060, 1029, 995, 953, 915, 876, and 803 cm^{-1} (Found: C, 87.85; H, 12.05. Calculated for $C_{15}H_{24}$ C, 88.16; H, 11.84%).

Reduction of β-agarofuran (28) with lithium ethylenediamine

To a stirred solution of β-agarofuran (400 mg) in anhydrous ethylenediamine (25 ml), lithium (150 mg) was added during 1.5 hr at a temperature of 90-100° in an atmosphere of anhydrous oxygen-free nitrogen. After all the lithium had been added the mixture was refluxed for 12 hrs. The product was then cooled in ice and carefully decomposed by addition of an adequate quantity of water and repeatedly extracted with ether. The combined organic layer was washed with dilute hydrochloric acid, sodium bicarbonate solution, then with distilled water, dried over anhydrous sodium sulphate, filtered and solvent removed to furnish a viscous residue (360 mg). This was chromatographed over 50 fold alumina (grade II, 18 g., elution with 50 ml. benzene) to furnish the alcohol (21) (300 mg), which on distillation afforded analytical specimen (241 mg), b.p. 115-120° at 0.3 mm., n_D^{25} 1.5045, $(\alpha)_D^{30}$ + 10.33° (c, 4.55); no colouration with tetranitromethane; IR bands at: 3480, 1136, and 933 cm^{-1} (Found: C, 80.50; H, 12.26. $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%).

Preparation of hydrocarbon (22)

(i) Benzoyl chloride (0.1 ml) was added to the solution of alcohol (21) (80 mg) in pyridine (0.5 ml) and the

mixture kept at room temperature for 24 hrs and then heated for 2 hrs on water bath. After working up the product in the customary way, the benzoate (65 mg) was obtained, which was chromatographed over alumina (grade II, 5 g), to furnish benzoate (54.6 mg); IR bands at: 1721, 1600, 1580, 1383, 1366, 1314, 1282, 1171, 1111, 1067, 1027, 807, and 710 cm-1 (characteristic bands for benzoate 1721, 1600, 1580, 1282, and 710 cm-1).

(ii) The pyrolysis of benzoate (50 mg) was carried out under a pressure of 100 mm. and at a temperature 200-210° for 15 minutes. The pyrolysed product was dissolved in pet.ether (2 ml) and transferred to a column of alumina (grade I, 1 g) and eluted with pet.ether(10 ml). After removing the solvent, it afforded hydrocarbon (22) (6.8 mg) which was sufficient for IR spectrum and showed bands at 2874, 1634, 1439, 1362, and 887 cm-1.

Dehydrogenation of ∝-agarofuran (29) with selenium

∝-Agarofuran (211 mg), mixed with Se (220 mg) was dehydrogenated and processed following the procedure as employed in the case of β-agarofuran to furnish eudalene (1) (150.8 mg), characterised through its picrate, m.p. and mixed m.p. with an authentic sample, 94° (Found: C, 57.90; H, 4.90; N, 10.38 . Calculated for $C_{20}H_{19}O_7N_3$ C, 58.11; H, 4.63; N, 10.17%).

Dihydro-∝-agarofuran

∝-Agarofuran (780 mg) dissolved in glacial acetic acid (25 ml) was stirred in an atmosphere of hydrogen with pre-reduced Adams PtO_2 catalyst (50 mg). The hydrogen (100 ml, 1.05 mole) was absorbed in 3 hrs. The catalyst was filtered and solvent was removed in vacuum to furnish dihydro-∝-agarofuran (710 mg), which on chromatography over alumina (grade I, 72.0 g) followed by distillation gave the analytical specimen (680 mg), b.p. 135° at 8 mm., n_D^{27} 1.4923, $(\alpha)_D^{28}$ - 60.8° (c, 6.0); no colouration with tetranitromethane; IR bands at: 1389, 1370, 1307, 1236, 1217, 1155, 1149, 1119, 1075, 1049, 1025, 1002, 963, 925, 888, and 872 cm^{-1}; no band at 1653 and 838 cm^{-1} (Found: C, 81.13; H, 11.75 . $C_{15}H_{26}O$ requires: C, 81.02; H,11.79%).

Treatment of dihydro-∝-agarofuran with borontrifluoride ethereate in acetone

Freshly distilled borontrifluoride ethereate(1 ml) was added to a solution of dihydro-∝-agarofuran (320 mg) in acetone (15 ml) and kept at room temperature for 48 hrs. Addition of ice cold water (10 ml) and working up the product in the customary manner furnished the viscous material (280 mg) which was purified by chromatography over alumina (grade I, 30 g., elution with 30 ml. pet.ether) followed by distillation to afford diene (15) (190 mg), b.p. 115-120° at 8 mm., n_D^{30} 1.5173; $(\alpha)_D^{30}$ - 182° (c, 3.72); UV spectrum: λmax.241,247,256 mμ (ε 18826, 19605, 14312),

IR bands at: 2900, 1645 (w), 1623, 1389, 1379, 1353, 1295, 1270, 1217, 1175, 1066, 1033, 994, 952, 877, and 806 cm^{-1} (Found: C, 87.82; H, 12.05. $C_{15}H_{24}$ requires: C, 88.16; H, 11.84%).

Prolonged hydrogenation of ∝-agarofuran (29)

∝-Agarofuran (800 mg) dissolved in glacial acetic acid (25 ml) was stirred in an atmosphere of hydrogen with pre-reduced Adams PtO_2 catalyst (40 mg), with absorption of hydrogen (170 ml., 1.78 mole) in 72 hrs. The catalyst was filtered off and the solvent was removed under vacuum, on a steam bath. The residue (720 mg) was chromatographed over alumina (grade II, 72 g). Pet.ether (50 ml) and benzene (125 ml) eluted two fractions: Fraction A (100 mg); Fraction B (511 mg).

Fraction A: On distillation furnished dihydro-∝-agarofuran, b.p. 135° at 8 mm., n_D^{27} 1.4921, $(\alpha)_D^{28}$ -60.02° (c, 2.5), with characteristic IR spectrum as previously described. (Found: C, 81.35; H, 11.82. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%).

Fraction B: On distillation gave a mixture of alcohols (30 and 31) (408 mg); b.p. 115-117° at 0.3 mm., n_D^{26} 1.5020, $(\alpha)_D^{30}$ - 1.38° (c, 2.88); no colouration with tetranitromethane, IR bands at: 3500 (s), 2933, 1451, 1381, 1284, 1250, 1151 (s), 1032, 985, 945, and 935 cm^{-1} (Found: C, 80.05; H, 12.27. $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%).

Preparation of hydrocarbon (32, 33) from alcohol(30,31)

(i) Benzoyl chloride (0.2 ml) was added to a solution of alcohol (200 mg) in pyridine (1 ml) and the mixture kept at room temperature for 24 hrs and then heated for 2 hrs. on a water bath. The product was worked up in the customary manner to furnish a viscous benzoate(180 mg), which showed the following IR spectrum: 1721, 1600, 1581, 1384, 1364, 1313, 1282, 1172, 1111, 1066, 1025, 807, and 709 cm^{-1} (characteristic bands for benzoate 1721, 1600, 1581, 1282, and 709 cm^{-1}).

(ii) The pyrolysis of the benzoate (150 mg) was carried out under a pressure of 100 mm. and at 200-210° for 15 minutes. The pyrolysed product was dissolved in pet.ether (5 ml) and transferred to a column of alumina (grade I, 7 g) and eluted with pet.ether (50 ml). After removal of solvent followed by distillation over sodium afforded the mixture of hydrocarbons (32 and 33) (70 mg); b.p. 140-145° at 2 mm., n_D^{25} 1.4998; yellow colouration with tetranitromethane; IR bands at: 2950, 1653, 1637, 1462, 1389, 1271, 1174, 1111, 1038, 891, and 811 cm^{-1}. (Found: C, 87.22; H, 12.82. $C_{15}H_{26}$ requires: C, 87.30; H,12.70%).

Preparation of epoxide (34) from ∝-agarofuran

To ∝-agarofuran (530.7 mg) dissolved in chloroform (5 ml) 10 ml. of perbenzoic acid (0.6 N approx.) was added and the mixture kept at 0° for 24 hrs; perbenzoic acid

equivalent 0.9 double bond was absorbed. The product was neutralised with sodium bicarbonate solution, washed with water, dried (Na_2SO_4) and the solvent removed. The residue (519 mg) was viscous, which on chromatography over alumina (grade II, 20 g., elution with 50 ml. benzene) afforded a white crystalline epoxide (34) (508 mg), m.p. 88°. It was further purified by sublimation (90° at 0.4 mm.), but the m.p. could not be raised further; $(\alpha)_D^{30}$ - 39.6°, (c, 4.09); IR bands at: 1389, 1370, 1309, 1287, 1241, 1160, 1144, 1121, 1089, 1067, 1058, 1049, 1024, 970, 961, 930, 898, 882, 844, 828, 808, 780, and 759 cm^{-1} (Found: C, 75.90; H, 10.00. $C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.24%).

Treatment of epoxide (34) with borontrifluoride ethereate in acetone

Freshly distilled borontrifluoride ethereate (1 ml) was added to a solution of epoxide (415 mg) in acetone (25 ml) and kept for 60 hrs at 0°. Addition of ice cold water (10 ml) and working up the product in the customary manner furnished a viscous material (390 mg), showing UV spectrum: λmax. 297 mμ (ε 1427). The viscous material was carefully chromatographed over alumina (grade II, 17 g). Pet.ether (50 ml), benzene (50 ml) and ether (25 ml) eluted three fractions A (60 mg), B (100 mg), and C (180 mg) respectively.

Fraction A: was further purified by chromatography over alumina (grade II, 2 g) and distillation to give the keto-oxide (35) (49 mg); b.p. 150° at 4 mm., n_D^{26} 1.4995;

$(\alpha)_D^{29}$ - 53.6° (c, 0.84); IR bands at: 2933, 1712, 1456, 1420, 1383, 1377, 1304, 1279, 1235, 1111, 1076, 1047, 1015, 961, and 889 cm^{-1}; no absorption in the UV spectrum (Found: C, 75.99; H, 10.45. $C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.24%). Semicarbazone m.p. 210° was not analysed due to paucity of material.

Fraction B was a yellowish viscous material, which showed UV absorption at λmax. 300mμ (ε 4934). It was further purified by chromatography over alumina (grade II, 5 g., elution with 30 ml of benzene) to give impure (-)-β-cyperone (36) (25%); n_D^{28} 1.5524, $(\alpha)_D^{29}$ -164.2° (c, 0.335), UV spectrum: λmax. 300 mμ (ε 6774); IR bands at: 2950, 1667, 1618, 1458, 1425, 1389, and 1362 cm^{-1}. Due to paucity of material further purification was not attempted.

Fraction C was a white solid, which on crystallization from hot pet.ether gave crystals, m.p.172°. The crystalline material was further purified by vacuum sublimation and two crystallizations from ethyl acetate to furnish needles of diol-oxide (37) (80 mg) having a constant m.p. 176°, which could not be raised by further crystallizations; $(\alpha)_D^{30}$ - 40.6° (c, 0.32); no absorption in UV spectrum; IR bands at: 3520, 2976, 1473, 1431, 1397, 1389, 1316, 1295, 1235, 1140, 1106, 1089, 1075, 1038, 1015, 983, 962, 945, 933, 909, and 881 cm^{-1} (Found: C, 70.84; H, 10.51. $C_{15}H_{26}O_3$ requires: C, 70.83; H, 10.30%).

REFERENCES

1. M.L.Maheshwari, T.C.Jain, R.B.Bates and S.C.Bhattacharyya, Tetrahedron, 19, 1079 (1963).

2. L.Ruzicka, Experientia, 9, 357 (1953); Proc.Chem.Soc., 341 (1959).

3. W.Sandermann in "Proceedings of the Fourth International Congress of Biochemistry", Vol.II, "Biochemistry of Wood" (Edited by K. Kratzl and G.Billek), p. 29, Pergamon Press, N.Y. (1959).

4. D.H.R.Barton, P. de Mayo and M.Shafiq, J.Chem.Soc., 929 (1957); O.Motl, V.Herout and F.Sorm, Coll.Czech. Chem.Commun., 23, 1293 (1958).

