TERPENOIDS

A

THESIS

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C O N T E N T S

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INTRODUCTION

The work incorporated in the thesis deals mainly with the four important aspects of costus root oil chemistry:

(i) The partial composition of the Punjab costus root oil, of which a critical examination of the ketone rich fraction has been described.

(ii) Synthesis of optically active hexahydrogermacrol, hexahydrogermacrone and germacrane from both costunolide and solid dihydrocostunolide the lactonic constituents of costus root oil. These have not been reported so far in optically active form.

(iii) Structure and some interesting transformations of dehydrocostus lactone, a crystalline guaianolide containing a conjugated lactone grouping, which is one of the major constituents of costus root oil obtained from both Punjab and Kashmir varieties.

(iv) Stereochemistry of 12-methoxydihydrocostunolide a Michael addition product of costunolide at the position C_{11}. The investigation resulted in the revision of the stereochemical feature of the methoxymethylene group at C11. Contrary to the accepted belief it has been shown to be β-oriented.

Costus root oil, obtained by both the steam distillation[1] and solvent extraction procedure[2-5] from the roots of the plant Saussurea lappa Clarke has been studied in past by many workers. Semmler and Feldstein[6] first studied the oil of commerce. By subjecting it to vacuum distillation at 11 mm, they collected fractions boiling with in a range of 10° and by examining the various fractions they reported the presence of following compounds. Camphene, phellandrene, α-costene, β-costene, aplotaxene, costol, costus acid ($C_{15}H_{22}O_2$), costus lactone ($C_{15}H_{20}O_2$), dihydrocostus lactone ($C_{15}H_{18}O_2$), but they were not able to isolate any ketone from the oil. Later, Naves[7] reported the presence of α- and β- ionones and cis-dihydroionone in the oil, obtained by the solvent extraction procedure using benzene. The first crystalline lactone isolated from costus root oil was dehydrocostuslactone (1). This was reported by Ukita[2] and later confirmed by Crabalona,[8] the oil used by them was obtained by solvent extraction procedure using pet.ether. Naves, later on studied the structure of this lactone but the correct structure for the same was given only in 1956 by Czech workers,[9] on the basis of spectral data and quantitative ozonolysis. Recently the oil of commerce (steam distilled) was again studied by Sorm et al[9-12], who reported the presence of following compounds in it, viz. p-cymene,

myrcene, humulene, β-elemene, caryophyllene, cedrene, aplotaxene, cedrol, costol, palmitic acid and dehydro-costuslactone.

The Czech workers, however, did not report the presence of any ketone in the oil.

The earlier workers did not take adequate precautions as regards the control of temperature during the isolation and processing of the oil. As a result of this several thermolabile lactonic constituents present in the oil were polymerised or denatured. Consequently, the results obtained by different workers were thus somewhat contradictory. This discrepancy was realised by Bhattacharyya et al[13] during the early stages of their investigation on the oil from costus roots of Kashmir origin. They therefore developed a new low temperature solvent extraction procedure for the isolation of the oil using pet.ether (40-60°) as the solvent. Extraction was carried out at room temperature and at no stage during the processing viz. concentration, decolourisation etc., the temperature was allowed to exceed 40 ± 2°. This method was first applied for the extraction of oil from the Kashmir roots. The yields were 6.5% (as against 1-2% by earlier workers). During the study[13-15A] of Kashmir costus root oil thus obtained in our laboratory, the

following compounds have been isolated and characterised: Costunolide, dehydrocostus lactone, dihydrodehydrocostus-lactone, 12-methoxydihydrocostunolide, β-selinene, aplotaxene, β-elemene, costol, costic acid, β-sitosterol, stigmasterol, betulin, a solid keto alcohol, m.p. 189°, palmitic acid and behenic acids, and some unidentified C_{13} ketones.

But it was not possible to isolate any ketone in pure condition from the oil, although the presence of α- and β-ionones, along with possibly the dihydroionones was inferred[15A,17] from the spectral data of certain fractions obtained during the chromatography of the oil.

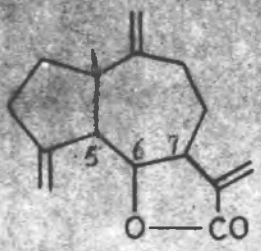
Following a procedure similar to that adopted for Kashmir roots, the costus root oil of Punjab origin could be obtained in 4% yield. The free acids were removed from the oil with alcoholic alkali and the neutral oil chromatographed on alumina and eluted with pet.ether, pet.ether-benzene (3:1), pet.ether-benzene (1:1), chloroform, ether and methanol.

The pet.ether-benzene (3:1) fraction, which contained ketones, esters and lactones was saponified and further chromatographed over alumina and silica gel and following compounds were isolated; dehydrocostus-lactone, dihydrodehydrocostus lactone, a C_{18} acid, probably

oleic acid (as ester), a C_{14}-ketone, a triterpenoid ketone, m.p. 252°, a triterpenic alcohol, m.p. 230°, caryophyllene monoxide and β-sitosterol.

The examination of this fraction has been described in Part I of this thesis.

The second objective was to synthesise the optically active hexahydrogermacrol (2), hexahydrogermacrone (3) and germacrane (4). These three compounds had been obtained earlier[16] from the naturally occurring α,β-unsaturated ketone, germacrone (5), which was isolated from the Bulgarian zdravets oil (Geranium macrorhyzum L) by Sorm and co-workers. These saturated compounds, although containing three to four asymmetric centres, were not optically active. Our aim was to synthesise these three compounds in optically active form, from costunolide (6) and solid dihydrocostunolide (7), the crystalline lactones isolated from costus root oil. The solid dihydrocostunolide on pressure hydrogenation gave solid hexahydrocostunolide (8), while costunolide gave a liquid material under similar conditions. Both solid and liquid hexahydrocostunolides were subjected to controlled LAH reduction followed by Huang-Minlon reduction, to get the same optically active hexahydrogermacrol (2). Dehydration of 2 with $KHSO_4$ followed by catalytic hydrogenation of the resulting product, however,



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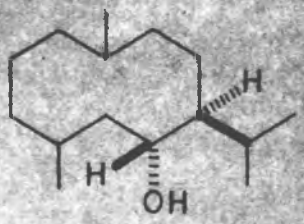

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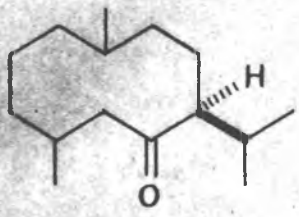

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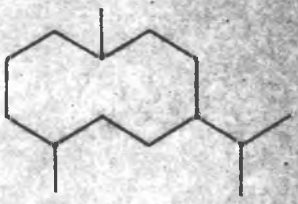

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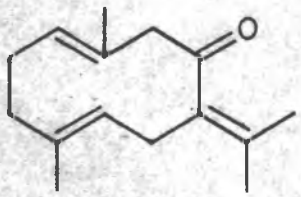

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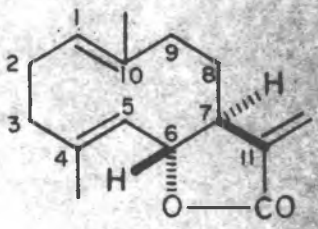

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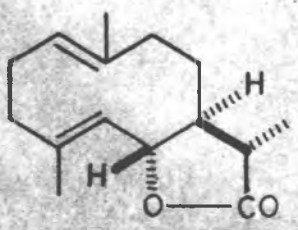

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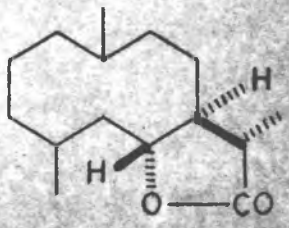

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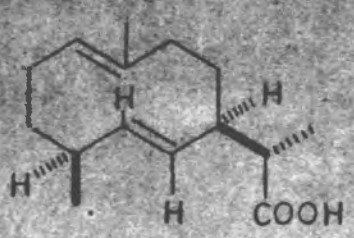
failed to give optically active germacrane (4). In order to obtain germacrane in the optically active form solid dihydrocostunolide was processed as follows:

Solid dihydrocostunolide was subjected to metal-amine reduction, which furnished an acid 9, the structure and stereochemistry of which at C_4, C_7 and C_{11} have been established.[17] The ester was hydrogenated under pressure and the saturated optically active ester 10 was reduced by LAH to the corresponding alcohol 11, which on tosylation followed by LAH reduction gave germacrane showing optical activity.

This piece of work has been described in Part II of the thesis.

Dehydrocostus lactone (1), the crystalline sesquiterpenic lactone, $C_{15}H_{18}O_2$, was first isolated from costus roots by Ukita[2] in 1939 and later by Crabalona[8] who isolated the same lactone from the oil extracted from the roots using pet.ether, after several years of standing. Among the lactones reported to occur in costus root oil, dehydrocostus lactone is the only crystalline lactone, the isolation of which has been consistantly reported by several workers though in varying and poor yields.

The guaianolide nature of the lactone was first indicated by Naves,[3] who on the basis of Se-dehydrogenation of the saturated lactone, came to the conclusion that it



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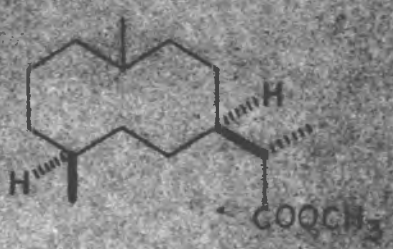

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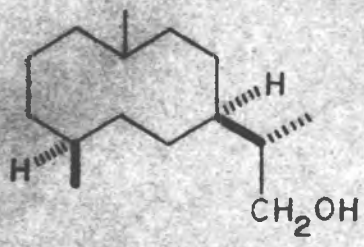

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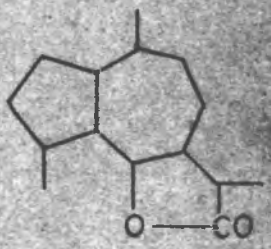

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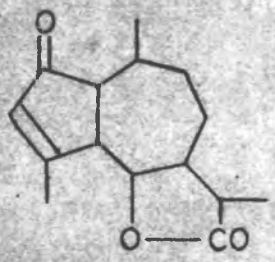

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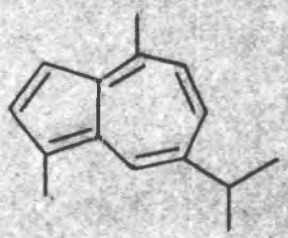

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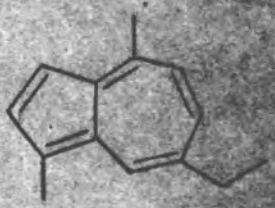

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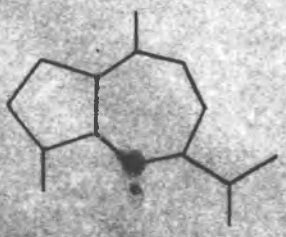

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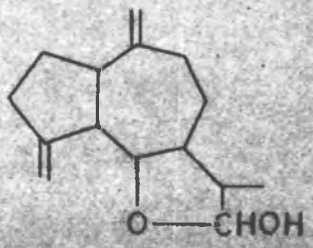

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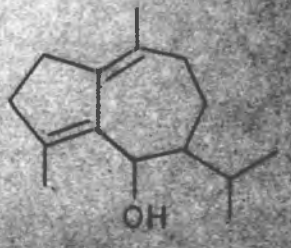

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possesses a gualane skeleton. On the basis of catalytic hydrogenation and quantitative ozonolysis, he concluded that dehydrocostus lactone is a bicyclic lactone containing two double bonds. In the year 1956, Sorm and co-workers[9] repeated the experiments of Naves and found that dehydrocostus lactone contains in fact three double bonds, all of which are present as $>C=CH_2$ groups. Out of these one of the double bonds is conjugated with lactone carbonyl as indicated by IR and UV data. On the basis of quantitative ozonolysis they were able to show that unlike Naves, three molecules of formaldehyde are obtained instead of two. These conclusions were further supported by IR spectrum of dehydrocostus lactone which when taken in chloroform solution, did not show the characteristic methyl bending vibrations round about 1380 cm^{-1} indicating the absence of any methyl group in the compound. Further the IR spectrum of the completely saturated lactone namely, hexahydrodehydrocostus lactone (12), was comparable with that of deoxodihydrocarpesia lactone (13). The Se-dehydrogenation of the saturated lactone was also repeated by the Czech workers when they were able to get a mixture of azulenes in low yields from which S-guaiazulene (14) and S-chamazulene (15) have been identified and characterised. On the basis of these results the structure of dehydrocostus lactone was given as 1.

Although the structure 1 appears to be reasonably satisfactory, there are certain points which need to be established by more rigorous chemical methods. For example, the low yield of azulenes formed during dehydrogenation did not lend sufficient support to the structure. To draw conclusions about the carbon skeleton of the lactone, based on such low yield, appears not so satisfactory, especially when many non-azulenic compounds give comparable amounts of azulenes on dehydrogenation. Secondly, the lactone attachment at C_6-C_7 was based mainly on the comparison of IR spectrum of a liquid material of doubtful homogeneity.

In addition, the stereochemistry at the four asymmetric centres, C_1, C_5, C_6 and C_7 is to be established.

As already indicated in Part I of this thesis, dehydrocostus lactone (1) is one of the major constituents of costus root oil of both the Punjab and Kashmir origin. It occurs to the extent of 35% of the oil. We were interested in preparing some useful products from this lactone for possible application in perfumery and medicine. Since azulenes are used as antiinflammatory agents in cosmetics, one such obvious possibility was to devise a method for getting reasonably good yield of the azulenes from dehydrocostus lactone. Before making attempts in

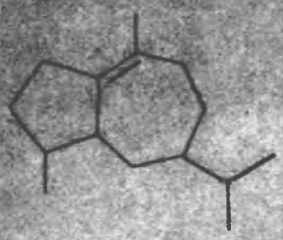
this direction it was felt desirable first to establish the guaianolide structure on firm basis. Since certain other points about the structure were to be established, it was felt desirable to review the entire structure and give more rigid proof in support of the same.

For this purpose experiments have been carried out[18] to prove the guaianolide nature of the lactone by (i) converting it into guaiane (16), the basic hydrocarbon, the identity being established with the help of IR spectrum, GLC analysis and physical constants; (ii) trying to get better yields of the azulene from some of the compounds prepared from dehydrocostus lactone and (iii) showing the lactone attachment at C_6-C_7 by chemical methods.

The formation of a lactol (17) from dehydrocostus lactone by $NaBH_4$ reduction has been reported by Japanese workers.[19] The lactol was found to be temperature sensitive and gave different products when subjected to Huang-Minlon reduction at different temperatures. One such alcohol (18) contained two tetraalkylated double bonds between C_1-C_{10} and C_4-C_5, which resulted due to migration of the originally occurring exocyclic double bonds at C4 and C_{10}. This alcohol will be definitely an allylic alcohol if the lactone attachment were to be between C_6 and C7; as expected it gave rise to a hydrocarbon (19) on metal-amine reduction, due to hydrogenolysis and reduction, thus confirming

the allylic nature of the -OH group and consequently the lactone attachment at C_6 and C_7. An attempt was also made to elucidate the stereochemistry at C_1 and C_5 by oxidative degradation of the saturated alcohol (20) prepared from hexahydrodehydrocostus lactone (12), but since the oxidation did not proceed according to our expectations it was not possible to get a compound related to nepetalinic acid (21). This work has been described in Part III of the thesis.

12-Methoxydihydrocostunolide (22), a crystalline sesquiterpenic lactone isolated[20] from both Kashmir and Punjab costus root oils is an artefact formed from costunolide (6) by the action of methanol under mild alkaline conditions. Its structure as well as its stereochemistry at C_6 and C_7 have been established both by degradative experiments and also by its synthesis from costunolide. The later shows that the stereochemistry at C_6 and C_7 is the same as in costunolide (6), but does not indicate the stereochemistry of $-CH_2OCH_3$ group at the position C_{11}. In analogy[21] with santonin group of compounds and also solid dihydrocostunolide and on thermodynamic considerations, the methoxy methylene group at C11 was previously assumed to be β. But recent observations,[22] as regards the stereochemistry of α-santonin (23) at C_{11}, indicating the methyl group at C_{11} to be α-oriented, led us initially to infer that the $-CH_2\overset{O}{\overset{\wedge}{}}CH_3$ group in the



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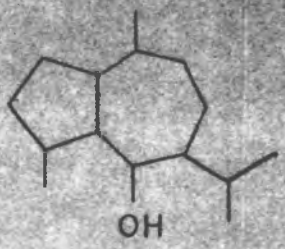

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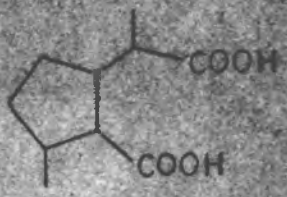

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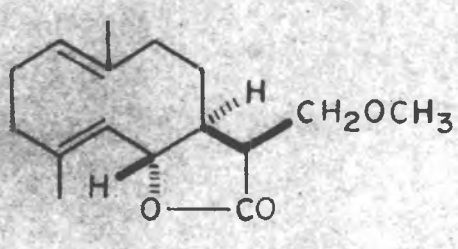

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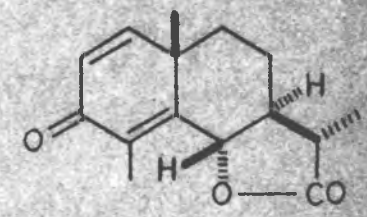

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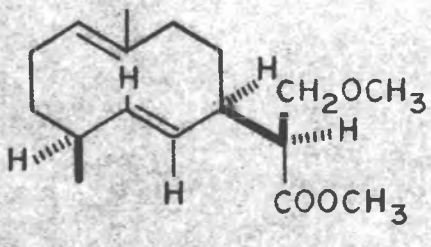

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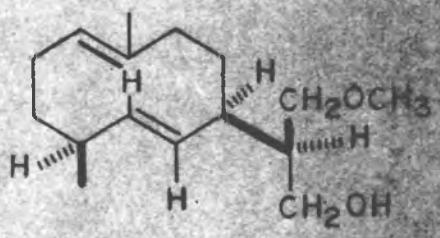

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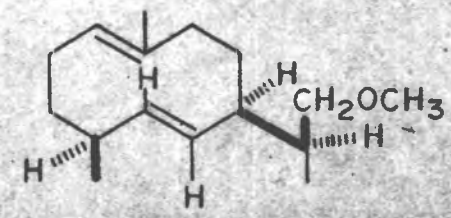

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methoxy lactone also is α-oriented. Such an assignment, however, is based on the comparison of rotation contributions of dihydrocostunolide (7) and 12-methoxy dihydrocostunolide (22), which possess identical stereochemistry at C_6 and C_7 and can possibly differ only as regards the position at C_{11}. Since a definite chemical proof to decide this point has not been given earlier, it was decided to prove the same by an unambigous chemical evidence.

For this purpose, the methoxylactone (22) was subjected to metal amine reduction to get the ester (24), which was subsequently converted into the alcohol 25, and then into the methyl ether 26.

Solid dihydrocostunolide (7) with the stereochemistry established at every optical centre, could also be converted into the same methyl ether 26 by means of mild chemical reactions already known in literature. This identity established without any doubt that the methoxymethylene group in 12-methoxydihydrocostunolide (22) is β-oriented.

The work carried out in this connection has been described in Part IV of the thesis.

During the course of these investigations, liberal uses of modern techniques such as infrared, ultraviolet spectroscopy, nuclear magnetic resonance, optical rotatory dispersion, gas liquid chromatography, thin layer chromatography etc. have been made.

For the sake of brevity a general introduction to terpenoids has been avoided in this thesis. The subject has already been covered adequately by a number of well known publications, of which special mention may be made of the following:

1. Terpenes by J.L. Simonsen and co-workers, Vol. I-V (University Press, Cambridge).
2. Chemistry of carbon compounds (Edited by E.H.Rodd), Vol. IIB, 1953 edition (Elsevier Publishing Co.).
3. Mono and Sesquiterpenoids by P. de Mayo (Interscience Publishers, N.Y.)
4. Progress in the Chemistry of Natural Products, Zechmeister, Vol. XII (Springer-Vienna).
5. The Essential Oils (6 volumes) by E. Guenther (D. Van Nostrand Co. Inc., N.Y.).

The author gratefully acknowledges the help he received from these publications, during the course of the investigations.

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GENERAL REMARKS

(1) The melting points and boiling points are uncorrected.

(2) All temperatures are recorded on the Centigrade scale.

(3) Unless otherwise stated, all rotations were taken in chloroform solution.

(4) The ultraviolet spectra were recorded in ethanol (and in some cases methanol) solution on a Beckman DK- II ratio recording spectrophotometer.

(5) The infrared spectra were recorded on a Perkin-Elmer infracord spectrophotometer, Model 137B and Model 221, with sodium chloride optics.

(6) The NMR spectra were measured in carbon-tetrachloride solution unless otherwise mentioned using tetramethylsilane as the internal reference on a 60 Mc Varian instrument and the chemical shifts were measured in τ units.

(7) The acid washed activated alumina standardised as per Brockmann's procedure was employed for column chromatography.

(8) Gas liquid chromatographic analyses were carried out on a Griffin & George VPC apparatus MK IIA with polyester column employing hydrogen as carrier gas.

(9) The numbers given to the structure and charts and also the numbers given to the figures in each chapter of the thesis refer to that particular part only.

(10) The list of references pertaining to each part has been given at the end of that part.

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PART I

COMPOSITION OF THE PUNJAB COSTUS ROOT OIL

A B S T R A C T

The new low-temperature solvent extraction procedure developed for the extraction of Kashmir costus root oil has been successfully applied for the isolation of Punjab costus root oil as well. The oil in the present case is obtained in a yield of approximately 4%. It consists of about 50% of its own weight of a mixture of crystalline lactones, which can be obtained by stagewise cooling of the pet.ether solution of the oil at 0° and -18°. This has been shown to consist mainly of dehydrocostus lactone (2) and costunolide(1). The Punjab costus root oil appears to contain a somewhat higher percentage of costunolide than the Kashmir costus root oil.

The partially delactonised oil, thus obtained, contained a substantial quantity of free acids which could be removed by extraction with alcoholic alkali at low temperature.

The neutral oil on chromatography over alumina furnished six fractions, which have been obtained by the elution with the following solvents: (i) pet.ether, (ii) pet.ether-benzene (3:1), (iii) pet.ether-benzene (1:1), (iv) chloroform, (v) ether, and (vi) methanol.

The fraction eluted with pet.ether-benzene (3:1) on saponification and chromatography over alumina and

silica gel, has been shown to consist of: (i) dehydrocostus lactone, (ii) dihydrodehydrocostus lactone, (iii) a C_{18} acid, (iv) a C_{14}-ketone, (v) caryophyllene monoxide, (vi) a triterpenoid ketone, m.p. 252°, (vii) a triterpenic alcohol, m.p. 230°, (viii) an alcohol, m.p. 115-120°, (ix) β-sitosterol and several other unidentified compounds, including oxides and ketones.

The costus plant, Saussurea lappa Clarke, belongs to the compositae family and is a valuable Indian raw material. It grows mainly in Kashmir and also in Lahaul and Spiti areas of the hill districts of the Punjab at an altitude of approximately 10,000 ft.[1] It has been used in the indigenous system of medicine,[2] the roots are tonic, stomachic, carminative, stimulant and useful in asthama, cough and cholera. It is alterative in chronic skin diseases and reheumatism. It is said to have remarkable effect in controlling bronchial asthama, specially of vagatonic type.[3-4] The root powder is used for preserving wollen clothes against moth attacks. Large quantities of roots used to be exported to China and Japan for use in temples[1] and to Europe for the distillation of essential oil. In India the roots are known by various names such as kashmiraja, kuth, kustha[5,6] etc. The roots contain a fragrant principle and the essential oil contained in the roots has attracted the attention of perfumers in India and abroad. Consequently the oil has found wide use in high grade perfumery preparations.

The two important methods applicable for the isolation of essential oil from costus roots are (i) steam distillation,[7] (ii) solvent extraction using different solvents. The oil has been known to be rich in lactonic constituents, some of which are conjugated and hence cannot stand high temperature,as they are easily

polymerised. The yield of the oil obtained by steam distillation of the roots is quite low, as many of the constituents which are present in the roots are not so volatile in steam and some of them are probably denatured by contact with water at high temperature. The oil has been extracted also, by solvent extraction procedure, using solvents like pet.ether,[8] benzene,[9,10] ether, alcohol[11] etc. by the previous workers, but no adequate precuations were taken as regards the control of temperature during extraction. As a result, some of the valuable constituents which are thermolabile were lost owing to polymerisation. Taking into consideration these facts, a procedure was standardised for the extraction of the oil from the costus roots of Kashmir origin using pet.ether (40-60°) under mild conditions in our laboratory. The process was so designed that at no stage during extraction, removal of solvents, decolourisation etc., the outside temperature was allowed to exceed 40 ± 2°. The oil obtained by this procedure is free from polymerisation and denaturing and contained a new sesquiterpenic lactone having a ten-membered carbocyclic ring, namely costunolide[12] (1) to the extent of 15%. The oil thus obtained has been critically examined[13-15] for its composition which formed the subject matter of several publications from this laboratory, the results of which are included in the thesis of some of my colleagues.

Following an exactly similar procedure, we have now been able to extract an essential oil in about 4% yield from the Punjab costus roots. As in the case of the Kashmir costus root oil, it also contains about 50% of its own weight of a mixture of crystalline lactones, which has been shown to consist mainly of costunolide (1) and dehydrocostus lactone (2). The partially delactonised oil which has been accepted by perfumers abroad is found to be somewhat different as regards the physico-chemical constants from the Kashmir oil. The comparative properties of the costus root oil from the two sources have been shown in the following table:

Origin	$(\alpha)_D^{25}$	n^{28}	d_{25}^{25}	Solubility	Acid value	Sap. value
Kashmir costus root oil	+28.96 (c,2.5)	1.5082	0.9977	98.62%	21.90	133.91
Punjab costus root oil	+29.65 (c,1.5)	1.5100	1.001	98.64%	29.80	133.91

Since large quantities of costus roots were cultivated in Punjab, a project was sponsored by the Punjab Government to find commercial uses for the oil and its constituents.

As a part of this programme, the study of the composition of the Punjab costus root oil was undertaken.