5. A.R.H.Cole in Zechmeister's "Progress in the Chemistry of Organic Natural Products", Vol.XIII, p.44, Wien Springer (1956).

6. G.M.Barrow and S.Searles, J.Amer.Chem.Soc., 75,1175 (1953).

7. S.Rangaswami and K.Sambamurthy, Proc.Indian Acad.Sci., 54A, 132 (1961).

8. L.Panizzi, L, Mangoni and M.Belardini, Tetrahedron Letters, No.11, 376-81 (1961).

9. R.B.Woodward, J.Amer.Chem.Soc., 64, 72 (1942).

10. G.C. Harris and T.F.Sanderson, J.Amer.Chem.Soc., 70, 339 (1948).

11. E.R.H.Jones and T.G.Halsall in L.Zechmeister, "Progress in the Chemistry of Organic Natural Products", Vol.XII, Wien Springer, p.53 (1955).

12. G.Büchi, M.Schach V.Wittenau and Dwain M. White, J.Amer.Chem.Soc., 81, 1968 (1959).

13. L. Ruzicka and M.Stoll, Helv.Chim.Acta, 6, 846(1923).

14. S.K.Paknikar and S.C.Bhattacharyya, Tetrahedron, 18, 1509 (1962).

15. L.Reggel, R.A.Friedel and I.Wender, J.Org.Chem., 22, 891 (1957).

16. K.G.O'Brien, A.R.Penfold, M.D.Sutherland, and R.L. Werner, Austr.J.Chem., 7, 298 (1954).

17. C.Djerassi, and D.Marshall, J.Amer.Chem.Soc., 80, 3986 (1958).

18. J.I.Musher, J.Amer.Chem.Soc., 83, 1146 (1961).

19. K.R.Varma, T.C.Jain, and S.C.Bhattacharyya, Tetrahedron, 18, 979 (1962).

20. R.Howe and F.J.McQuillin, J.Chem.Soc., 2423(1955).

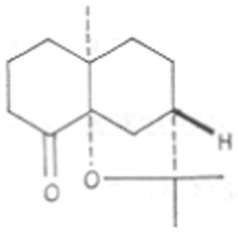
21. D.H.R.Barton, Chem. & Ind., 664 (1953).

CHAPTER III

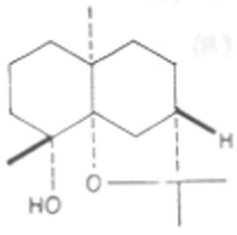
STRUCTURE AND ABSOLUTE CONFIGURATION OF NOR-KETOAGAROFURAN, 4-HYDROXY DIHYDRO-AGAROFURAN AND 3,4-DIHYDROXYDIHYDROAGAROFURAN

SUMMARY

In addition to ∝-, β- and dihydroagarofurans three other crystalline furanoids of the selinanic group are also obtained from agarwood oil. These are nor-ketoagarofuran, 4-hydroxydihydroagarofuran, and 3,4-dihydroxydihydroagarofuran. Their structure and absolute configurations are represented by the structures (I, II, and III) respectively. These conclusions were drawn by interconversion of these into known compounds described in Chapter II, during the course of which β-agarofuran could be converted into ∝-agarofuran.



I



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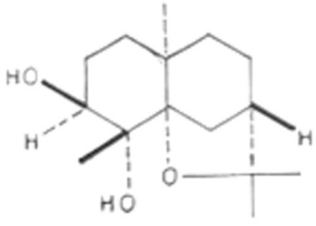


III

In addition to the previously reported ∝-, β-, and dihydroagarofurans (1, 2, 3) (Chapter II, p. 31) three new crystalline sesquiterpenic furans of the selinane group have also been obtained in small quantities by elaborate chromatography of the appropriate fractions of agarwood oil from infected Aquillaria agallocha Roxb. (Chapter I, p. 17). Experiments leading to the determination of their structure and absolute configurations[1] are described in this chapter.

As these have been found to be derivatives of agarofuran, they have been assigned the names on the basis of the positions of their functional groups:

(i) Nor-ketoagarofuran, $C_{14}H_{22}O_2$ (4)

(ii) 4-Hydroxydihydroagarofuran, $C_{15}H_{26}O_2$ (5)

(iii) 3,4-Dihydroxydihydroagarofuran, $C_{15}H_{26}O_3$ (8).

Nor-ketoagarofuran

Nor-ketoagarofuran (4) was obtained in the form of a crystalline material (m.p. 56-57°) after cooling the appropriate chromatographic fraction in the freeze for a long time and subsequent crystallization from pet.ether. Its m.p. remained unaltered after regeneration from the semicarbazone, m.p. 216°. It analyses correctly for the molecular formula, $C_{14}H_{22}O_2$ and also shows the expected molecular weight. The occurrence of a C_{14}- terpenoid in nature is somewhat unusual.[2]

Nor-ketoagarofuran is a saturated compound as shown by negative colour test with tetranitromethane. Its IR spectrum (Fig. 3.1) shows the presence of a keto-group on a six-membered ring with a methylene group next to the keto-function (1712 and 1418 cm^{-1}) and gem-dimethyl group (doublet at 1379 and 1361 cm^{-1}). The other oxygen function present in nor-ketoagarofuran was suspected to be in the form of a tetrahydrofuran ring from its IR spectrum (1295, 1229, 1149, 1110, 1067, 1013, and 888 cm^{-1}) and was identical with the spectral characteristics of the previously reported agarofurans in the finger print region. Superimposability of its IR spectrum with that of the previously reported liquid C_{14}-keto-oxide obtained by ozonisation of β-agarofuran[3] (2) (cf. Chapter II, Fig. 2.2) suggested their possible identity. The optical rotation of the liquid C_{14}-keto-oxide $\left[(\alpha)_D^{30} - 69.2^\circ\right]$ from β-agarofuran was considerably lower than that of the crystalline nor-ketoagarofuran $\left[(\alpha)_D^{30} - 118.86^\circ\right]$. However, on seeding with a trace of the crystalline nor-ketoagarofuran, the liquid C_{14}-keto-oxide crystallized immediately. On further crystallizations from pet.ether (40-60°), the crystals melted at 56-57°; $(\alpha)_D^{30} - 116.54^\circ$ (c, 0.35); mixed m.p. with crystalline nor-ketoagarofuran remained undepressed. The low rotation of the liquid C14-keto-oxide was presumably due to the presence of impurities having high dextro-rotation.

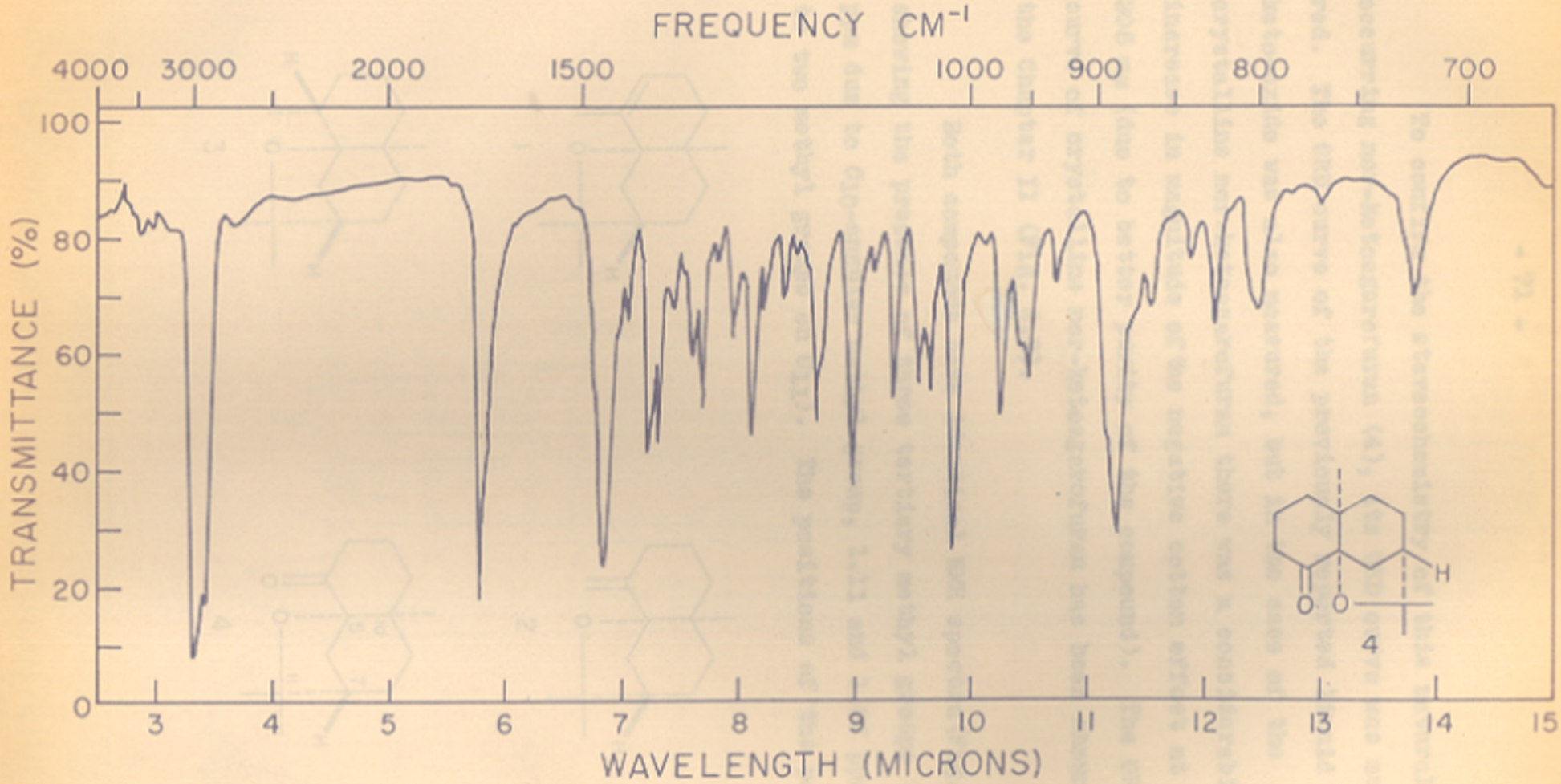
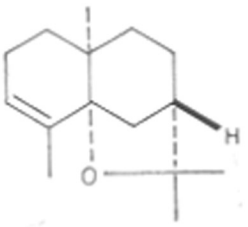


FIG. 3.1 IR SPECTRUM (IN NUJOL) OF NOR-KETO AGAROFURAN (4)

To confirm the stereochemistry of this naturally occurring nor-ketoagarofuran (4), its ORD curve was measured. The ORD curve of the previously reported liquid C_{14}-keto-oxide was also measured, but in the case of the crystalline nor-ketoagarofuran there was a considerable increase in magnitude of the negative cotton effect at 305 mμ (due to better purity of the compound). The ORD curve of crystalline nor-ketoagarofuran has been shown in the Chapter II (Fig. 2.8).

Both compounds have identical NMR spectra(Fig.3.2) showing the presence of three tertiary methyl groups (0.86 ppm due to C_{10}-angular methyl group, 1.11 and 1.37 ppm due to two methyl groups on C_{11}). The positions of the peaks



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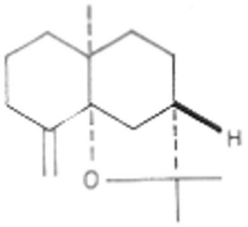

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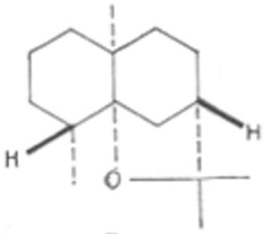

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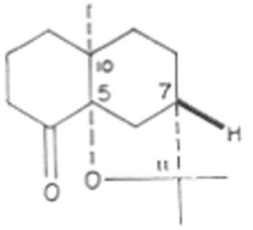

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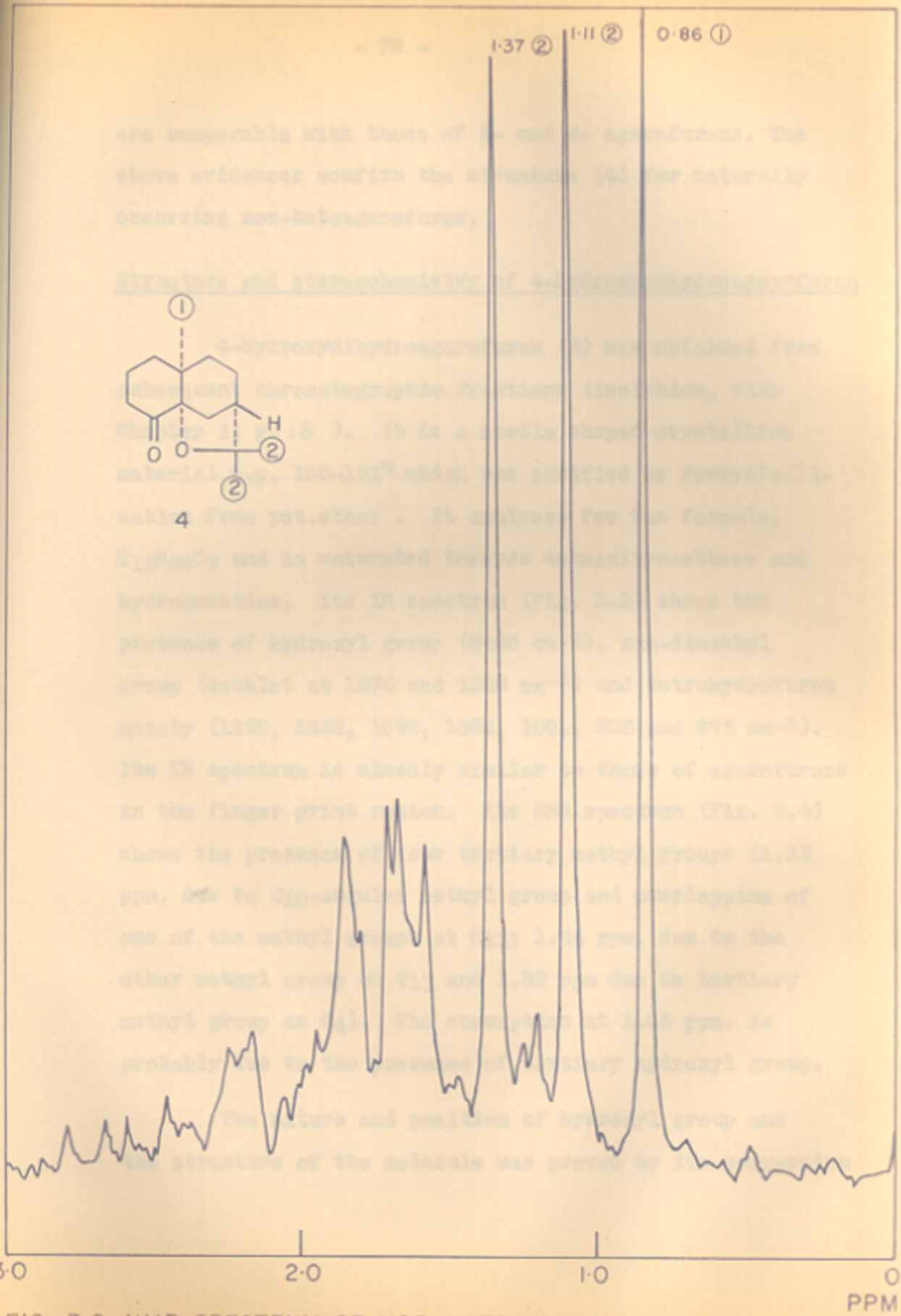

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FIG. 3.2 NMR SPECTRUM OF NOR-KETO AGAROFURAN (4)

are comparable with those of β- and ∝- agarofurans. The above evidences confirm the structure (4) for naturally occurring nor-ketoagarofuran.

Structure and stereochemistry of 4-hydroxydihydroagarofuran

4-Hydroxydihydroagarofuran (5) was obtained from subsequent chromatographic fractions (isolation, vide Chapter I, p. 18). It is a needle shaped crystalline material m.p. 130-131° which was purified by recrystallization from pet.ether . It analyses for the formula, $C_{15}H_{26}O_2$ and is saturated towards tetranitromethane and hydrogenation. Its IR spectrum (Fig. 3.3) shows the presence of hydroxyl group (3460 cm^{-1}), gem-dimethyl group (doublet at 1374 and 1359 cm^{-1}) and tetrahydrofuran moiety (1290, 1242, 1099, 1082, 1001, 885 and 875 cm^{-1}). Its IR spectrum is closely similar to those of agarofurans in the finger print region. Its NMR spectrum (Fig. 3.4) shows the presence of four tertiary methyl groups (1.15 ppm. due to C_{10}-angular methyl group and overlapping of one of the methyl groups at C_{11}; 1.24 ppm. due to the other methyl group on C_{11} and 1.32 ppm due to tertiary methyl group on C_4). The absorption at 1.65 ppm. is probably due to the presence of tertiary hydroxyl group.

The nature and position of hydroxyl group and the structure of the molecule was proved by its conversion

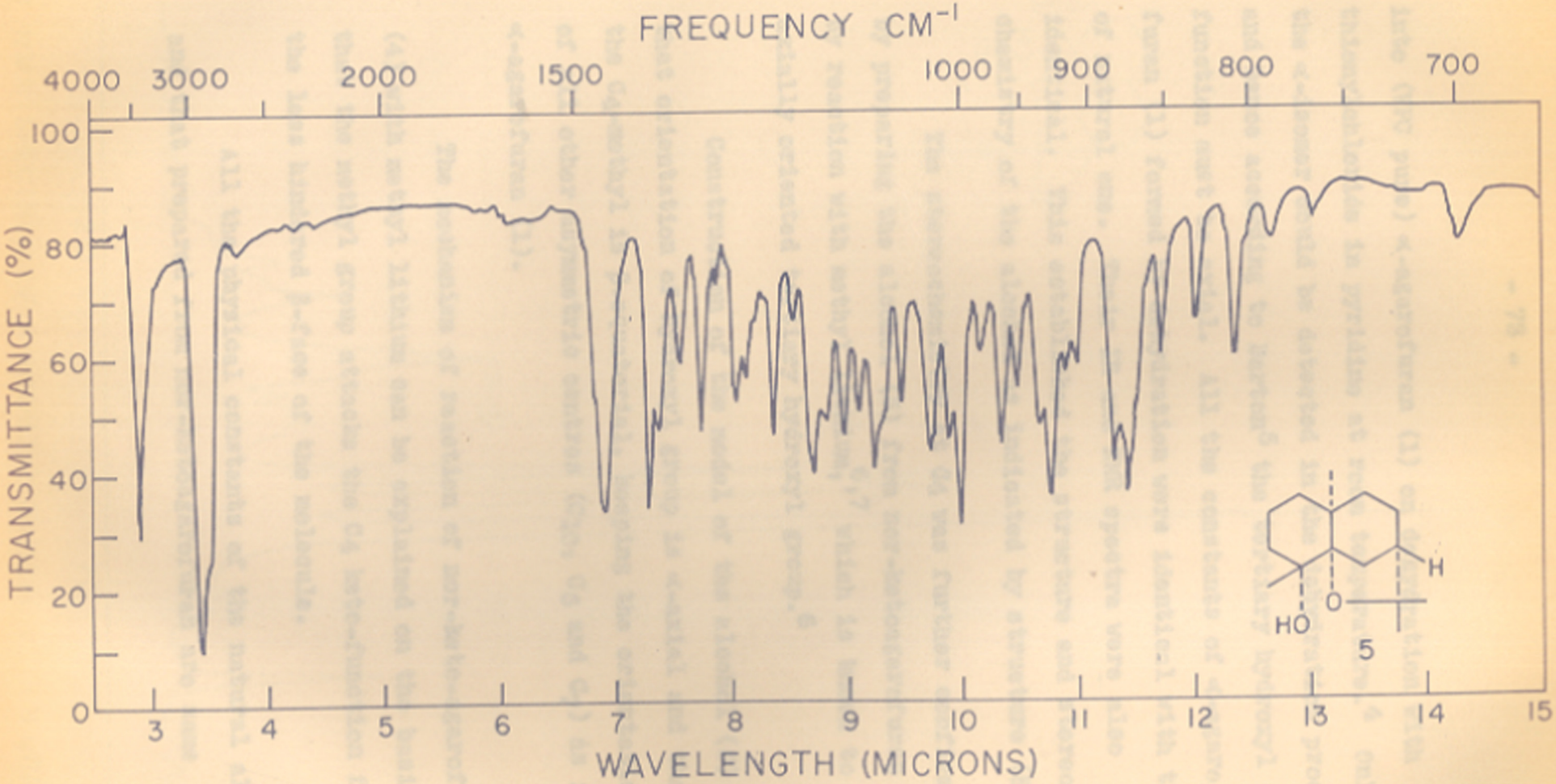

FIG. 3.3 IR SPECTRUM (IN NUJOL) OF 4-HYDROXY DIHYDROAGAROFURAN (5)

into (VPC pure) ∝-agarofuran (1) on dehydration with thionylchloride in pyridine at room temperature.[4] Only the ∝-isomer could be detected in the dehydration product and hence according to Barton[5] the tertiary hydroxyl function must be axial. All the constants of ∝-agarofuran (1) formed by dehydration were identical with those of natural one. Their IR and NMR spectra were also identical. This established the structure and stereochemistry of the alcohol as indicated by structure (5).

The stereochemistry at C_4 was further confirmed by preparing the alcohol (5) from nor-ketoagarofuran (4) by reaction with methyl lithium,[6,7] which is known to give axially oriented tertiary hydroxyl group.[6]

Construction of the model of the alcohol (5) shows that orientation of hydroxyl group is ∝-axial and that of the C_4-methyl is β-equatorial, keeping the orientations of all other asymmetric centres (C_{10}, C_5 and C_7) as in ∝-agarofuran (1).

The mechanism of reaction of nor-keto-agarofuran (4) with methyl lithium can be explained on the basis that the methyl group attacks the C_4 keto-function from the less hindered β-face of the molecule.

All the physical constants of the natural alcohol and that prepared from nor-ketoagarofuran are same and