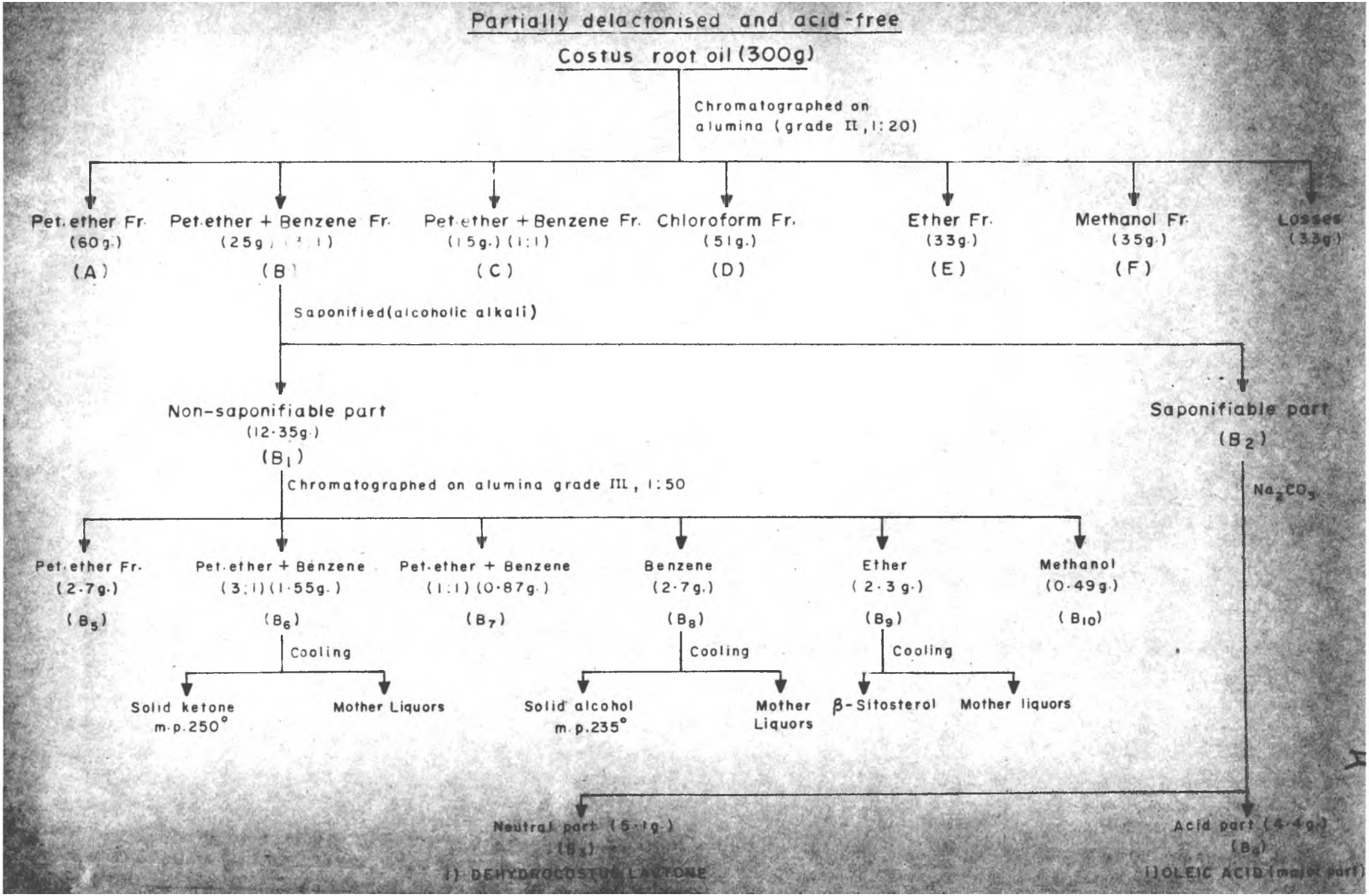
The partially delactonised oil contained free acids, as indicated by its acid value. These were initially removed by extraction of the oil with alcoholic alkali. The quantity of the acids obtained amounts to about 5%. For the critical examination of the constituents, the neutral oil was initially chromatographed on neutral alumina (gr.II) and collected into various fractions by using the following solvents for elution (see Chart I): (i) pet.ether (A), (ii) pet.ether-benzene (3:1) (B), (iii) pet.ether-benzene (1:1) (C), (iv) chloroform (D), (v) ether (E), and (vi) methanol (F). In this thesis the critical examination of the pet.ether-benzene (3:1) fraction (B) which was found to contain ketones has been described.

Among the ketones reported to occur in costus root oil, α- and β-ionones seem to be the only known compounds. Although Semmler and Feldstein first studied[16] the oil of commerce for its composition, no ketone was reported to occur in this oil. It was only Naves[17] who showed the presence of α- and β-ionones along with cis-dihydroionones in the oil, obtained by solvent extraction procedure using benzene. In a series of recent communications[18-21] by Czech

workers the composition of costus root oil of commerce (steam distilled) was again examined, but no information about the ketones of the oil was mentioned.

While dealing with the composition of the Kashmir costus root oil,[13-15] it was observed that a similar fraction eluted with pet.ether-benzene (1:1) contained some ketones, in which the presence of a C13-ketone, α- and β-ionones have been indicated. It was not possible however to isolate any of these compounds in pure condition. The same fraction also contained substantial quantities of lactones and esters, which made the isolation of ketones rather difficult. This is probably because a lower ratio of alumina (gr.III) was used in the main chromatography, as a result better separation between the constituents did not take place. For this reason while studying the composition of the Punjab costus root oil, twenty times grade II alumina was used and a fraction with pet.ether-benzene (3:1) (Fr.B) was taken, so as to get a fraction rich in ketones (see Chart I).

Initial examination of the IR spectrum of the pet. ether-benzene (3:1) fraction(B) indicated in it, the presence of only carbonyl compounds (the presence of a broad band near 1760 cm^{-1} and also at 1710 cm^{-1} respectively), which is probably due to esters, ketones and lactones. The


Partially delactonised and acid-free
Costus root oil (300g)
Chromatographed on
alumina (grade II, 1:20)
Pet.ether Fr.
(60g.)
(A)
Pet.ether + Benzene Fr.
(25g.) (1:1)
(B)
Pet.ether + Benzene Fr.
(15g.) (1:1)
(C)
Chloroform Fr.
(51g.)
(D)
Ether Fr.
(33g.)
(E)
Methanol Fr.
(35g.)
(F)
Losses
(3.3g.)
Saponified (alcoholic alkali)
Non-saponifiable part
(12.35g.)
(B1)
Saponifiable part
(B2)
Chromatographed on alumina grade III, 1:50
Na2CO3
Pet.ether Fr.
(2.7g.)
(B5)
Pet.ether + Benzene
(3:1) (1.55g.)
(B6)
Pet.ether + Benzene
(1:1) (0.87g.)
(B7)
Benzene
(2.7g.)
(B8)
Ether
(2.3g.)
(B9)
Methanol
(0.49g.)
(B10)
Cooling
Cooling
Cooling
Solid ketone
m.p. 250°
Mother Liquors
Solid alcohol
m.p. 235°
Mother
Liquors
β-Sitosterol
Mother liquors
Neutral part (5.1g.)
(B3)
1) DEHYDROCOSTUS LACTONE
Acid part (4.4g.)
(B4)
1) OLEIC ACID (major part)

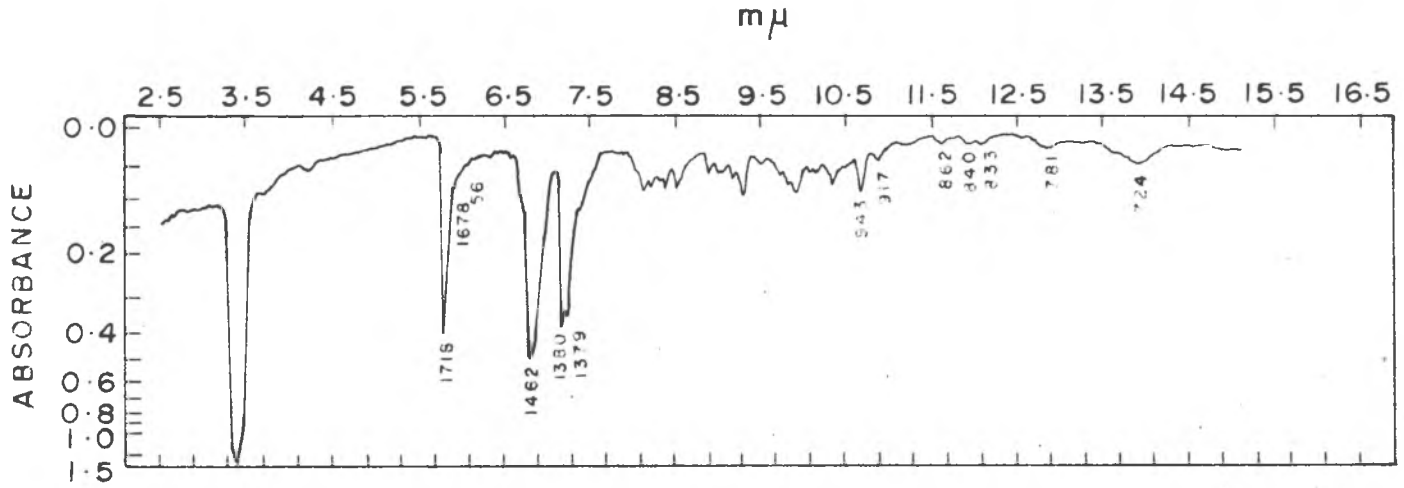
IR spectrum did not show any peak for -OH stretching indicating the absence of free alcohols in the fraction. An attempt to separate the various constituents of the fraction by column chromatography over alumina was, however, unsuccessful as the various fractions isolated were indicated to be mixtures of three to four components from TLC analysis. Since the presence of lactones was indicated in the IR spectrum of the fraction B), it was felt desirable to separate the fraction into saponifiable acidic and neutral portions by saponification.

For this purpose the fraction was saponified using 5% alcoholic KOH for 12 hr. and separated into non-saponifiable part (B_1) and saponifiable part (B_2) in the usual way. From the saponifiable part (B_2) some quantity of the acid could be recovered by extraction with aqueous Na_2CO_3. The three portions thus obtained by the above procedure have been separately examined as described below.

(I) Examination of the non-saponifiable part (B_1)
(See Chart I)

The non-saponifiable part (B_1) was chromatographed on fifty times (gr.III) alumina and eluted with (i) pet. ether (B_5), (ii) pet.ether-benzene (3:1) (B_6), (iii) pet. ether-benzene (1:1) (B_7), (iv) benzene (B_8), (v) ether (B_9) and (vi) methanol (B_{10}).

The fraction (B_5) eluted with pet.ether showed in its TLC analysis, the presence of four components, two of these, which are less polar were observed close to one another. The fraction (B_6) eluted with pet.ether-benzene (3:1) gave a solid on cooling at -18° for a day. It was filtered off and purified by crystallisation with pet.ether to give a solid m.p. 249-250°, $(\alpha)_D$ - 25°, which was indicated by its IR spectrum (Fig.1) to be a ketone (1715 cm^{-1}). The UV spectrum of this ketone also confirmed the presence of a ketone group ($\lambda_{max.}$ 275; $\epsilon_{max.}$ 70) and in addition showed an end absorption (ϵ 210,450). An idea about the unsaturation was also obtained by the tetranitromethane test which indicated it to be a saturated compound. The elemental analysis ($C_{30}H_{48}O$) of the compound indicated it to be a triterpenoid ketone, which is probably pentacyclic in nature, although the other possibilities like $C_{30}H_{50}O$ and $C_{30}H_{52}O$ are not completely ruled out. Owing to the paucity of the material further examination of the ketone was not undertaken. The low end absorption of the UV spectrum and also a close examination of the m.p. and $(\alpha)_D$ of the compound initially suggested it to be freidlin (3), but the mixed m.p. determination with an authentic sample of freidlion was depressed by 25°. The mother liquors from the fraction (B_6) along with the fraction (B_5) eluted with pet.ether



FIG·1. IR SPECTRUM OF KETONE m.p.250°(IN NUJOL)

were chromatographed on silica gel (1:25) (see Chart II) and eluted with (i) pet.ether (B_{11}), (ii) pet.ether-benzene (1:1) (B_{12}), (iii) benzene (B_{13}) and (iv) ether (B_{14}).

The fraction (B_{11}) eluted with pet.ether was obtained in nearly pure condition as indicated by its TLC analysis. The IR spectrum did not indicate it to be either a carbonyl or hydroxyl compound, but the elemental analysis showed that it contained one oxygen function. This is probably an oxide. The pet.ether-benzene (1:1) fraction (B_{12}) showed four spots on TLC analysis, since the fractions (B_{11}) and (B_{12}) were small in quantities, they were not critically examined.

The benzene fraction (B_{13}) was further chromatographed over silica gel impregnated with silver nitrate[22] (1:30) and eluted with (i) pet.ether-benzene (2:1) (B_{15}); (ii) benzene (B_{16}) and (iii) ether (B_{17}).

The fraction (B_{15}) was found to be almost pure (TLC) and was identical with the fraction (B_{11}) (R_f values comparable). The fraction eluted with benzene (B_{16}) was purified by distillation and the distillate on cooling gave a low melting solid, which was purified by crystallisation from pet.ether to give a crystalline solid m.p. 60-61°, $(\alpha)_D$ - 74°. It analysed for $C_{15}H_{24}O$ and the IR spectrum (Fig. 2) was found to be identical with caryophyllene monoxide (4). This was further confirmed by taking a mixed melting point with an authentic sample of

CHART II

FRACTION (B_5) AND MOTHER LIQUORS OF (B_6)

Chromatographed
Silica gel 1:25

- Pet.ether Fr. (0·16 g) TLC pure (B_{11})
- Pet.ether + Benzene Fr. (0·38 g.) (1:1) (B_{12})
- Benzene Fr. (1·91 g.) (B_{13})
  - Chromatography on $SiO_2 - AgNO_3$ (1:30)
    - Pet.ether + Benzene (2:1) (0·38 g.) TLC pure (B_{15})
    - Benzene (0·158 g.) Caryophylleneoxide (B_{16})
    - Ether (0·857 g) Ketone (B_{17})
- Ether Fr. (1·17 g.) (B_{14}) (IDENTICAL WITH B_{17})

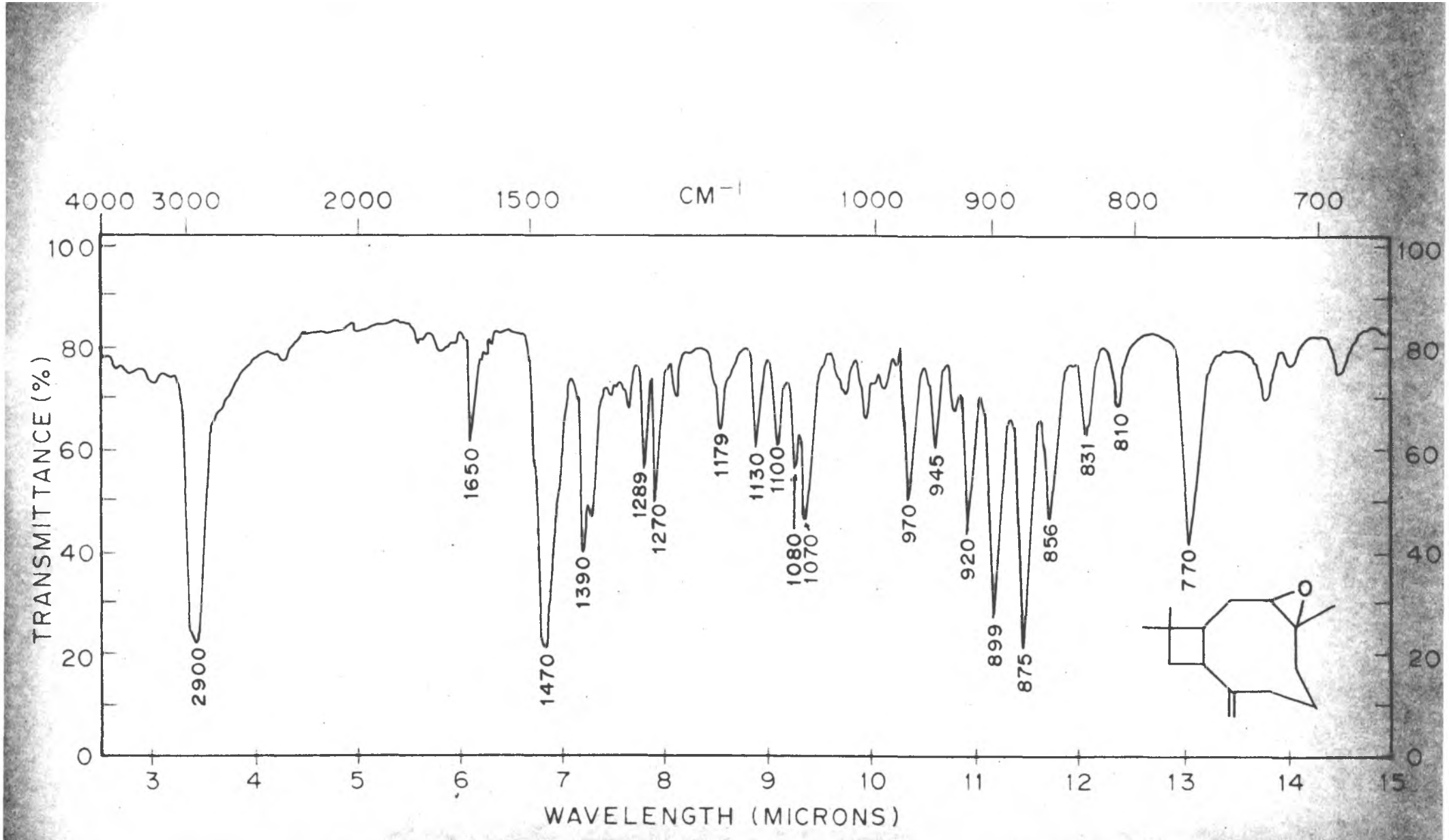


FIG. 2. IR SPECTRUM OF CARYOPHYLLENE MONOXIDE.