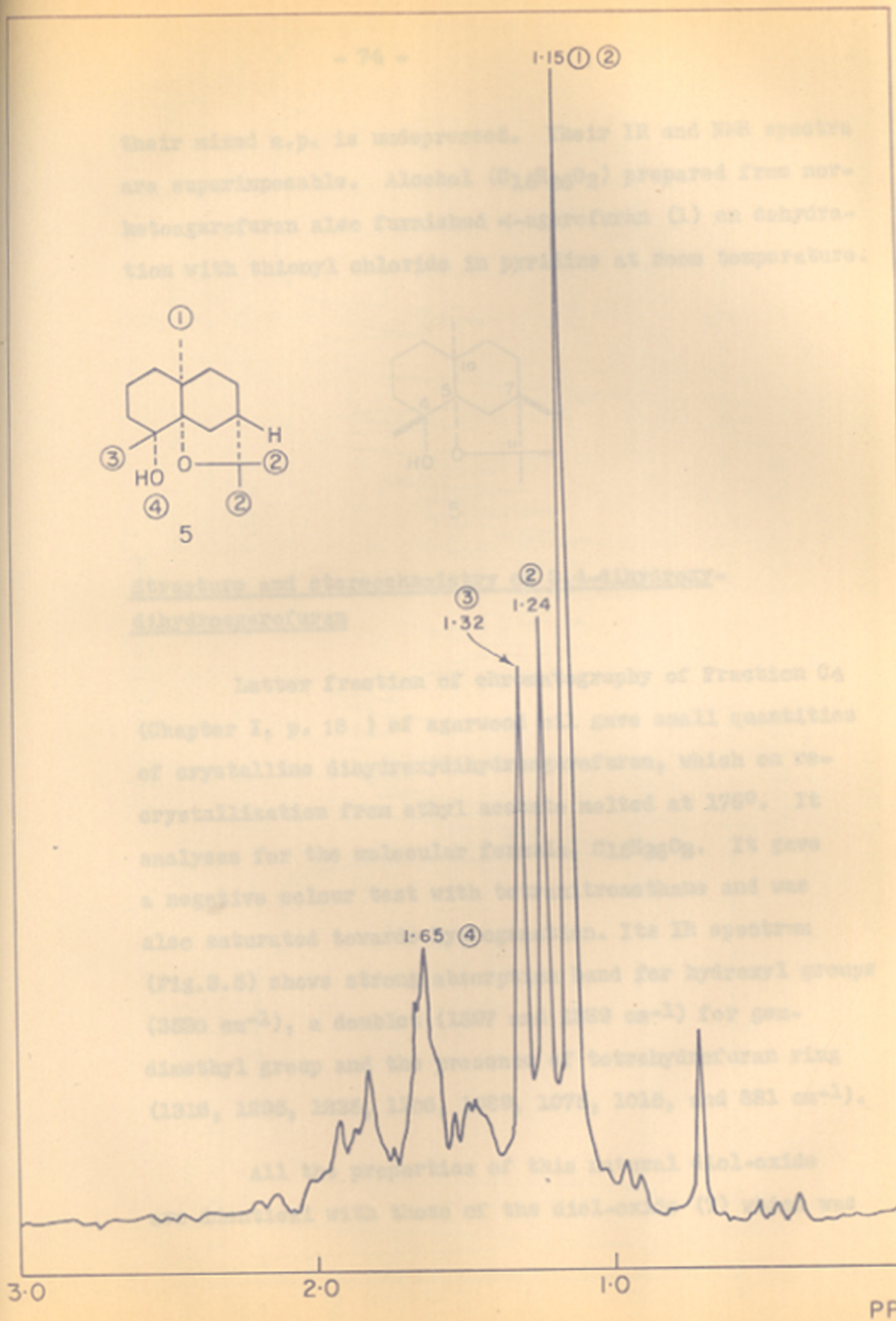

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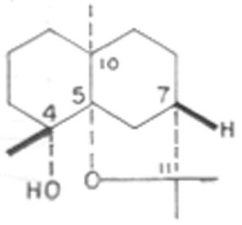
FIG. 3.4 NMR SPECTRUM OF 4-HYDROXY DIHYDROAGAROFURAN (5)

their mixed m.p. is undepressed. Their IR and NMR spectra are superimposable. Alcohol ($C_{15}H_{26}O_2$) prepared from norketoagarofuran also furnished α-agarofuran (1) on dehydration with thionyl chloride in pyridine at room temperature.



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Structure and stereochemistry of 3,4-dihydroxydihydroagarofuran

Latter fraction of chromatography of Fraction C_4 (Chapter I, p. 18) of agarwood oil gave small quantities of crystalline dihydroxydihydroagarofuran, which on recrystallization from ethyl acetate melted at 176°. It analyses for the molecular formula, $C_{15}H_{26}O_3$. It gave a negative colour test with tetranitromethane and was also saturated towards hydrogenation. Its IR spectrum (Fig.3.5) shows strong absorption band for hydroxyl groups (3520 cm^{-1}), a doublet (1397 and 1389 cm^{-1}) for gem-dimethyl group and the presence of tetrahydrofuran ring (1316, 1295, 1235, 1106, 1089, 1075, 1015, and 881 cm^{-1}).

All the properties of this natural diol-oxide are identical with those of the diol-oxide (7) which was

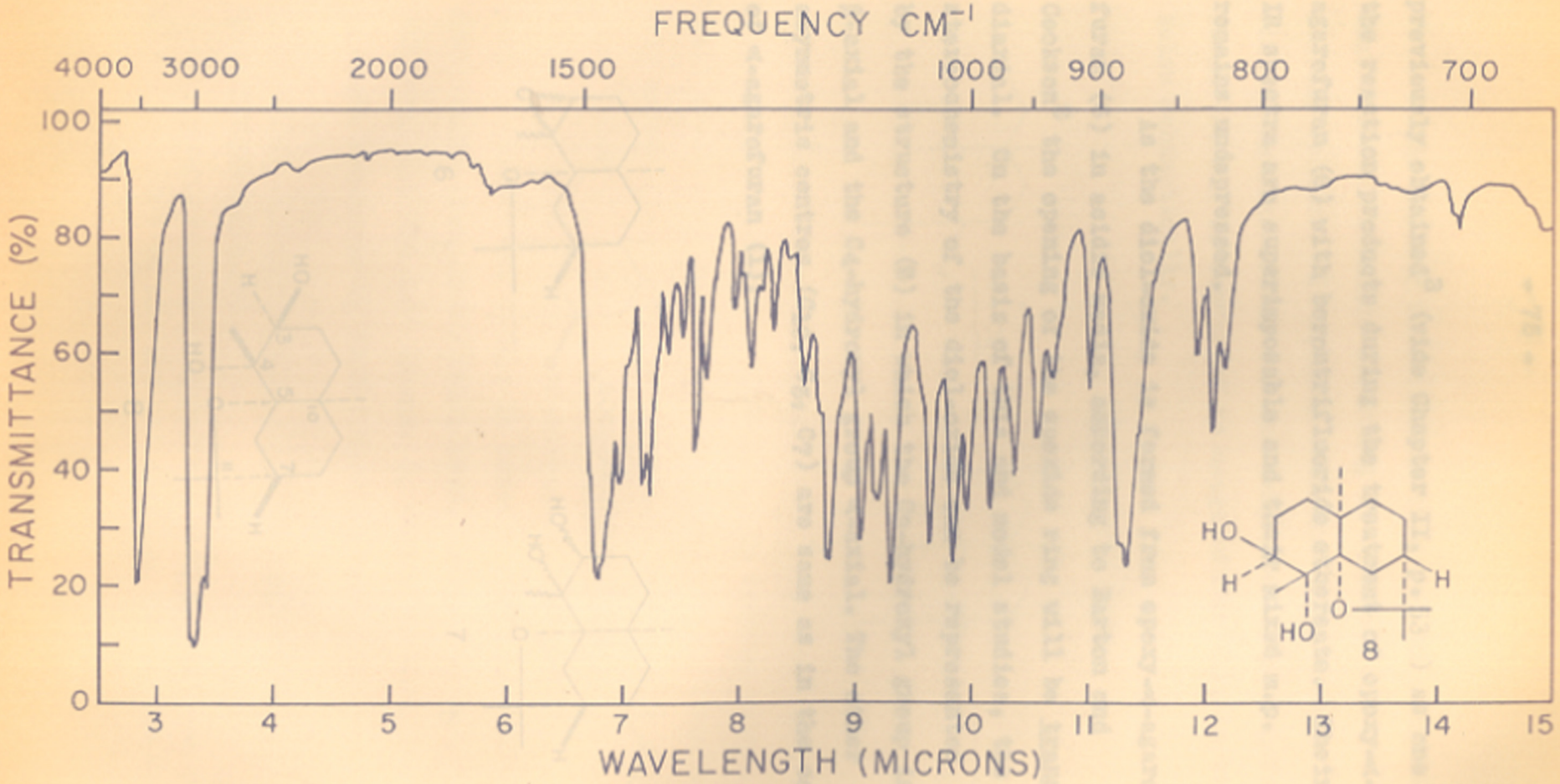
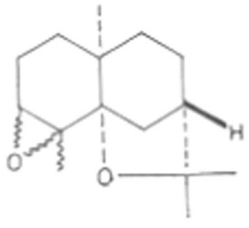


FIG. 3.5 IR SPECTRUM (IN NUJOL) OF 3,4–DIHYDROXY DIHYDRO AGAROFURAN (8)

previously obtained[3] (vide Chapter II, p.53) as one of the reaction products during the treatment of epoxy-∝-agarofuran (6) with borontrifluoride ethereate. Their IR spectra are superimposable and their mixed m.p. remains undepressed.

As the diol-oxide is formed from epoxy-∝-agarofuran (6) in acidic media, according to Barton and Cookson[8] the opening of the epoxide ring will be trans-diaxial. On the basis of this and model studies, the stereochemistry of the diol-oxide can be represented by the structure (8) in which the C_3-hydroxyl group is β-axial and the C_4-hydroxyl group ∝-axial. The other asymmetric centres (C_{10}, C_5, C_7) are same as in the case of ∝-agarofuran (1).



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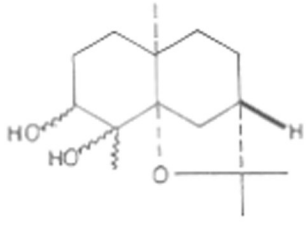

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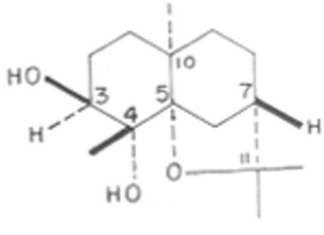

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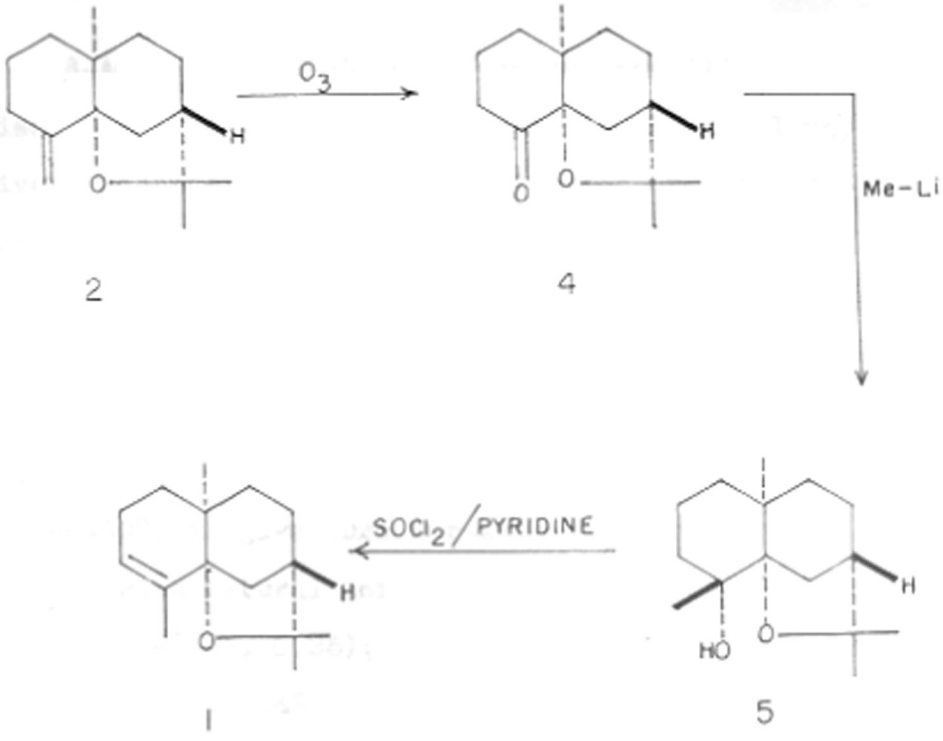
The results described in this chapter establish that various furanoids occurring in agarwood oil are closely related. It is also seen that β-agarofuran (2) via nor-ketoagarofuran (4) and 4-hydroxy-dihydroagarofuran (5) can be converted to ∝-agarofuran (1), as shown below schematically.



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E X P E R I M E N T A L

Isolation of nor-ketoagarofuran (4), 4-hydroxydihydroagarofuran (5) and 3,4-dihydroxydihydroagarofuran (8)

For their isolation and properties please refer to Chapter I, p. 27 of this thesis.

Preparation of crystalline nor-ketoagarofuran (4) from β-agarofuran (2)

β-Agarofuran (200 mg) in chloroform (40 ml) was ozonised for 1 hr at 0° and processed in the usual way to give a neutral fraction(173 mg), which on chromatography over alumina (grade II, 10 g., elution by 50 ml. of benzene) followed by distillation (b.p. 145° at 4 mm) gave a liquid keto-oxide (100 mg). On seeding with a small crystal of natural nor-ketoagarofuran, it immediately crystallized out. On recrystallization from pet. ether (40-60°) it gave pure nor-ketoagarofuran, m.p. mixed m.p. with natural nor-ketoagarofuran 56-57°; $(\alpha)_D^{30}$ - 116.54° (c, 0.35); IR bands at: 1712, 1418, 1379, 1361, 1295, 1229, 1149, 1110, 1067, 1013, 980, 950, 838, 826, 803, and 725 cm^{-1} (Found: C, 75.71; H, 9.75. $C_{14}H_{22}O_2$ requires: C, 75.63; H, 9.97%).

Preparation of α-agarofuran (1) from 4-hydroxydihydroagarofuran (5)

4-Hydroxydihydroagarofuran (150 mg) was dissolved in dry pyridine (6 ml) and the solution was cooled to about

5°. Thionyl chloride (0.75 g) was added dropwise with shaking during 10 minutes. After standing for 2 hrs, sodium carbonate (0.45 g) in water (5 ml) was added slowly. The mixture was extracted three times with ether and the ether-layer was washed with saturated tartaric acid solution until all pyridine was removed. After washing with water and drying the ethereal solution was worked up to give a mobile liquid (110 mg), which was purified by chromatography over alumina (grade I, 10 g., elution with 40 ml pet.ether) followed by distillation to afford ∝-agarofuran (1) (80 mg), b.p. 134° at 4 mm., n_D^{30} 1.5062, $(\alpha)_D^{30}$ + 39.8° (c, 2.1); yellow colouration with tetranitromethane; IR bands at: 1667, 1389, 1370, 1326, 1307, 1285, 1250, 1239, 1202, 1164, 1155, 1136, 1099, 1080, 1046, 1012, 965, 950, 935, 887, 856, 838(s), 825, 805, 770, and 703 cm^{-1} (Found: C, 81.60; H, 11.14. $C_{15}H_{24}O$ requires: C, 81.70; H, 10.98%). NMR spectrum identical with that of ∝-agarofuran reported previously[3] (vide Chapter II, p.49).

Preparation of 4-hydroxydihydroagarofuran (5) from nor-ketoagarofuran (4)

Methyl lithium was prepared by adding methyl iodide (6 ml) to lithium (1.8 g., in 6 pieces) in dry ether (40 ml), with stirring and cooling in ice. When the initial vigorous reaction had subsided, the mixture was refluxed with stirring for 4 hrs. The mixture was then cooled and the unreacted lithium was mechanically removed.

The nor-ketoagarofuran (4) (440 mg) in dry ether (20 ml) was added slowly to the solution of methyl lithium and the mixture was refluxed with stirring for 19 hrs. Excess of methyl lithium was decomposed by adding aqueous sodium sulphate solution containing some sodium thiosulphate to the stirred, ice-cooled solution. Additional amount of water was added and the ether-layer was separated and washed well with water, dried over anhydrous sodium sulphate and evaporated to give crude alcohol (430 mg), m.p. 127°. It was purified by repeated crystallization from pet.ether to furnish needle shaped crystalline 4-hydroxydihydroagarofuran (5) (m.p. and mixed m.p. 130-131°; 360mg); $(\alpha)_D^{30}$ - 74.34° (c, 1.52); IR bands at: 3460, 1374, 1359, 1323, 1290, 1242, 1229, 1195, 1148, 1136, 1114, 1099, 1082, 1054, 1028, 1011, 1001, 985, 966, 954, 939, 929, 918, 910, 885, 875, 852, 831, 810, 789, 768, and 700 cm-1 (Found: C, 75.77; H, 10.94. $C_{15}H_{26}O_2$ requires: C, 75.58; H, 11.00%). IR and NMR spectra are identical with those of natural 4-hydroxydihydroagarofuran.

REFERENCES

1. M.L.Maheshwari, K.R.Varma, and S.C.Bhattacharyya, Tetrahedron - in press.

2. A.Romo De Vivar and J.Romo, J.Amer.Chem.Soc., 83, 2326 (1961).

3. M.L.Maheshwari, T.C.Jain, R.B.Bates and S.C. Bhattacharyya, Tetrahedron, 19, 1079 (1963).

4. E. von Rudloff, Canadian J.Chem., 41, 1 (1963).

5. D.H.R.Barton, A. Campos-Neves and R.C.Cookson, J.Chem.Soc., 3500 (1956).

6. G.Stork and F.H.Clarke,Jr., J.Amer.Chem.Soc., 83, 3114 (1961).

7. E.J.Corey, Masaji Ohno, Paul A.Vatakancherry, and Rajat B.Mitra, J.Am.Chem.Soc., 83, 1251-3 (1961).

8. D.H.R.Barton and R.C.Cookson, Quarterly Rev., 10, 44 (1956).

C H A P T E R IV

THE COMPOSITION OF THE OIL FROM UNINFECTED AGARWOOD

SUMMARY

The oil from the uninfected variety of Aquillaria agallocha Roxb. was also obtained by low temperature solvent extraction procedure, which was used in the case of the infected variety of agarwood (Chapter I, p. 12). Compared to the oil-content of the infected agarwood (0.4%), the uninfected wood gave a poor yield of the oil (0.08%).

It was predominantly composed of hydrocarbons as against the oil from the infected wood which was mainly composed of oxygenated bodies.

This oil has been found to contain[1] elemental sulphur, selinane, two unidentified sesquiterpene hydrocarbons, agarol, a crystalline sesquiterpene alcohol and a crystalline hydroxyketone. In addition the fractions containing ketonic and conjugated hydroxy ketonic functions have also been isolated.

The occurrence of five isomeric decenes in the oil has been established by gas liquid chromatography.

It has been mentioned in Chapter I (p. 11) of this thesis, that the oil from infected variety of Aquillaria agallocha Roxb. contains mainly oxygenated bodies. The formation of oxygenated bodies in the fungus infected wood has considerable bearing on plant physiology and enzyme chemistry. From mycological experiments[2] it has been shown that quality and quantity of the oil from the wood is dependent on the extent of fungus infestation. Abnormal metabolism of the plant induced by the fungus infestation thus seems to be responsible for the formation of oxygenated bodies in agarwood. Such phenomena are well-known in the literature.[3]

We were interested in the chemical aspects of this problem and consequently for comparison purposes isolated the essential oil from the infected as well as the uninfected agarwood. For extraction of uninfected wood the same procedure as previously employed was used. Uninfected agarwood was very tough and difficult to disintegrate in comparison to infected wood. It was converted into a fine powder with considerable difficulty.

Compared to the oil-content of the infected agarwood (0.4%), the uninfected wood gave a poor yield of the oil (0.08%). It was predominantly composed of hydrocarbons in contrast to the oil from the infected wood which was mainly composed of oxygenated bodies, as shown in the previous chapters of this thesis.

In this chapter, we describe the results of our investigation on the oil obtained from the healthy plant. Because of paucity of material and somewhat unstable nature of some of the constituents, we avoided distillation as far as possible and tried to separate the constituents through column chromatography, which was also accompanied by a considerable loss of material.

On account of very limited amount of material at our disposal, systematic examination was not always possible and often we had to take recourse to spectral studies for general characterisation of the constituents.

On preliminary chromatography of the oil on grade II alumina, twenty seven fractions were collected. These were then combined together on the basis of their refractive indices, optical rotations, IR and UV spectra to form seven major fractions (A - G), as described in Table I. These fractions were then further sub-divided through rechromatography and different fractions were then examined for characterisation of the constituents.

T A B L E I

Major fraction.	Fraction No.	Eluent	Volume (l)	Weight (g)
A	1-7	Pet.ether	0.7	13.8
B	8-9	Pet.ether	1.1	1.1
C	10-17	Pet.ether + Benzene (1:1)	5.0	5.3
D	18-20	Benzene	2.6	2.5
E	21-23	Benzene + Ether (1:1)	4.2	5.7
F	24	Ether	0.5	1.06
G	25-27	Ether + Ethanol (1:1)	4.0	2.4

Fraction A. This fraction on cooling in the freeze deposited a yellow crystalline material, m.p. 113-114°, which was identified as sulphur.

The filtrate left after the separation of sulphur was subjected to chromatography on grade II alumina. The pet.ether elutions of chromatography were grouped into five fractions, which after distillation over sodium showed the properties recorded in Table II.

T A B L E II

Fraction	Weight (g)	n_D^{26}	F o u n d* C%	H%
1	0.15	1.4537	86.30	14.00
2	0.21	1.4473	-	-
3	1.07	1.4759	86.70	12.80
4	0.01	1.4837	86.30	13.50
5	0.35	1.4568	86.40	13.32

The IR spectrum of Fraction 1 and chemical tests showed the presence of a saturated carbon skeleton which seems to be new as none of its constants agreed with any of the sesquiterpenes described in the literature. Because of paucity of material we could not pursue its investigation further.

All other Fractions (2-5) were also saturated in nature. Fractions 3 and 4 were essentially composed of selinane. Traces of unsaturated impurities present therein (weak IR absorption at 1604 and 884 cm^{-1}) were removed by shaking with concentrated sulphuric acid. Properties of the saturated hydrocarbon thus obtained were in good agreement with those of selinane described in the literature,[4,5]

* $C_{15}H_{28}$ requires: C, 86.46; H, 13.54 %.

except that the intensities of some of the bands in the infrared spectrum were somewhat different. Physical constants and IR spectrum of this selinane sample were identical with those of selinane obtained from infected agarwood oil (Chapter I, p. 16).

Fraction 5 showed identical spectral feature with that of Fraction 1, but its elution behaviour on the column would suggest skeletal isomerism.

Fraction B. This (1.1 g) was extremely unstable and polymerised during distillation under vacuum.

Fraction C The Fraction C was resolved into a ketone and a hydroxy-ketone by means of chromatography and treatment with Girard's reagent(T). The ketonic fraction absorbed strongly at 1724 cm^{-1} in its IR spectrum, indicating it to be a six-membered ring ketone, with a methylene group next to carbonyl group (1418 cm^{-1}). Due to its IR absorption at 888 cm^{-1}, this ketonic fraction was initially supposed by us[1] to have unsaturation in the form of methylenic group. But recent elucidation of the structure of nor-ketoagarofuran (4) (Chapter III, p. 68) would suggest that it is composed mainly of nor-ketoagarofuran alongwith some conjugated impurities and supported by its infrared (1600 cm^{-1}) and ultra-violet absorption (λmax. 259 mμ, E 1%, 1 cm, 98). Its IR spectrum is identical with that of nor-ketoagarofuran

(Fig. 3.1, Chapter III). The band 888 cm^{-1} is not due to the methylenic double bond but due to the presence of tetrahydrofuran ring. Due to paucity of material, no further purification was attempted.

The hydroxy ketone seemed to contain a hindered carbonyl function since it did not react with Girard's reagent.