caryophyllene oxide, which did not show any depression. The fraction (B_{17}) (see Chart II) was further purified by chromatography over silica gel and the fraction eluted with pet.ether gave a pale yellow oil. The IR spectrum (Fig.3) showed absorption at 1715 cm^{-1} indicating the presence of a non-conjugated ketone grouping in the molecule; another peak was also observed at 1648 (and 890 cm^{-1}) showing that it also contains an exocyclic double bond. The UV spectrum showed only the end absorption at 210 mμ (εmax. 4,450), from which it can be inferred that it contains also a tri-substituted double bond. The NMR spectrum (Fig. 4) indicated the presence of $-COCH_3$ group (signal at 7.91τ, 3H) and also the presence of olefinic protons (3H) (signals at 4.85, 4.98, 5.3 and 5.35τ). It also showed signals at 8.95 , 9.05τ(3H) due to a secondary methyl group. From these spectral data, it can be inferred that it is a methyl ketone. It, however, failed to give a solid semicarbazone.

The IR, as well as, NMR spectra did not give any indication of an oxide or ether group in the molecule and hence it was considered probable that the compound contains only one oxygen function which is present as ketone group.

Although the elemental analysis of the ketone did not agree exactly with $C_{14}H_{24}O$, the elemental analysis of the corresponding alcohol prepared by $NaBH_4$ reduction agreed

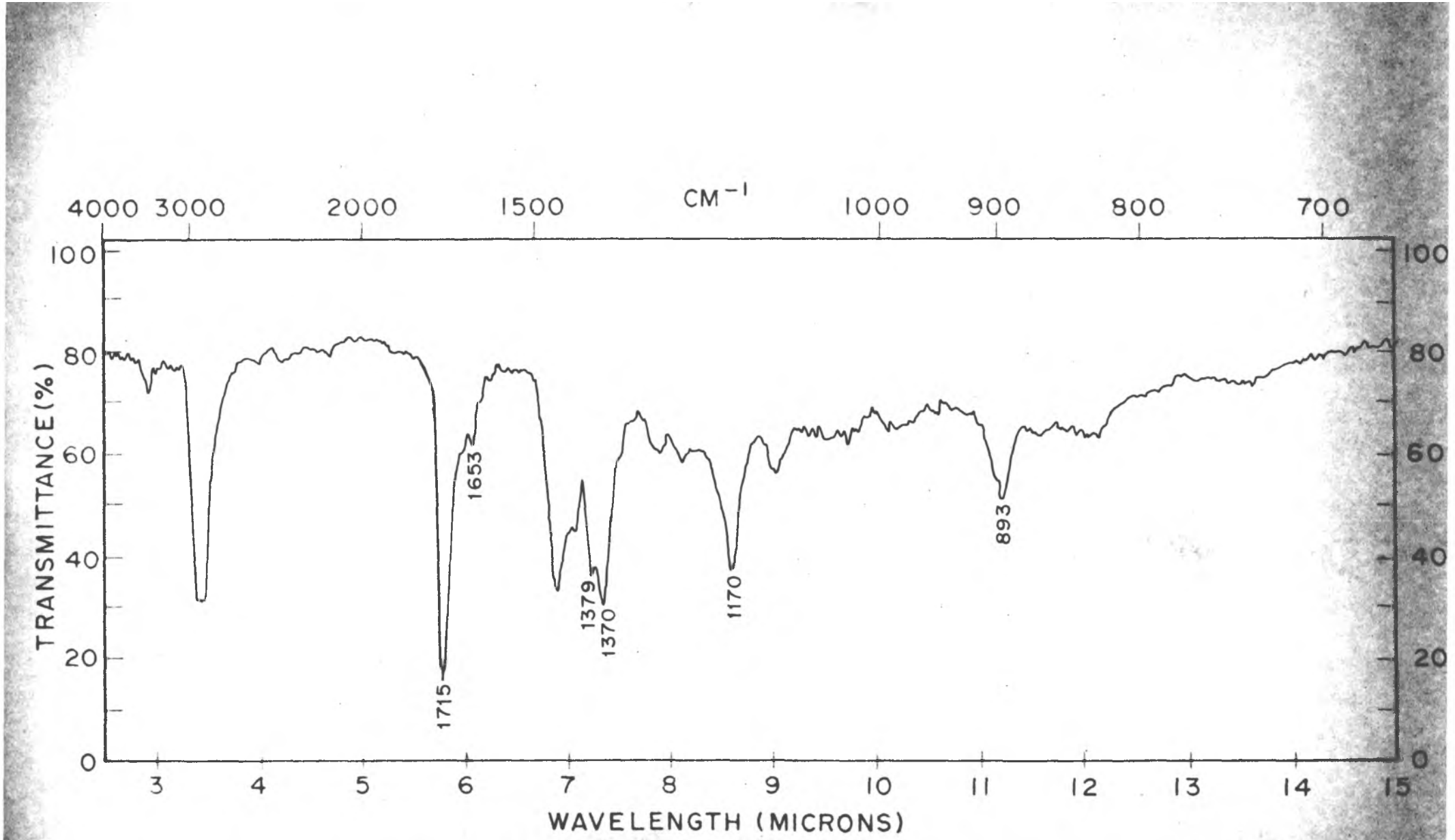
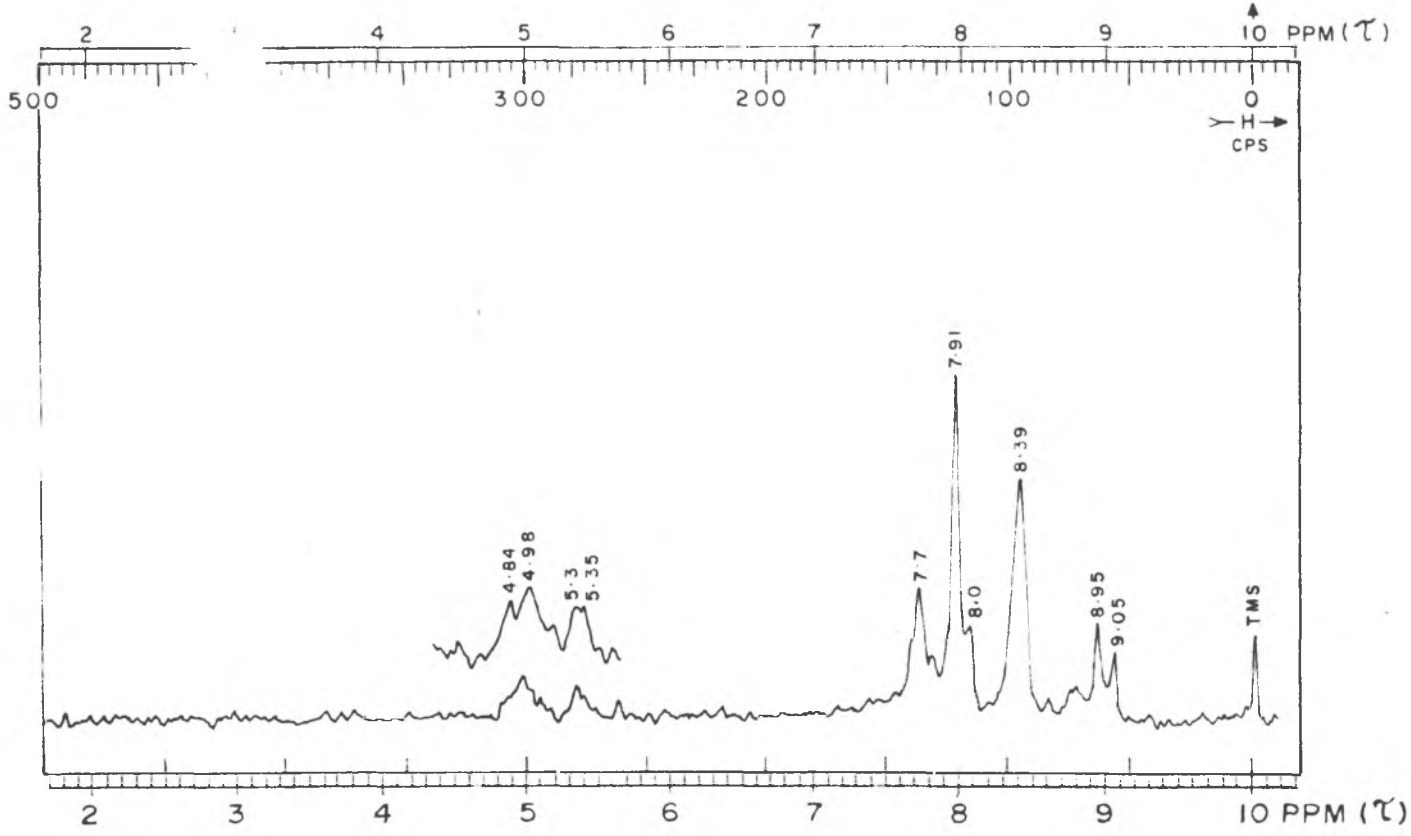


FIG. 3. IR SPECTRUM OF C_{14} - KETONE.