Fraction D. The dextrorotatory Fraction D, after preliminary purification by chromatography, was subjected to Girard treatment, whereupon a laevorotatory and an optically inactive fraction were obtained. The former contained agarol[6] identified by its constants and infrared spectrum. The other fraction was a hydroxy-ketone possessing α-, β- unsaturated keto system as indicated by its IR spectrum (1667 and 1613 cm^{-1}), further confirmed by its UV spectrum (λmax. 237 mμ, ε 7975). High end absorption values for this ketol suggest that the olefinic linkage in the molecule is heavily substituted.[7]

Fraction E. Crystallization of the viscous Fraction E from pet.ether and sublimation of the product in vacuo furnished a mono-ethynoid bicyclic sesquiterpene alcohol, m.p. 133°, corresponding to the molecular formula, $C_{15}H_{26}O$. Infrared spectrum of this compound (Fig.4.1) revealed the absence of methylenic grouping

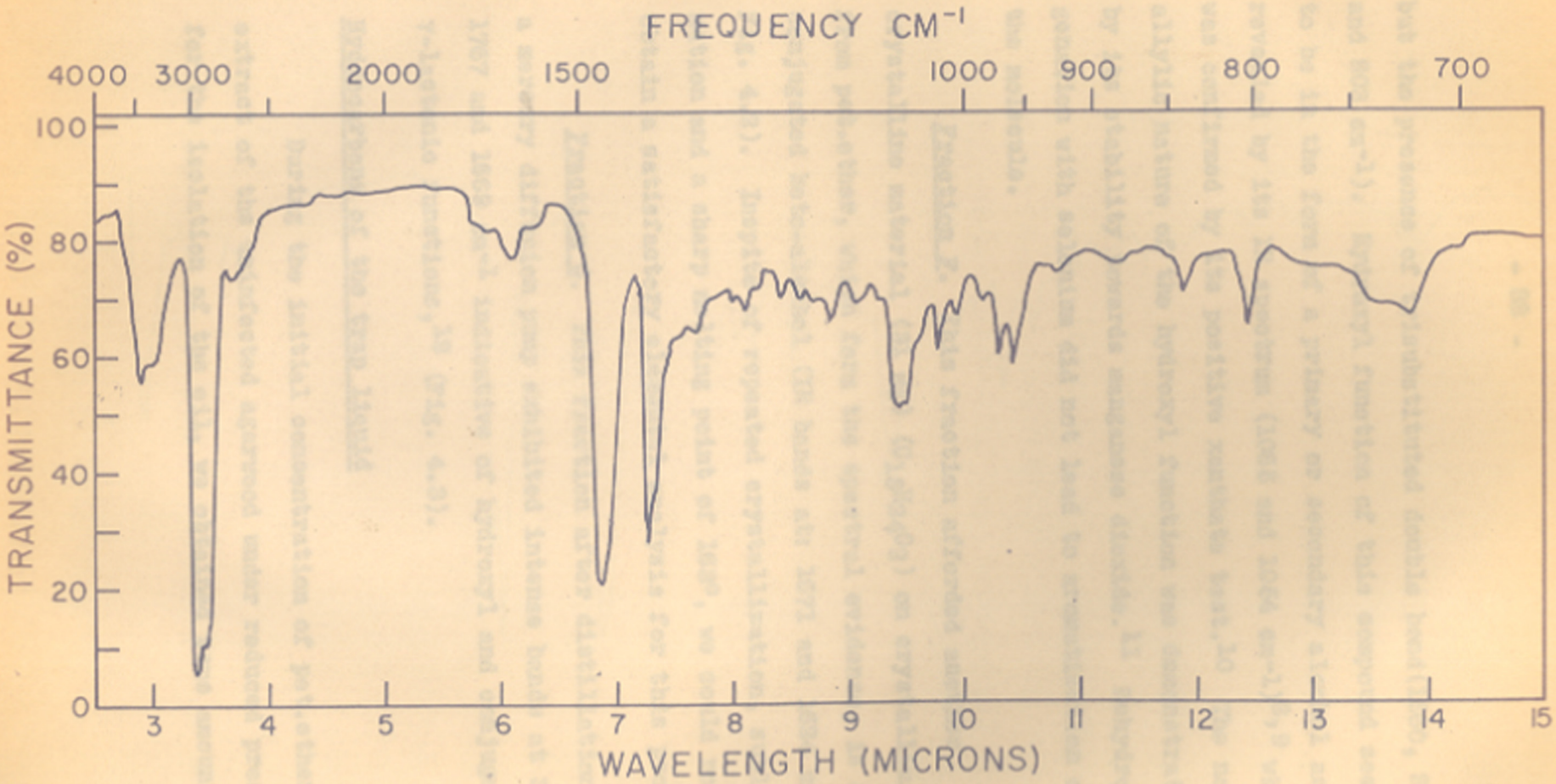

FIG. 4.1 IR SPECTRUM (IN NUJOL) OF ALCOHOL ($C_{15}H_{26}O$) FROM FRACTION E.

but the presence of trisubstituted double bond(1660, 837 and 803 cm^{-1}). Hydroxyl function of this compound seems to be in the form of a primary or secondary alcohol as revealed by its IR spectrum (1065 and 1054 cm^{-1})[8,9] which was confirmed by its positive xanthate test.[10] The non-allylic nature of the hydroxyl function was demonstrated by its stability towards manganese dioxide.[11] Dehydrogenation with selenium did not lead to aromatisation of the molecule.

Fraction F. This fraction afforded another crystalline material (31 mg) ($C_{15}H_{24}O_2$) on crystallizing from pet.ether, which form the spectral evidence, is a conjugated keto-alcohol (IR bands at: 1671 and 1624 cm^{-1}; Fig. 4.2). Inspite of repeated crystallization, sublimation and a sharp melting point of 163°, we could not obtain a satisfactory elemental analysis for this product.

Fraction G. This fraction after distillation at a mercury diffusion pump exhibited intense bands at 3480, 1767 and 1669 cm^{-1} indicative of hydroxyl and conjugated γ-lactonic functions,[12] (Fig. 4.3).

Hydrocarbons of the trap liquid

During the initial concentration of pet.ether extract of the uninfected agarwood under reduced pressure for the isolation of the oil, we obtained some amount of

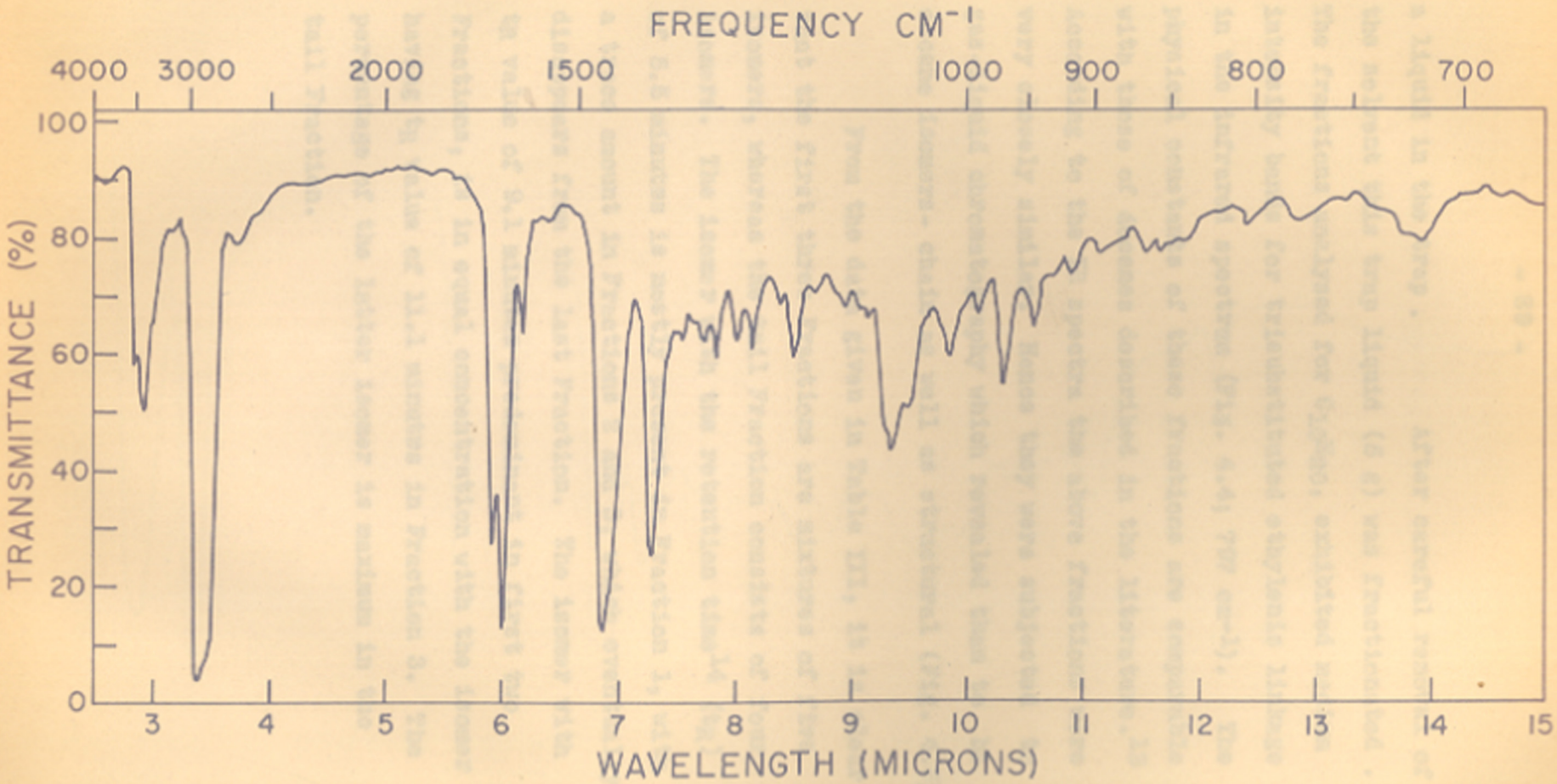

FIG. 4.2 IR SPECTRUM (IN NUJOL) OF KETO-ALCOHOL FROM FRACTION F.

a liquid in the trap . After careful removal of the solvent this trap liquid (5 g) was fractionated . The fractions analysed for $C_{10}H_{20}$, exhibited medium intensity bands for trisubstituted ethylenic linkage in the infrared spectrum (Fig. 4.4; 797 cm^{-1}). The physical constants of these fractions are comparable with those of decenes described in the literature.[13] According to the IR spectra the above fractions were very closely similar. Hence they were subjected to gas-liquid chromatography which revealed them to be decene isomers- chain as well as structural (Fig. 4.5).

From the data given in Table III, it is clear that the first three Fractions are mixtures of five isomers, whereas the tail Fraction consists of four isomers. The isomer with the retention time[14] (t_R) of 5.5 minutes is mostly present in Fraction 1, with a trace amount in Fractions 2 and 3, which eventually disappears from the last Fraction. The isomer with t_R value of 9.1 minutes predominant in first two Fractions, is in equal concentration with the isomer having t_R value of 11.1 minutes in Fraction 3. The percentage of the latter isomer is maximum in the tail Fraction.

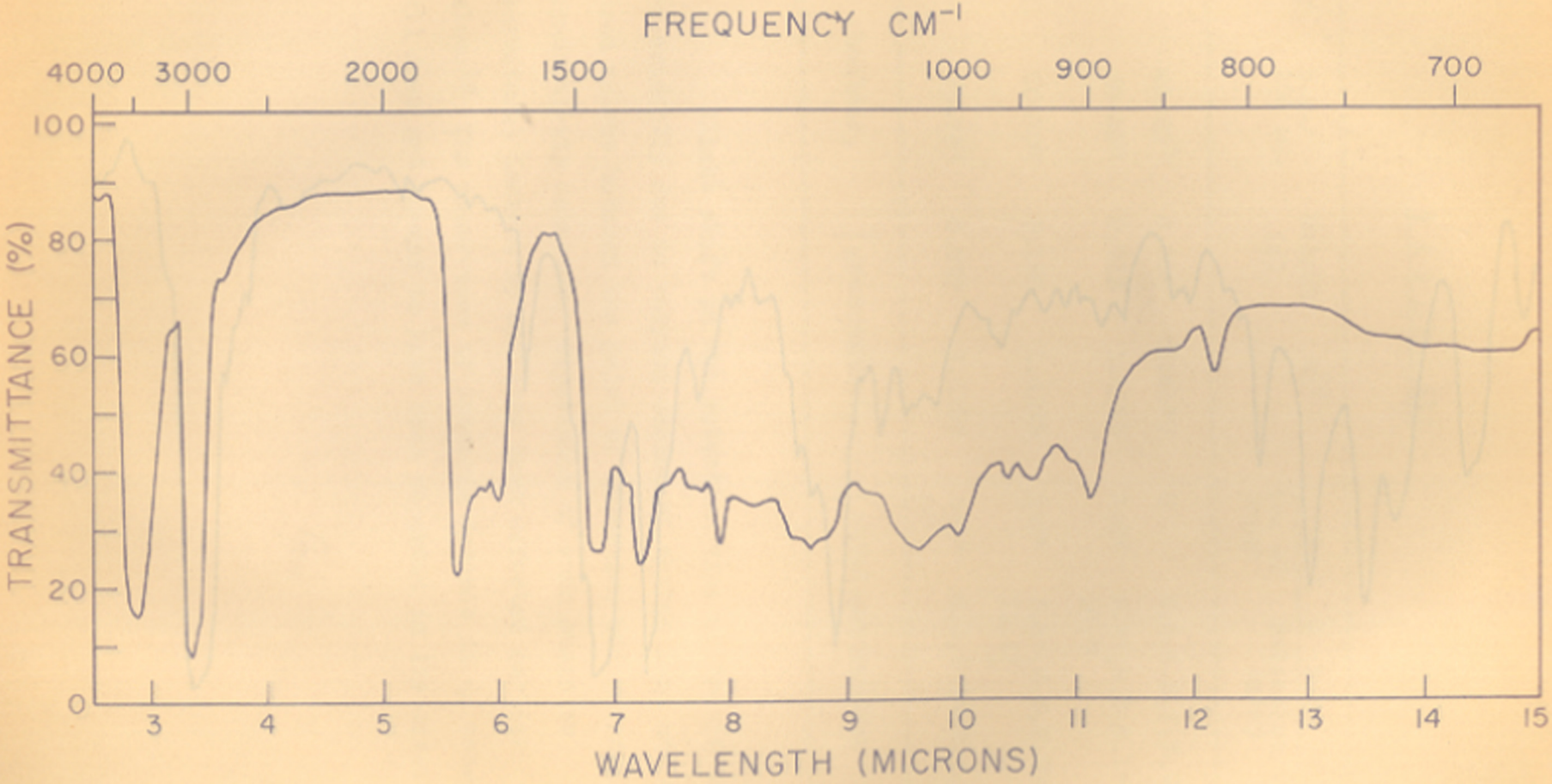

FIG. 4.3 IR SPECTRUM (LIQUID FILM) OF HYDROXY-γ-LACTONE (FRACTION G)

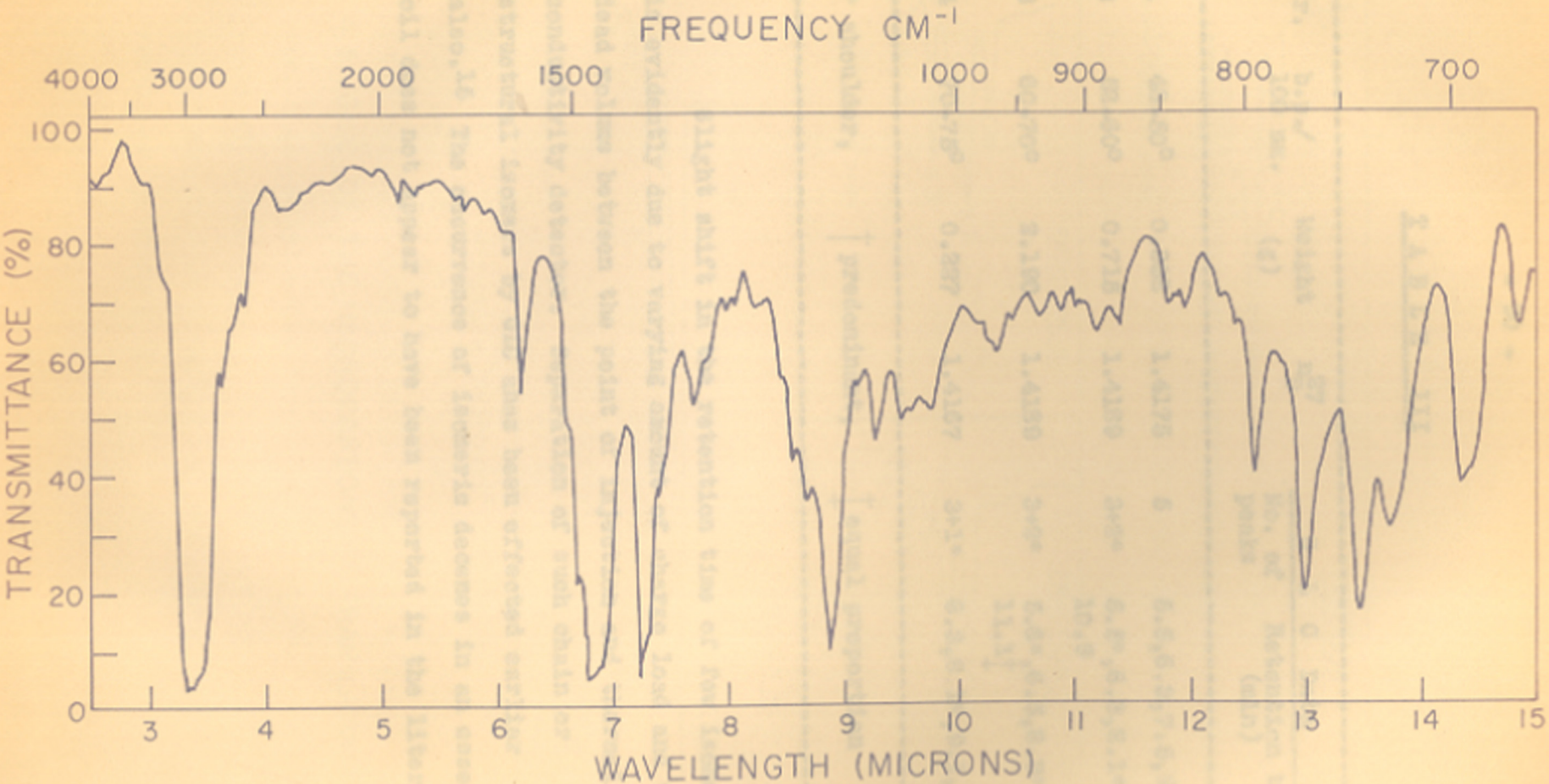

FIG. 4.4 IR SPECTRUM (IN 0·1 MM CELL) OF TRAP HYDROCARBONS (DECENES)

TABLE III

Fr.	b.p./ 100 mm.	Weight (g)	n_D^{27}	G L C Data	
				No. of peaks	Retention time (min)
1	48-50°	0.338	1.4175	5	5.5,6.3,7.5,9.1†,11.0
2	52-60°	0.715	1.4189	3+2*	5.5*,6.3,8.1*,9.2†, 10.9
3	60-70°	2.190	1.4189	3+2*	5.6*,6.5,8.3*,9.3‡, 11.1‡
4	70-75°	0.227	1.4167	3+1*	6.3,8.1*,9.1,11.1†.

* shoulder, † predominant, ‡ equal proportion

Slight shift in the retention time of few isomers is evidently due to varying amount of charge load and the dead volume between the point of injection and thermal conductivity detector. Separation of such chain or structural isomers by GLC ahas been effected earlier also.[15] The occurrence of isomeric decenes in an essential oil does not appear to have been reported in the literature.

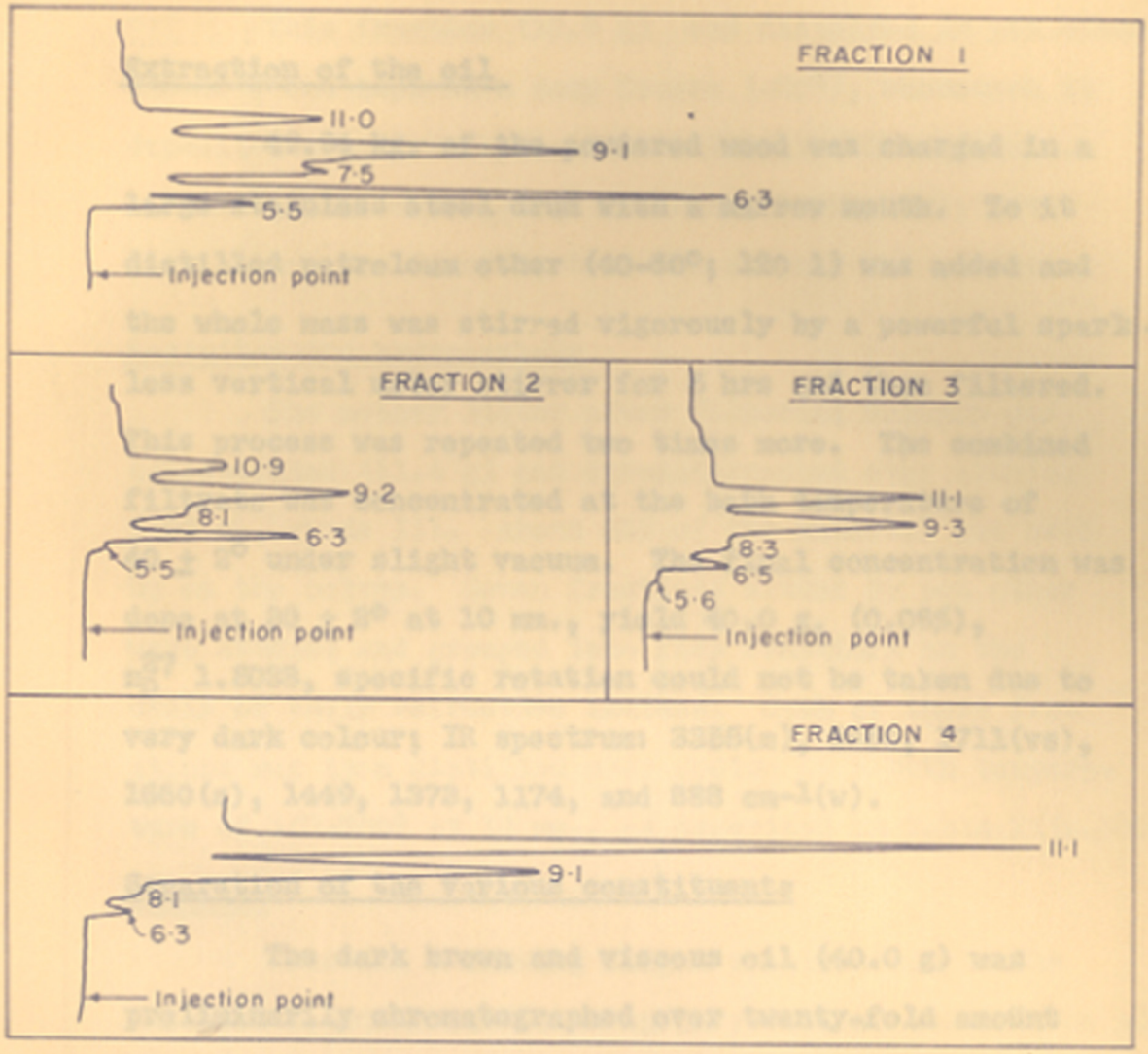

FIG. 4.5 GLC OF THE HYDROCARBONS OF TRAP LIQUID.

E X P E R I M E N T A L

Extraction of the oil

49.94 kg. of the powdered wood was charged in a large stainless steel drum with a narrow mouth. To it distilled petroleum ether (40-60°; 120 l) was added and the whole mass was stirred vigorously by a powerful sparkless vertical motor stirrer for 5 hrs and then filtered. This process was repeated two times more. The combined filtrate was concentrated at the bath temperature of 40 ± 2° under slight vacuum. The final concentration was done at 30 ± 2° at 10 mm., yield 40.0 g. (0.08%), n_D^{27} 1.5038, specific rotation could not be taken due to very dark colour; IR spectrum: 3355(m), 2860, 1711(vs), 1660(s), 1449, 1373, 1174, and 888 cm^{-1}(w).

Separation of the various constituents

The dark brown and viscous oil (40.0 g) was preliminarily chromatographed over twenty-fold amount of grade II alumina (800 g). The chromatogram was eluted with pet.ether, pet.ether-benzene, benzene, benzene-ether, ether and ether-ethanol in the sequence indicated to afford twenty seven fractions. Similar fractions were then combined together into seven major fractions on the basis of observations of their physical constants. All the fractions were somewhat coloured. Details are mentioned in Table I (p. 84).

Fraction A

This fraction (13.8 g) was dissolved in pet.ether (40-60°) and kept in a deep freeze (-20°), whereupon it deposited a yellow crystalline material (200 mg), m.p. 113-114° (lit. m.p. 112.8° for rhombic sulphur); gave zero values for C and H; test for sulphur was positive.

Sesquiterpene hydrocarbons

The mother liquor after filtering sulphur was concentrated (11.4 g) and chromatographed over alumina (500 g., grade II). About 60% of the material was held up on the column. Seven fractions eluted by pet.ether were studied and grouped into five fractions on the basis of their refractive indices. Each of these fractions was then distilled over sodium at a bath temperature of 150-200° at 10 mm., as described in Table II(p.85).