FIG·4· NMR SPECTRUM OF C_{14}-KETONE

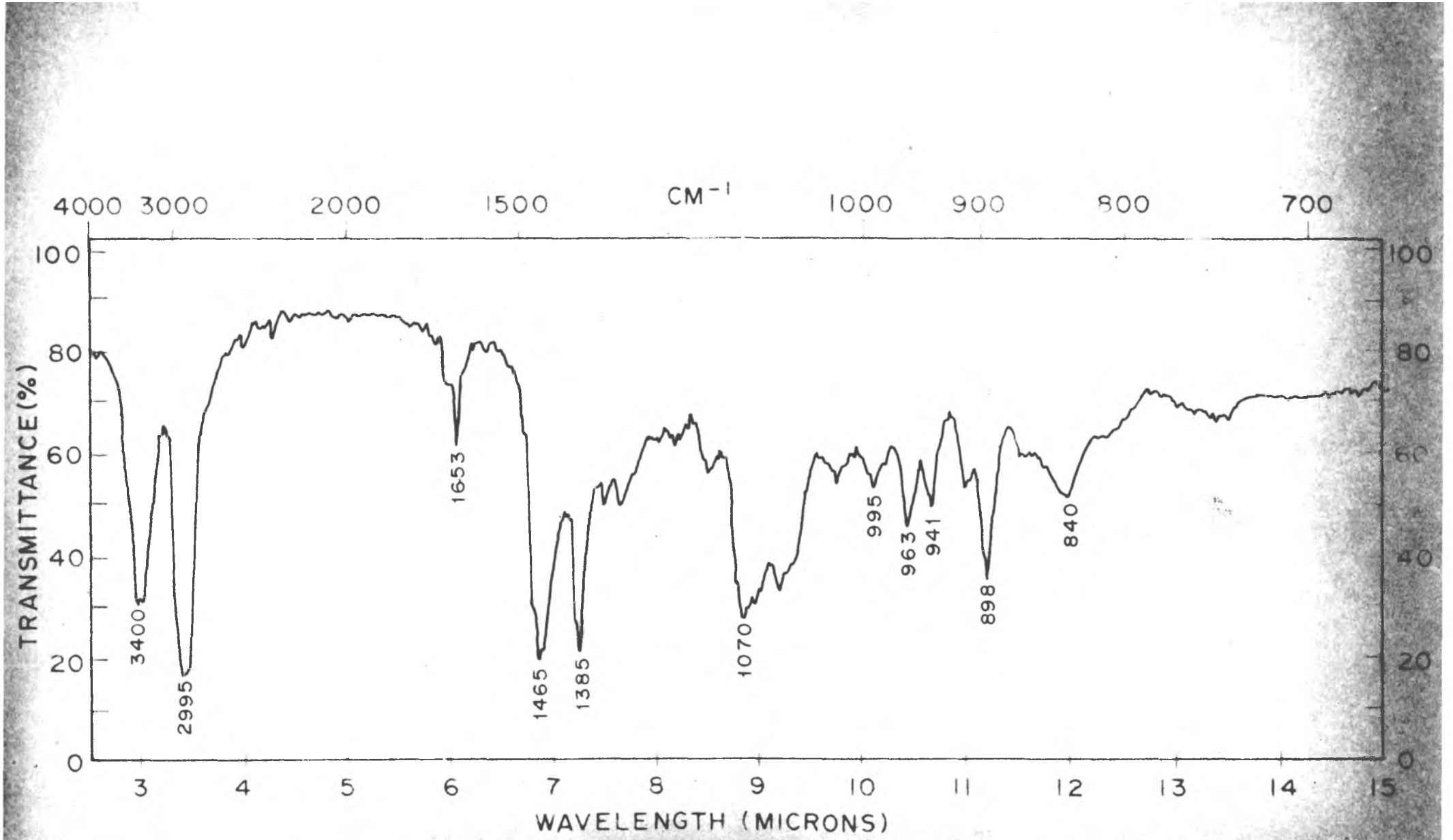


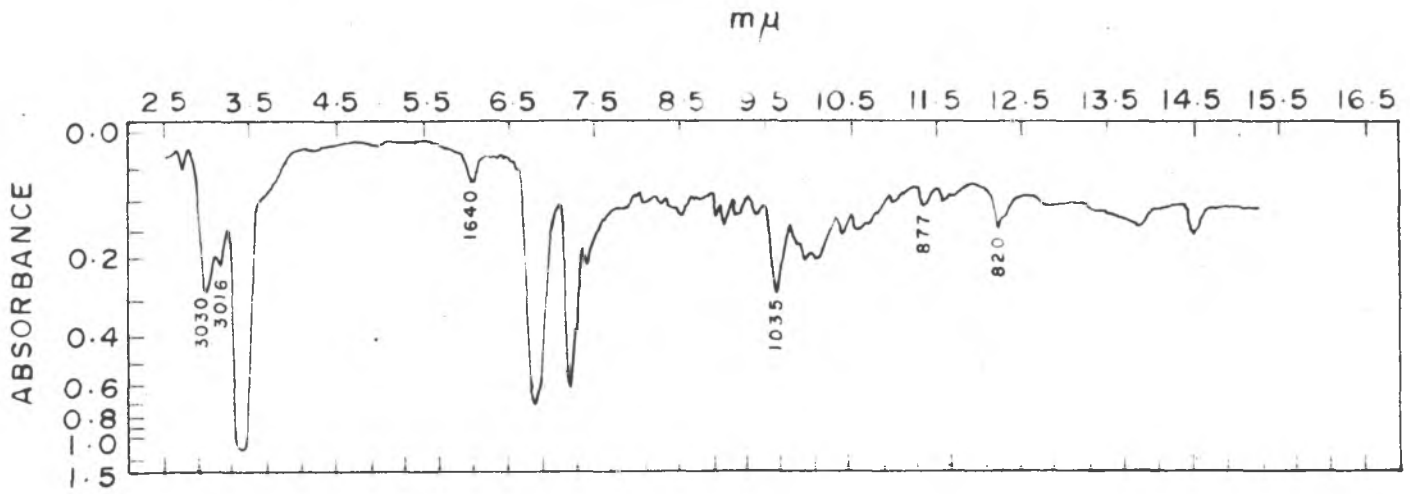
FIG. 5. IR SPECTRUM OF REDUCED PRODUCT OF C_{14} - KETONE.

for $C_{14}H_{26}O$. Also a rough idea of the molecular weight of the ketone by Rast's method indicated it to be either a C_{14} or C_{15} ketone. In view of the presence of a methyl ketone grouping ($-CH_3CO$) in the molecule, which is somewhat uncommon in a C_{15} carbon skeleton, a C_{14} carbon skeleton was preferred.

Examination of benzene fraction (B_8)

The fraction B_8 was dissolved in pet.ether and cooled to get a solid, which on crystallisation with pet. ether gave m.p. 230°. The TLC analysis of the compound on silver nitrate impregnated silica gel indicated the presence of two compounds which were situated very close to each other. The IR spectrum (Fig. 6) indicated to be alcohol(s) and the elemental analysis corresponded to $C_{30}H_{52}O$. Further work in this connection was not undertaken due to paucity of the material.

The mother liquor left after the removal of the solid was further chromatographed on alumina gr.III (1:30) and eluted with pet.ether (B_{18}), pet.ether-benzene (3:1) (B_{19}), pet.ether-benzene (1:1) (B_{20}), benzene (B_{21}) and ether (B_{22}). The pet.ether-benzene fraction (1:1) (B_{20}) on cooling gave a solid m.p. 115-120° indicated by its IR spectrum to be an alcohol (3333 and 1031 cm^{-1} due to hydroxyl group) containing an exocyclic double bond



FIG·6· IR SPECTRUM OF ALCOHOL m·p·230° (IN NUJOL)

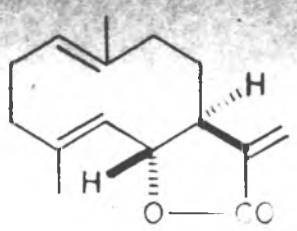
(1630 and 877 cm^{-1}). This alcohol showed $(\alpha)_D$ + 48.60°. Elemental analysis $C_{15}H_{26}O$ indicated it to be a sesquiterpenic alcohol. Further work is in progress.

Examination of fraction B_9

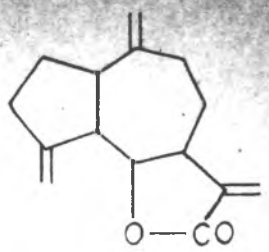
The fraction (B_9) eluted with ether on cooling gave a solid, which on crystallisation with methanol furnished a pure compound, $C_{29}H_{50}O$, m.p. 135-36°, $(\alpha)_D$ - 37°. It was identified as β-sitosterol (7), from m.p., mixed m.p. and IR spectrum. The saponifiable part B_2 (see Chart I) was separated into neutral part (B_3) and the acid part (B_4) by extraction with Na_2CO_3.

Examination of the neutral part (B_3)

The neutral part B_3 was chromatographed on alumina grade III (1:25) and eluted with pet.ether, pet.ether-benzene (3:1), pet.ether-benzene (1:1), benzene and ether. The second fraction which was eluted with pet.ether-benzene (3:1) on cooling gave a solid which was filtered and purified by crystallisation from methanol to give a solid, m.p. 59-60° and $(\alpha)_D$ - 11.2°. The IR spectrum was superimposable with that of dehydrocostus lactone (2). It was identified as dehydrocostus lactone. The remaining fractions were mixed together and the conjugated lactones present in it were converted into the ammonia adduct in the manner as described[23] already. This was then treated with picric



1



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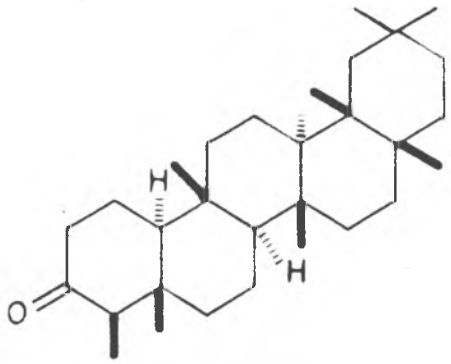

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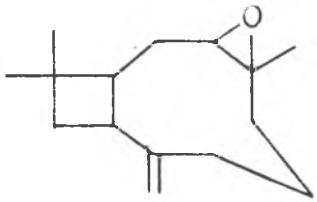

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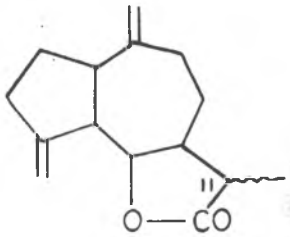
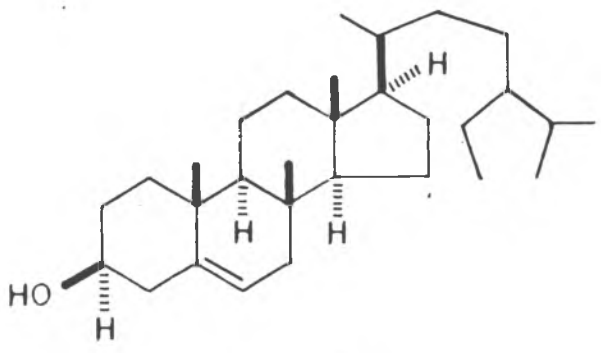


5

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOCH_3$$

6



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acid when the picrate derivative of ammonia adduct of the conjugated lactones separated out. The derivative formed was identical with that of the ammonia adduct of dehydrocostus lactone, thus confirming the presence of dehydrocostus lactone in the fraction.

The mixture of lactones which did not form the ammonia adduct derivative showed three spots on TLC analysis (two major and one in very small proportion). When a comparative TLC of this mixture was taken with the dihydrodehydrocostus lactone (5), prepared by $NaBH_4$ reduction of dehydrocostus lactone, the two major spots were identical in R_f values with the C_{11} epimeric mixture of dihydrodehydrocostus lactone. This mixture was further chromatographed on alumina gr.III (1:30) and eluted with pet.ether, pet.ether-benzene (3:1), benzene and ether. The IR spectrum of the fractions eluted with pet.ether-benzene (3:1) and benzene were identical with dihydrodehydrocostus lactone prepared from dehydrocostus lactone and TLC behaviour was also similar. It analysed correctly for $C_{15}H_{20}O_2$, thus confirming that it is a mixture of C_{11}-epimeric dihydrodehydrocostus lactones. There was, however, some difference between the two as regards their optical rotations.

Examination of the acid part (B_4) see Chart I)

The Na_2CO_3 extract on acidification gave a mixture of acids, which was converted into a mixture of esters by

diazomethane. The methyl esters were purified by chromatography over alumina gr.II (1:20) and eluted with pet.ether, benzene, ether and methanol. The major part of the ester was eluted with pet.ether and was found to be pure (TLC). The IR spectrum (Fig.7) indicated the presence of ester grouping (1750 cm^{-1}). The indication of a fatty acid was also inferred by the characteristic band at 720 cm^{-1} in the IR spectrum. The NMR spectrum (Fig. 8) showed a triplet centred at 9.11 τ (3H) which can be ascribed to the presence of a primary methyl group in the molecule. A sharp signal centred at 8.7 τ with approximate intensity of about 22 protons indicated the presence of eleven $-CH_2$ groups which are in the vicinity of saturated carbon atoms. Signals centred at 7.7 7.89 τ(6H) indicated the presence of three methylene groups which are in the vicinity of the double bond. A signal at 6.4 τ(3H) is due to the ester methyl group. In the olefinic region a multiplet centred at 4.65 was observed due to two (or possibly three) olefinic protons. The elemental analysis and the spectral data thus suggested it to be oleic acid methyl ester (6), which was further supported by the TLC analysis (R_f values comparable). However, it did not furnish any solid amide from which the possibility of either oleic acid (cis-isomer) or eladic acid (trans-isomer) could not be inferred with certainty. Saponification of the ester gave a low melting solid (m.p. 40°). Further work in this connection is in progress.

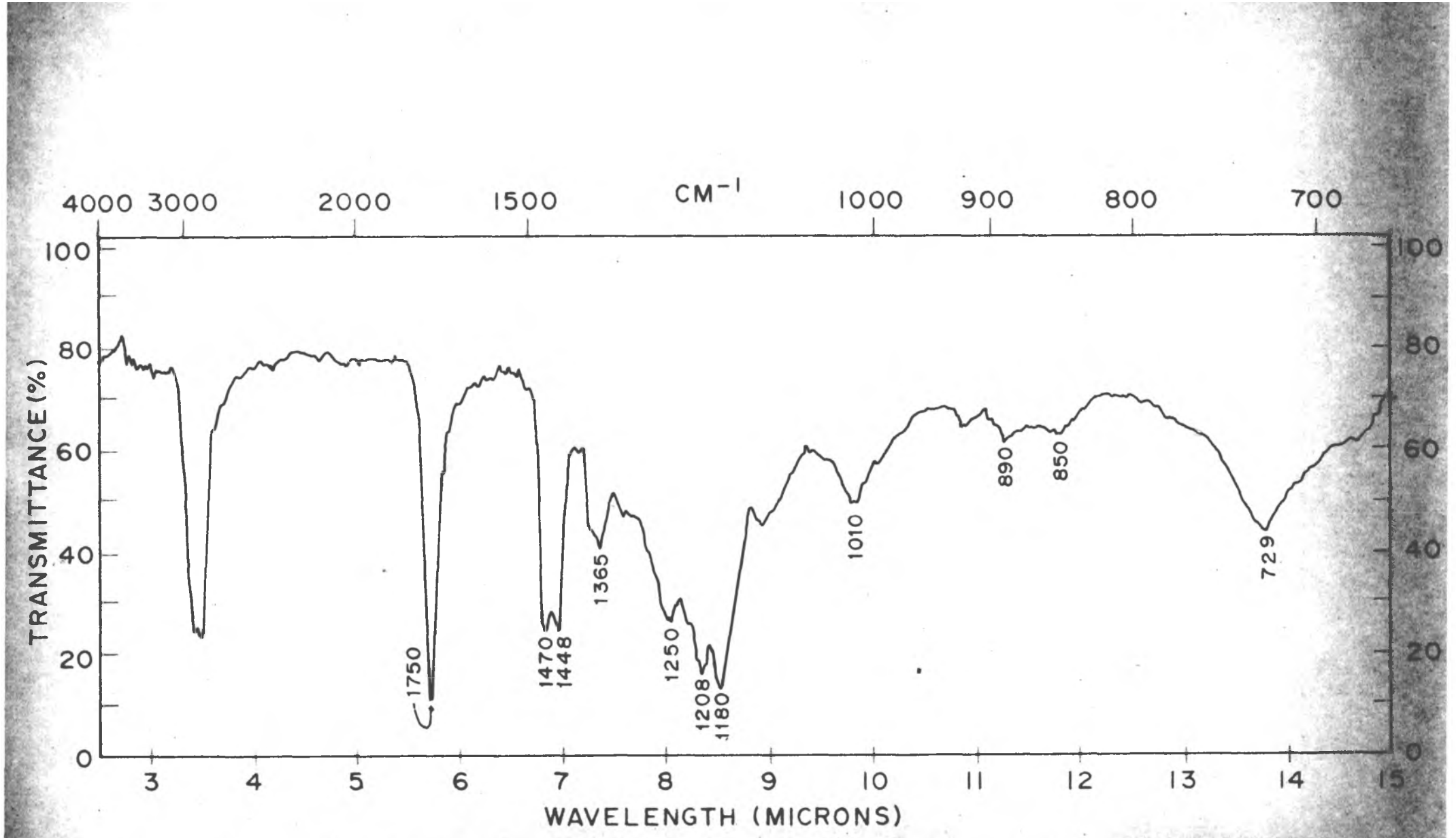


FIG. 7. IR SPECTRUM OF METHYL ESTER OF C_{18}-ACID.

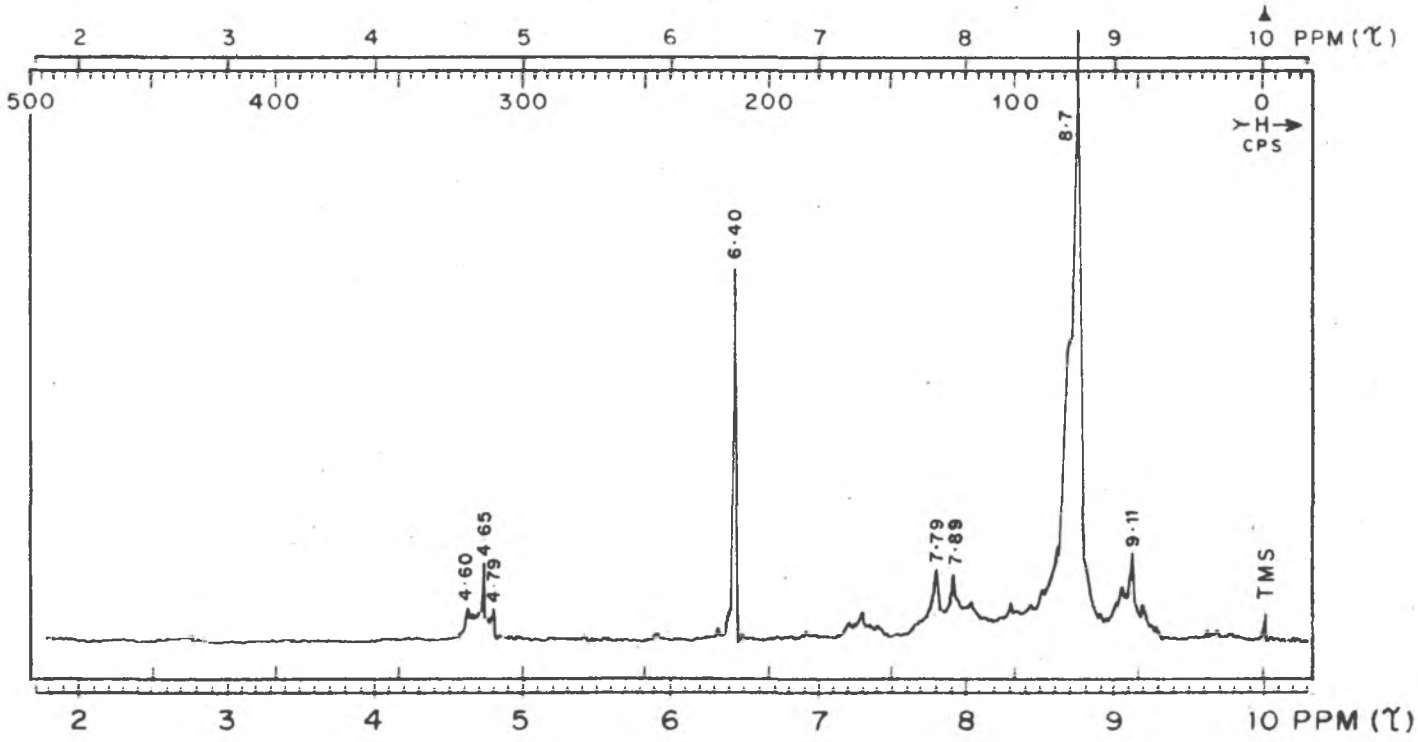


FIG. 8. NMR SPECTRUM OF METHYL ESTER OF C_{18}-ACID

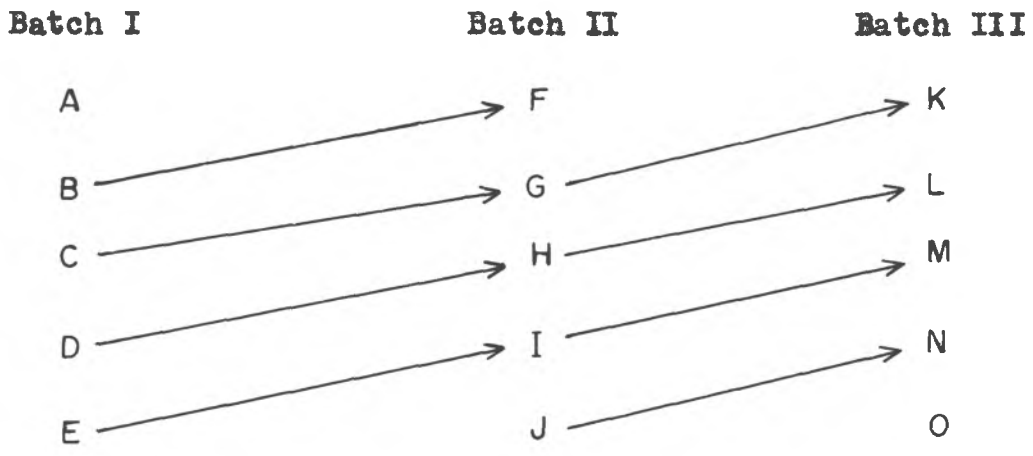
E X P E R I M E N T A L

Preparation of partially delactonised oil from Punjab costus roots

The costus root oil was extracted from the Punjab costus roots, by the solvent extraction procedure using pet.ether (40-60°) at room temperature by employing counter current extraction principle. For this purpose, three consecutive batches each of 57.1 kg were taken together to form one lot.

Finely powdered costus roots (57.1 kg) were charged in a narrow mouthed stainless steel tank of adequate size, fitted with a flase bottom, a tap for drawing extract and a powerful spark proof vertical motor stirrer. Pet.ether (40-60°) 80 L., was then added to the root powder and the mixture stirred for 2 1/2 hr. It was then allowed to settle for 15 min. and drawn through the tap. The extract (A) thus obtained was taken for concentration at 40°. The residual plant pulp was then extracted similarly four times with pet.ether, taking 28 L each time, to get the extracts B, C, D and E. These extracts namely B, C, D and E, along with required quantities of pet.ether, were used, to extract a second batch of costus root powder (57.1 kg) similarly to get the extracts F, G, H and I. The extract J was then

taken by adding 28 L of fresh pet.ether. Of these, the extract F was taken for concentration and the remaining namely G. H, I and J, along with the required quantity of pet.ether were used for the extraction of the 3rd batch to get the extracts K, L, M and N. A fresh extract O was then taken by adding 28 L pet.ether. The total extracts namely A, F, K, L, M, N and O were then taken together and concentrated in a double jacketted stainless steel distillation vessel under vacuum at a bath temperature not exceeding 40 ± 2°, to get the total concentrate (about 7300 ml of about 800 g/lit. concentration). The scheme of extraction is shown below.



Delactonisation of the oil

The concentrated extract thus obtained was then kept at 0° for three days, when the crystals separated out 565 g., $(\alpha)_D$ + 117° , showing it to be almost pure costunolide .

The concentrate was then kept for another three days at 0° and the lactones removed . The extract was then kept at -20° for 15 days, when more lactones separated out (1493 g), $(\alpha)_D$ + 30.77°.

When no more lactones separated at -20° the extract was then subjected to charcoal treatment to remove the colouring material and other impurities. The extract in the form of pet.ether solution was treated with activated charcoal (5%, 257 g) and after filling in bottles, was shaken in a shaking machine for 3 hr. It was filtered using a filter paper and the solvent removed under reduced pressure. The last traces of solvent was removed under reduced pressure of 10 mm. at 40 $\pm$ 2°, when costus root oil (2.57 kg) was obtained (yield about 1.5%).

Removal of free acids from the delactonised costus root oil

Delactonised costus root oil (500 g) from normal commercial roots (acid value 23.17) was taken in a separating funnel and alcoholic potassium hydroxide (15 g, in 250 ml of alcohol;calculated quantity 11.6 g) was added. The homogeneous mixture was vigorously shaken for a short time and ether(2.5 l) was then added. After vigorous shaking the ether solution was extracted three times with sodium chloride solution (5%) (3 X 1 L). The ether layer was separated, dried over anhydrous Na_2SO_4. One more batch of the oil was similarly treated.

From the combined ether extracts of both the batches, ether was removed at 40° under reduced pressure till the vacuum was 10 mm., when the neutral portion of the oil (920 g) was obtained. Part of this oil (300 g) was subjected to chromatography as shown in Table 1.

The combined aqueous alkaline extract from the above experiment was then acidified with ice cold dilute sulphuric acid and extracted with ether (2 times). The ethereal layer was washed twice with water and dried over anhydrous sodium sulphate. Ether was then removed under reduced pressure (10 mm) at a bath temperature of 40 ± 2° and the acidic part (53 g) was obtained.

Manali costus root oil (neutral) 300 g was chromatographed on alumina (gr.II; 6 kg) and eluted as follows:

T A B L E No.1

No.	Solvent	Volume (lit.)	Weight (g)	Fr. Nos. in Chart I.
1	Pet.ether	15	60	A
2	Pet.ether-benzene (3:1)	10	25	B
3	Pet.ether-benzene (1:1)	15	15	C
4	Chloroform	12	59	D
5	Ether	10	33	E
6	Methanol	10	35	F
7	Material held up in the column		73	

The pet.ether-benzene (3:1) fraction (B) which was found to be rich in ketones as indicated by its IR spectrum was taken for its critical examination.

Saponification of fraction (B)

The fraction (B) (25 g) was saponified by alcoholic potassium hydroxide (300 ml; 5%) by refluxing for 12 hr. on a steam bath. Excess of alcohol was removed under suction and the residue diluted with water and extracted with ether several times to remove the non-saponifiable part (B1), which amounted to 12.35 g.

The aqueous layer, thus left after extraction with ether, was acidified and kept at room temperature for 24 hr.(to complete lactonisation). The aqueous layer was then extracted with ether thoroughly. The ether extract was washed free of mineral acids, with water and it was then extracted with Na_2CO_3 solution (aq. 10%) several times to remove free organic acids. The ether extract was washed with water and dried over anhydrous Na_2SO_4 and evaporated to give neutral fraction (5.1 g) (B_3).

The Na_2CO_3 extract obtained above was acidified with dilute HCl (1:1) and extracted with ether. The ether layer was washed free of mineral acid, dried and evaporated to give acid fraction (4.4 g; B_4).

Examination of the saponifiable portions (B_3) and (B_4)

(i) Neutral part (5.1 g) B_3 (see Chart I)

The material (5.1 g) was chromatographed on alumina (gr.III; 130 g) and eluted as follows:

TABLE 2

No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	100	nil
2	Pet.ether-benzene (3:1)	250	1.108
3	Pet.ether-benzene (1:1)	300	0.88
4	Benzene	300	0.58
5	Ether	150	2.15

The fraction(2) on cooling at -18° for 12 hr. gave a solid, which was filtered and purified by crystallisation from methanol to give dehydrocostus lactone (2), m.p. and mixed m.p. 59-60°, $(\alpha)_D$ - 11.2° (c, 1.1).

IR bands at: 1779, 1639, 1264, 1195, 1151, 1136, 1081, 1015, 985, 952, 916, 894 and 819 cm^{-1}.

Analysis

Found: C, 78.47; H, 8.12.

$C_{15}H_{18}O_2$ requires: C, 78.23; H, 7.88%.

Isolation of dihydrodehydrocostus lactone

The combined fraction (2.9 g) from fractions 3, 4 and 5 (Table 2) was dissolved in ethanol (25 ml) and treated with liquor ammonia (15 ml). The mixture was kept at -18° for 24 hr. It was then diluted with water and worked up in the usual way to give a liquid product (2.1 g).

A solution of picric acid (1.1 g) in ethyl alcohol (50 ml) was added to the alcoholic solution of the ammonia adduct (2.1 g in 15 ml). The yellow solid which separated out was found to be picrate of the ammonia adduct of dehydrocostus lactone from its m.p. and mixed m.p. The mother liquor was diluted with water and extracted with ether. The ether layer was washed with NaOH solution to remove excess of picric acid. The ether extract was made free of alkali by washing repeatedly with water, dried over anhydrous Na_2SO_4 and ether evaporated to give a lactone mixture (1.7 g).

The mixture (1.7 g) was chromatographed on alumina (gr.III; 54 g) and eluted as follows:

TABLE 3

No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	150	0.056
2	Pet.ether-benzene (3:1)	200	0.73
3	Benzene	200	0.42
4	Ether	150	0.17

TLC analysis showed that the fractions 2 and 3 were identical and therefore mixed together. Comparative TLC of this combined fraction with that of dihydro-dehydrocostus lactone prepared from dehydrocostus lactone by $NaBH_4$ reduction, indicated that it is a mixture of C_{11} epimeric dihydrodehydrocostus lactones. The IR spectrum was identical with that of dihydrodehydrocostus lactone prepared from dehydrocostus lactone, b.p. 175-180°(bath)/ 0.5 mm., n_D^{25} 1.5245; $(\alpha)_D$ + 69.82° (c, 2.0).

IR bands at: 1770, 1639, 1449, 1205, 1175, 1117, 1010, 990 and 892 cm^{-1}.

Analysis

Found: C, 76.98; H, 8.69.

$C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.68%.

Thus the neutral part B_3 mainly consists of

(i) Dehydrocostus lactone (2), and

(ii) C_{11} epimeric dihydrodehydrocostus lactones(5).

Examination of acid part B_4

The mixture of acids obtained above was converted into a mixture of corresponding esters by diazomethane. The methyl esters (1.0 g) were chromatographed on alumina (gr.II, 20 g) and eluted as follows:

TABLE 4

No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	60	0.37
2	Benzene	120	0.20
3	Ether	70	0.097
4	Methanol	85	0.087

The fraction (1) was found to be TLC pure and showed the following properties: b.p. 160-62° (bath)/0.45 mm., n_D^{21} 1.4540; $(\alpha)_D \pm 0°$.

IR bands at: 1750, 1470, 1448, 1365, 1250, 1208, 1180, 1010, 890, 850 and 729 cm^{-1}.

Analysis

Found: C, 77.18; H, 11.84.

$C_{19}H_{36}O_2$ requires: C, 76.97; H, 12.24%.

The spectral data and the elemental analysis probably indicated it to be methyl oleate (6).