Selinane

Fraction 3 showing slight unsaturation in the infrared spectrum was taken up in pet.ether (40-60°; 30 ml) and shaken with conc. sulphuric acid. Pet.ether extract was washed with sodium bicarbonate solution, water and dried over anhydrous sodium sulphate. After solvent removal it was distilled over sodium under vacuum to afford an analytical specimen of selinane, b.p. 120° at 0.2 mm., n_D^{26} 1.4837; $(\alpha)_D^{28}$ + 2.19°, d_4^{30} 0.8769; no colouration with tetranitromethane. IR bands at:

1380, 1339, 1299, 1170, 1020, 972, 894, 816, 763, and 722 cm^{-1} (in 0.1 mm. cell). (Found: C, 86.22; H, 13.66. Calculated for $C_{15}H_{28}$ C, 86 46; H, 13.54%).

Fraction 4 was almost pure selinane.

Fraction C

The dark brownish coloured liquid was distilled at 170-190° at 1.5 X 10^{-3} mm. to get a yellowish viscous liquid (1.34 g), which was chromatographed over alumina (grade II, 75 g) with elution by pet.ether (500 ml) and benzene (250 ml) to furnish two fractions:

Pet.ether fraction (257 mg). It contained mainly nor-ketoagarofuran with a slight impurity of conjugated material (λmax. 259 mμ, E,1%, 1 cm, 98). It shows n_D^{24} 1.4979 and IR bands at: 1724, 1600, 1418, 1376, 1361, 1297, 1280, 1242, 1148, 1093, 1075, 1036, 1010, 963, 888, 840, 825, 805, 755, and 702 cm^{-1}. This IR spectrum is almost identical with that of nor-keto agarofuran (4) (Chapter III, Fig. 3.1). It did not form a derivative with 2:4-dinitrophenylhydrazine.

Benzene fraction (261 mg). It showed n_D^{24} 1.5099, IR bands at: 3540, 1724, 1669, 1387, 1304, 1155, 1018, 934, 892, 845, 807, and 759 cm^{-1}. It appears to be a mixture of alcohol and ketone hence subjected to Girard's treatment (reagent T).

Benzene fraction (0.2 g) dissolved in alcohol(3 ml) was refluxed for 1 hr with Girard's reagent T (0.32 g) in acetic acid (1 ml). After removal of the solvent in vacuo, the residue was taken up in ether and washed with water (15 ml). The aqueous layer was warmed with conc. hydrochloric acid (2 ml) and worked up in the customary manner to afford the ketone (30 mg) having identical IR spectrum with that of nor-ketoagarofuran.

The ether layer after washing with sodium carbonate and water, was dried over anhydrous sodium sulphate. Removal of the solvent afforded the ketol (147 mg), n_D^{24} 1.5115, IR bands at: 3500, 1709, 1667, 1376, 1153, 933, and 891 cm^{-1}.

Fraction D

The whole fraction was passed through a column of alumina (grade II, 62 g). Elution with pet.ether (200 ml) and benzene (200 ml) afforded a liquid (2.02 g), n_D^{27} 1.5160, $(\alpha)_D^{27}$ + 20.57°; UV spectrum: λmax. 237 mμ (E, 1%, 1 cm, 96); IR bands at: 3500, 2850, 1708(w), 1666, 1620, 1453, and 1378 cm^{-1}.

This liquid (2.0 g) in ethanol (20 ml) was refluxed for 1 hr with Girard's reagent T (1.6 g) dissolved in acetic acid (2.0 ml) and worked up in the manner described in the case of Fraction C.

The neutral material (1.38 g) from ether layer, after filtering through a column of alumina (grade II, 20 g) and distilling under vacuum, furnished the alcohol (0.7 g), b.p. 150° at 0.2 mm., n_D^{25} 1.5089, $(\alpha)_D^{28}$ - 10.55°; IR bands at: 3480, 2959, 1761(vw), 1634, 1449, 1370, 1276, 1202, 1143, 1047, 931, 912, 887, 842, and 810 cm^{-1}. (Found: C, 81.1; H, 12.1. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%). Its IR spectrum was very similar to that of agarol, the low rotation is evidently due to the presence of minor impurities, the presence of which was further supported by the elemental analysis.

The aqueous layer from the Girard treatment was worked up as usual to furnish the ketol (0.3 g), n_D^{26} 1.5320, $(\alpha)_D^{26}$ ± 0°; IR spectrum: 3509 cm^{-1} (-OH group), 1667 and 1613 cm^{-1} (conjugated >C=O); UV spectrum: λmax. 237 mμ (ε,7975), end absorption: ε_{210} 5316, ε_{215} 4915, ε_{220} 5087. (It approximated to a molecular formula, $C_{16}H_{24}O_2$, though a satisfactory analysis could not be obtained).

Fraction E

The orange coloured viscous mass (5.7 g) was dissolved in pet.ether and kept in deep freeze (-18°), whereupon a crystalline material (0.9 g), m.p.128° was obtained.

Sesquiterpene alcohol

The crystalline material (0.9 g) was chromatographed on alumina (grade II, 50 g). The eluate (1.5 l) obtained in benzene fractions was concentrated and crystallized to sharp m.p. of 133° which remained unchanged after three crystallizations and two high vacuum sublimations(0.45 g), $(\alpha)_D^{27}$ - 31° (c, 1.0); yellow colouration with tetranitromethane; IR bands at: 3420(m), 2860, 1660(w), 1641(vw), 1463, 1375, 1309, 1241, 1195, 1175, 1136, 1103, 1085, 1065, 1054, 1021, 1007, 981, 965, 955, 837, and 803 cm^{-1}; no specific absorption in the UV spectrum; perbenzoic acid titration showed the presence of one double bond, xanthate test positive (Found: C, 81.5; H, 12.1. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%).

It formed a 3,5-dinitrobenzoate, m.p. 204°, IR bands at: 3125(w), 2976, 1739, 1626, 1543, 1464, 1377, 1340, 1271, 1167, 1076, 1027, 991, 972, 920, 829, 802, 775, 732, and 719 cm^{-1}.

Hydrogenation of alcohol

The parent alcohol (96 mg) in glacial acetic acid (10 ml) was stirred in an atmosphere of hydrogen with Adams catalyst PtO_2 (20 mg) at room temperature (21°) and atmospheric pressure (714.5 mm). The uptake equivalent to one mole was over in 2 hrs, after that there was no further absorption. The catalyst was filtered and the

reaction product was worked up in the usual way to give dihydro-alcohol, crystallized from pet.ether and sublimed under vacuum, m.p. 133°, $(\alpha)_D^{28}$ - 25.66° (c, 1.87); IR bands at: 3470, 1458, 1376, 1127, 1064, 1051, 1018, 970, and 958 cm^{-1} (Found: C, 81.53; H, 12.12. $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%).

Attempted oxidation with manganese dioxide

The parent alcohol (21 mg) dissolved in chloroform (4 ml) was shaken with active manganese dioxide (210 mg) for 20 hrs. After working up in the customary manner the starting material was recovered unchanged.

Dehydrogenation of alcohol

The alcohol (130 mg) was mixed with equal amount of selenium and heated at 288° in an atmosphere of nitrogen for 16 hrs. The product, after working up in the usual manner, did not exhibit any aromatic character in UV and IR spectra.

Fraction F

1.06 g. of the brownish material was dissolved in pet.ether and put for crystallization at -18°, whereupon the crystalline keto-alcohol (129 mg), m.p. 158° was obtained. It was filtered through a column of alumina (grade II, 10 g., elution with 500 ml benzene) and after

several crystallizations followed by high vacuum sublimation, it (31 mg) melted at 163°, which could not be raised by further crystallization. It showed IR bands at: 3430, 1669, 1639, 1461, 1373, 1278, 1249, 1226, 1175, 1070, 966, 944, 840, 809, and 723 cm^{-1}; UV spectrum: λmax. 235 mμ (ε 5664) (Found: C, 78.8; H, 11.4. $C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.24%).

Fraction G

It was a very viscous and brown material, distilled at 150-180° at 1.5 X 10^{-3} mm (diffusion pump) to give the viscous liquid (626 mg), n_D^{23} 1.5238, $(\alpha)_D^{28}$ + 7.76° (c, 1.15), UV spectrum: λmax. 242.5 mμ (ε 3680), end absorption: ε_{215} 6700, ε_{220} 4559; IR spectrum: 3480 cm^{-1} (-OH group), 1767 and 1669 cm^{-1} (γ-lactone). It approximates to a molecular formula, $C_{15}H_{22}O_3$, but no dependable analysis could be obtained.

Trap liquid

During the removal of the last traces of solvent, from the oil extract under vacuum, the trap was found to contain some liquid. After careful removal of the solvent, the trap liquid (5 g) was examined, n_D^{27} 1.4161, $(\alpha)_D^{28}$ 0.08°; IR spectrum: 1608(w), 1123, 797, 769, 741, 726, and 694 cm^{-1} (liquid film). It was filtered through a column of alumina (grade II, 100 g) and the eluate, after concentration was

fractionally distilled over sodium and four main fractions were obtained (Table III, p. 90), which were examined by gas-liquid chromatography (The Griffin VPC apparatus MK-IIA). The operating conditions were as follows: temperature 122°, outlet pressure 248 mm., inlet pressure 700 mm., nitrogen flow rate 1.9 l/hr., stationary phase (silicone oil) impregnated over size-graded and washed celite[16] in the ratio of 20% w/w. Details are given in Table III (p. 90).

Analysis: Found: C, 85.4; H, 15.0 (Fraction 3). C, 85.4; H, 14.9 (Fraction 4). $C_{10}H_{20}$ requires: C, 85.63; H, 14.37%.

Fraction 3 was redistilled over sodium, b.p. 130-140° at 710 mm., n_D^{27} 1.4181; d_4^{30} 0.7439; IR spectrum: 2880, 1608, 1463, 1372, 1303, 1217, 1172, 1122, 1080, 1051, 971, 835, 797, 769, 743, 726, 694, and 672 cm^{-1} (in 0.1 mm. cell).

R E F E R E N C E S

1. T.C.Jain, M.L.Maheshwari, and S.C.Bhattacharyya, Perf. & Ess.Oil Rec., 53, 294 (1962).

2. S.R.Bose, Science & Culture, 2, 4 (1938).

3. M.Hayano, A.Saito and R.I.Dorfman; Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto (1957), Pergamon Press, N.Y.,1958, p.204.

4. J.L.Simonsen and D.H.R.Barton, The Terpenes, Vol.III, Cambridge University Press, p. 33 (1952).

5. (i) J.Pliva, V.Herout, B.Schneider and F.Šorm, Coll. Czech.Chem.Commun., 18, 500 (1953)
   (ii) O.Motl, V.Herout and F.Šorm, Coll.Czech.Chem.Comm., 23, 1293 (1958).

6. T.C.Jain and S.C.Bhattacharyya, Tetrahedron Letters, 9, 13 (1959).

7. P.Bladon, H.B.Henbest and G.W.Wood, J.Chem.Soc.,2737(1952).

8. P.S.Kalsi, K.K.Chakravarti and S.C.Bhattacharyya, Tetrahedron, 19, 1073 (1963).

9. A.A.Rao, K.L.Surve, K.K.Chakravarti and S.C.Bhattacharyya, Tetrahedron, 19, 233 (1963).

10. F.Feigl, Spot tests, Vol.II, Organic Applications, Elsevier Pub.Co., N.Y., p.129 (1954).

11. R.M.Evans, Quart.Rev., 13, 61 (1959).

12. L.J.Bellamy, The Infrared spectra of Complex Molecules, John Wiley & Sons Inc., N.Y., p. 186 (1958).

13. G.Egloff, Physical Constants of Hydrocarbons, Vol.V, Reinhold Pub.Co., N.Y., p.366 (1953).

14. R.L.Pecsok, Principles and Practice of Gas Chromatography, John Wiley & Sons Inc., N.Y., p. 12 (1959).

15. A.I.M.Keulemans, A.Kwantes, and G.W.A. Rijnders, Anal.Chim.Acta, 16, 29 (1957).

16. A.T.James and A.J.P.Martin, Biochem. J., 50, 679(1952).