Examination of the non-saponifiable part (B_1)

The fraction B_1 (12.3 g) was chromatographed on alumina (gr.III; 620 g) and eluted as follows:

TABLE 5

No.	Solvent	Volume (ml)	Weight (g)	Fr. Nos. in Chart I
1	Pet.ether	5500	2.73	B_5
2	Pet.ether-benzene (3:1)	1600	1.56	B_6
3	Pet.ether-benzene (1:1)	1200	0.87	B_7
4	Benzene	1500	2.70	B_8
5	Ether	1200	2.33	B_9
6	Methanol	1000	0.49	B_{10}

The fractions (B_5) and (B_6) were identical in TLC pattern. The fraction B_6 was chilled for 12 hr when fine needles separated out, m.p. 248°. The solid was further crystallised from pet.ether to a constant m.p. 252°, $(\alpha)_D$ - 25° (c, 0.8), mixed m.p. with friedlein 230°.

IR bands at: 1718, 1462, 1389, 1379, 943, 917, 862, 840, 833, 781 and 724 cm^{-1}.

UV spectrum $\varepsilon_{210,450}$.

Analysis

Found: C, 84.33; H, 11.87.

$C_{30}H_{50}O$ requires: C, 84.44; H, 11.81%.

Further characterisation of this ketone could not be done due to the paucity of material.

The mother liquor of fraction (B_6) was mixed with fraction B_5 and the total fraction (3.7 g) was chromatographed on silica gel (95 g) and eluted as follows:

TABLE 6

No.	Solvent	Volume (ml)	Weight (g)	Fr. Nos. in Chart II
1	Pet. ether	300	0.16	B_{11}
2	Pet.ether-benzene(1:1)	450	0.39	B_{12}
3	Benzene	450	1.92	B_{13}
4	Ether	300	1.17	B_{14}

Fraction (B_{11}) was TLC pure and its IR spectrum did not indicate the presence of any carbonyl or hydroxyl absorption. Its elemental analysis, however, showed it to be an oxygen containing compound, from which it was considered it to be an oxide.

The fraction B_{12} was found to be a complex mixture of four to five compounds (by TLC) and was not examined further.

Isolation of caryophyllene monoxide (4)

The fraction B_{13} (1.92 g) was chromatographed further on silver nitrate-impregnated silica gel (60 g) and eluted as follows:

T A B L E 7

No.	Solvent	Volume (ml)	Weight (g)	Fr.Nos. in Chart II
1	Pet. ether	500	-	-
2	Pet.ether-benzene (1:1)	450	0.38	B_{15}
3	Benzene	550	0.16	B16
4	Ether	300	0.86	B17

The fraction (B_{15}) was identical with fraction (B_{16}) in TLC pattern, but contained a minor compound as impurity. The fraction B16 which was TLC pure was distilled and the distillate on cooling gave a solid which was purified by crystallisation from pet.ether and showed the following properties: m.p. 60-61°, $(\alpha)_D$ - 74° (c, 1.1).

IR bands at: 2900, 1650, 1470, 1390, 1289, 1270, 1179, 1130, 1100, 1089, 1070, 970, 945, 920, 899, 875, 856, 831, 810 and 770 cm^{-1}.

Analysis

Found: C, 81.81; H, 10.85.

$C_{15}H_{24}O$ requires: C, 81.76; H, 10.98%.

Mixed m.p. with an authentic sample did not show any depression confirming that the compound is a caryophyllene monoxide (4).

Isolation of a C_{14}-ketone

The fraction B_{17} was identical with the fraction B_{14} (TLC). After mixing, the mixture (1.9 g) was purified by chromatography over silica gel. The pet.ether eluted fraction (0.52 g) was found to be pure and the IR spectrum (Fig.3) showed it to be a ketone (1715 cm^{-1}). It showed the following properties: b.p. 125-130°(bath)/0.15 mm., n_D^{25} 1.4670, $(\alpha)_D \pm 0°$.

UV spectrum: ε_{210},4667.

IR bands at: 1715, 1653, 1379, 1370, 1170 and 893 cm-1.

Analysis

Found: C, 78.50; H, 11.05.

$C_{14}H_{24}O$ requires: C, 80.71; H, 11.61%.

Molecular weight (Rast's method)

Found: 229; required($C_{14}H_{24}O$) 208.

$NaBH_4$ reduction of the ketone

The ketone (0.076 g) was dissolved in methanol (20 ml) and $NaBH_4$ (0.049 g) was added in instalments during a period of 15 min. The reaction mixture was kept at room temp. for 30 hr. and then worked up to give a liquid material (0.080 g) which was chromatographed over alumina (2 g) and eluted with pet.ether and benzene. The

benzene fraction gave pure alcohol (TLC) (0.0478 g) and further purified by distillation and showed the following properties: b.p. 125-130° (bath)/0.45 mm., n_D^{28} 1.4591; $(\alpha)_D \pm 0°$.

IR bands at: 3400, 2996, 1653, 1465, 1385, 1070, 995, 941, 898 and 840 cm^{-1}.

Analysis

Found: C, 79.58; H, 12.11.

$C_{14}H_{26}O$ requires: C, 79.93; H, 12.11%.

Isolation of the alcohols, m.p.230° and 115-120°

The benzene fraction B8 (2.7 g) was dissolved in pet.ether and kept for 24 hr. at -18°. A solid separated out which was crystallised with pet.ether to give a solid (0.15 g), m.p.230°. This showed on TLC analysis two spots (silver nitrate impregnated silica gel plate). The two spots were observed quite close to one another. The compound showed the following properties, m.p.230°, $(\alpha)_D$ + 15.53° (c, 1.1).

UV spectrum: ε_{210} 6056.

IR bands at: 3030, 3018, 1640, 1035, 877 and 820 cm^{-1}.

Analysis

Found: C, 83.30; H, 11.77.

$C_{30}H_{52}O$ requires: C, 84.04; H, 12.22%.

The mother liquors left after removal of the solid was chromatographed on alumina (gr.III; 60 g) and eluted as follows:

TABLE 8

No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	250	-
2	Pet.ether-benzene (3:1)	300	0.513
3	Pet.ether-benzene (1:1)	350	0.816
4	Benzene	250	0.361
5	Ether	200	0.091

The fractions 2 and 3 on cooling gave a solid which on crystallisation showed following properties: m.p. 115-120°; $(\alpha)_D$ + 48.60° (c, 1.07).

UV spectrum: $\varepsilon_{210,3229}$.

Analysis

Found: C, 81.51; H, 11.53.

$C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%.

Isolation of β-sitosterol

Fraction B9 was dissolved in methyl alcohol and cooled at -18° for a day, when some solid material separated out. This was filtered and crystallised twice from methanol to get a white crystalline solid, m.p. 135-36°, $(\alpha)_D$ - 37° (c, 1.2). It was identified as β-sitosterol by IR spectrum and mixed m.p. determination with an authentic sample.

IR bands at: 3448, 1667, 1639, 1460, 1372, 1064, 1080, 961, 840, 800 cm^{-1}.

Analysis

Found: C, 83.45; H, 11.85.

$C_{29}H_{50}O$ requires: C, 83.99; H, 12.15%.

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PART II

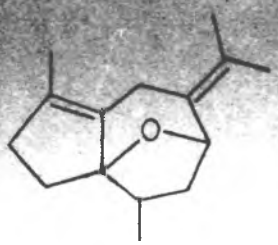
SYNTHESIS OF OPTICALLY ACTIVE GERMACRANE DERIVATIVES

A B S T R A C T

Optically inactive hexahydrogermacrol (6), hexahydrogermacrone (7) and germacrane (8), previously prepared from the naturally occurring α,β- unsaturated monocyclic ketone, germacrone (3), isolated from Bulgarian zdravets oil, have now been prepared in the optically active form. Both liquid and solid hexahydrocostunolide afforded the same optically active hexahydrogermacrol (16), when subjected to controlled LAH reduction followed by Huang-Minlon reduction. The alcohol on chromic acid oxidation afforded the optically active hexahydrogermacrone (17). The acid (23) obtained due to hydrogenolysis of solid dihydrocostunolide (10) by metal amine reduction, was converted into its methyl ester. This ester when hydrogenated initially in alcohol medium under pressure and subsequently in acetic acid medium furnished an optically active saturated ester (28). This has been reduced into the corresponding alcohol (29), which in turn has been converted into the optically active hydrocarbon germacrane (8b or 8c) by LAH reduction of its tosylate.

The essential oil of Bulgarian zdravets, Geranium macrorhizum L., was found to contain a crystalline solid, m.p. 56-57°, to which the composition $C_{16}H_{24}O$, was given by Weinhaus and Scholz.[1] They considered it to be an alcohol and named it as 'Germacrol'. Later Naves[2] contradicted these results and suggested that the compound is a bicyclic sesquiterpenic oxide with the composition $C_{15}H_{22}O$. Treibs[3] on the basis of degradative experiments assigned to it the structure (1).

During the course of their study on the composition of this oil, Sorm et al.[4] in collaboration with Bulgarian workers studied this compound in detail and showed that it is neither an oxide nor an alcohol, but is in fact an α,β- unsaturated sesquiterpenic ketone with a ten-membered ring. This conclusion was arrived at from the spectroscopic data. The IR spectrum showed a strong band at 1675 cm^{-1}, characteristic of an α,β- unsaturated ketone grouping, which was supported by the presence of a band at 1670 cm^{-1} in its Raman spectrum. In conformity with this, the UV spectrum showed absorption maxima at 242 mμ (log ε, 3.59) at 314 mμ (log ε, 2.69). On the basis of these spectral evidences and chemical reactions, the Czech workers assigned the structure (2), which was later on modified to (3) in order to explain satisfactorily certain reactions.[4A]



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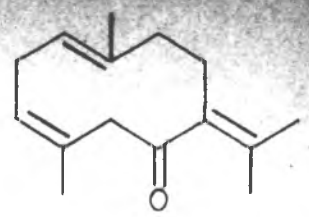

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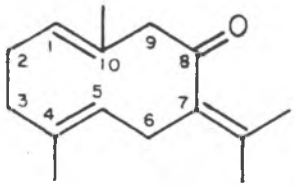
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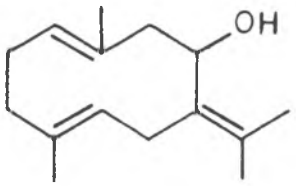
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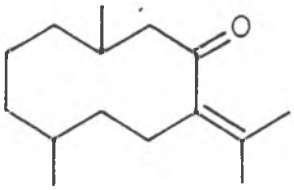
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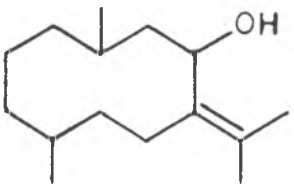
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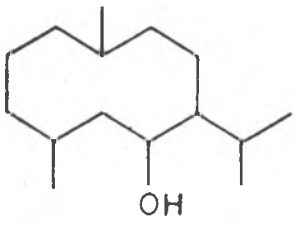
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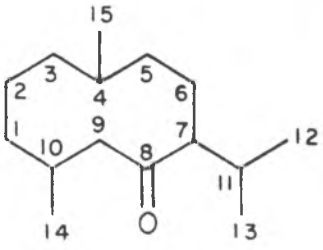
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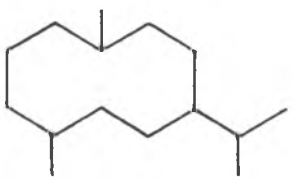

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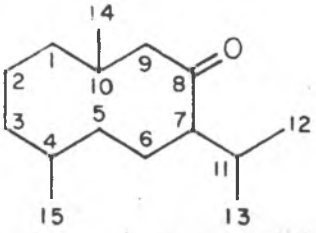
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In another series of experiments Sorm et al. hydrogenated germacrone to give hexahydrogermacrone (7), which was converted to hexahydrogermacrol (6) by LAH reduction. Dehydration of 6 with $KHSO_4$, followed by catalytic hydrogenation gave the saturated hydrocarbon germacrane (8). Alternatively, germacrone (3) was partially hydrogenated to tetrahydrogermacrone (5), which on reduction with LAH gave tetrahydrogermacrol (5a). Hydrogenation of this alcohol gave the hexahydrogermacrol (6) and the germacrane (8).

All these saturated compounds namely hexahydrogermacrol, hexahydrogermacrone, and germacrane obtained from natural germacrone showed no optical activity, inspite of the fact that they possess three to four asymmetric centres. Germacrane was later on synthesised by Sorm et al.[5] and also by Martin Smith[6] from aristolactone. Both these samples have been shown by GLC analysis, to consist of three stereoisomers. It was, therefore, felt desirable to synthesise these compounds in optically pure form. The crystalline lactone costunolide (9) (NR spectrum is shown in Fig. 1), isolated from costus root oil was considered to be suitable starting material for these compounds.

Costunolide was hydrogenated in alcohol medium under pressure to give liquid hexahydrocostunolide. The

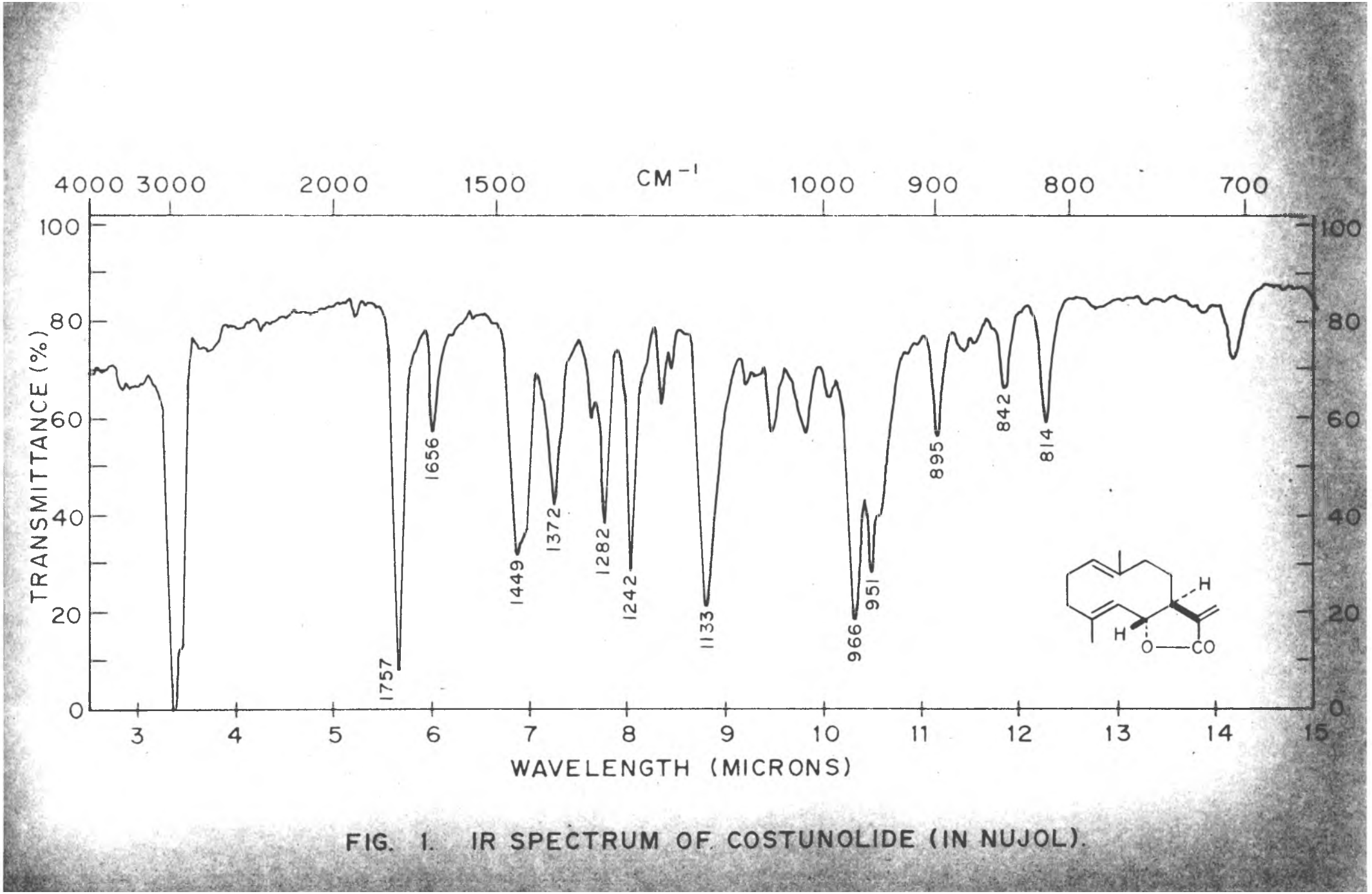
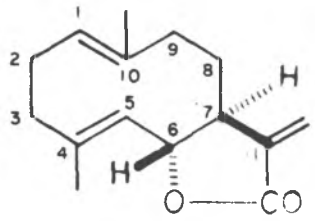


FIG. 1. IR SPECTRUM OF COSTUNOLIDE (IN NUJOL).



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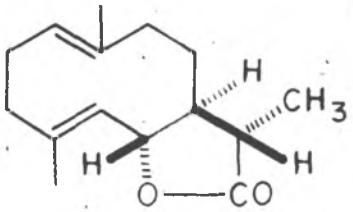

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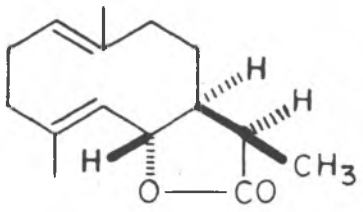

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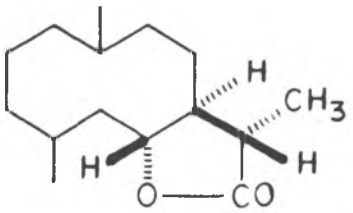

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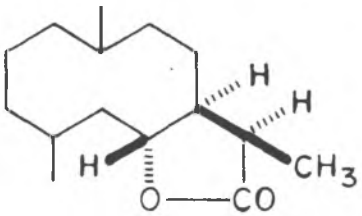

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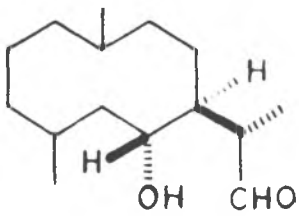

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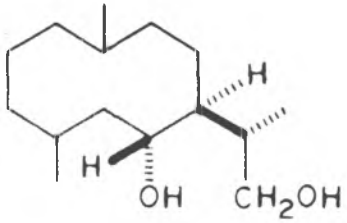

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distilled sample of hexahydrocostunolide on GLC analysis on silicon column at 250° indicated the presence of two components in almost equal proportions. The partial hydrogenation of costunolide is known to give[7] two dihydrocostunolides (10) and (11). One of them 10 is a solid, the structure of which has been decided by converting it to santanolide 'c', m.p. 154-55° of known stereochemistry.[8] It is therefore quite probable that the two hexahydrocostunolides indicated by GLC analysis are the two C_{11}-epimers 12 and 13.

Liquid hexahydrocostunolide was then reduced with lithium aluminium hydride under controlled conditions[9] to give the mixture of the hydroxy aldehyde (14) and the diol (15). The Huang-Minlon reduction of this mixture afforded the optically active saturated monohydric alcohol, $C_{15}H_{30}O$, which was further purified by chromatography to get the optically pure (GLC/TLC) hexahydrogermacrol (16) (IR spectrum has been shown in Fig. 2). The alcohol 16 on oxidation with Jones chromic acid reagent[10] yields the pure (GLC/TLC) optically active ketone, $C_{15}H_{28}O$ (17), identified by IR spectrum (Fig.3) and physical constants as hexahydrogermacrone. In agreement with the structure 17, the NMR spectrum (Fig. 6) shows signals at 9.19, 9.10 and 9.02 τ (12H) due to four methyl groups at C_4, C_{10} and C_{11} and a multiplet at 7.74, 7.71, 7.66, 7.55 and 7.42 τ (3H)

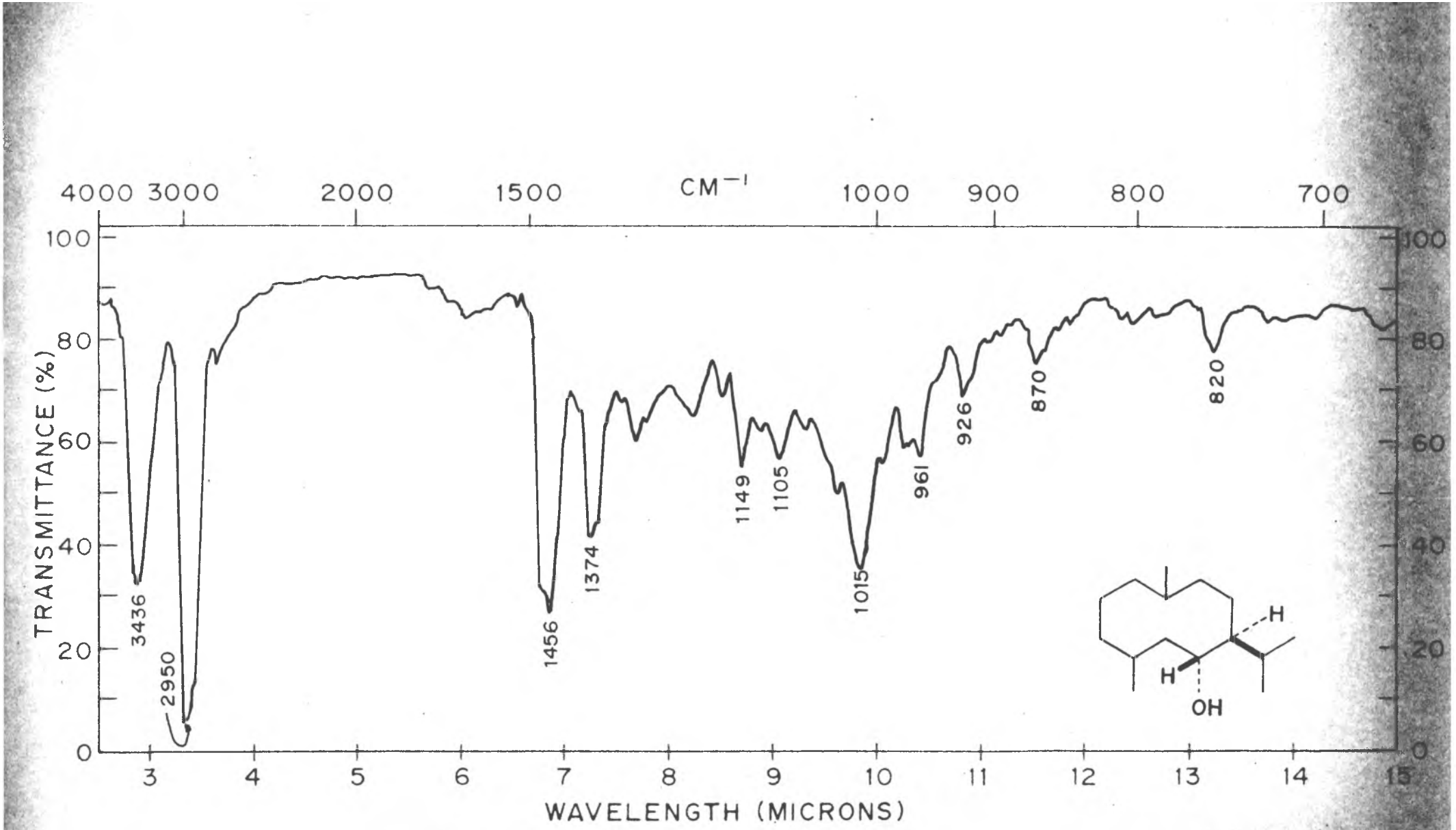


FIG. 2. IR SPECTRUM OF HEXAHYDROGERMACROL (LIQ. FILM).

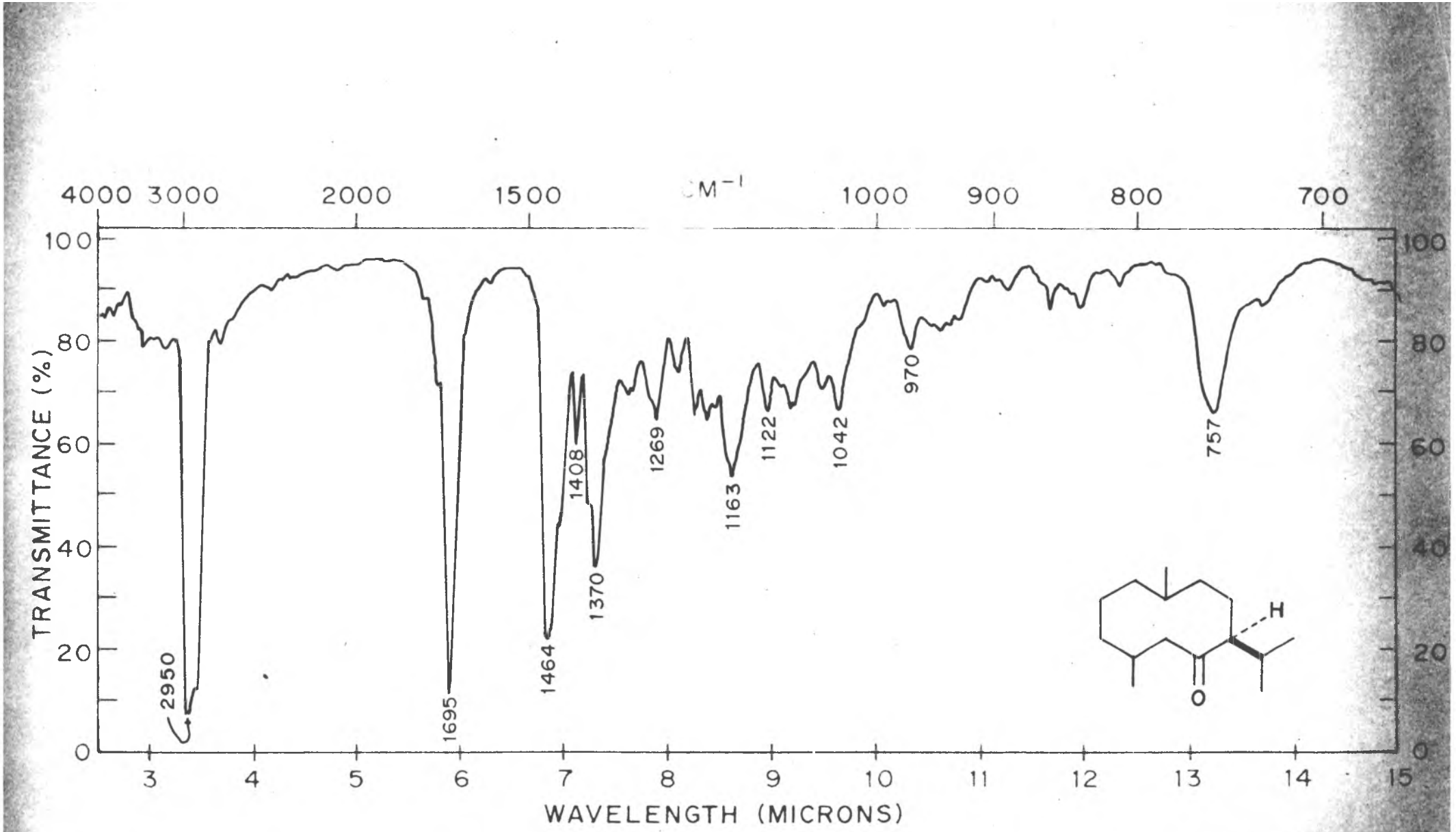
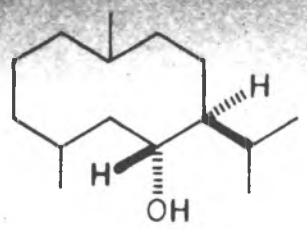


FIG. 3. IR SPECTRUM OF HEXAHYDROGERMACRONE (LIQ. FILM).

due to three protons adjacent to the carbonyl group at C_5 and C_7. We failed to obtain 2,4-DNP derivative as well as semicarbazone of this ketone. An attempt to obtain germacrane by direct Huang-Minlon reduction of the ketone 17 did not succeed.

Dehydration of the alcohol 16 with $KHSO_4$ gives a mixture of two hydrocarbons ($C_{15}H_{28}$) 18 and 19 in which 19 predominates as indicated by IR (Fig.4) and NMR spectra. The mixture on catalytic hydrogenation gives the pure, saturated, optically inactive hydrocarbon 8, which analysed correctly for $C_{15}H_{30}$. From its physical constants and IR spectrum (Fig. 5), it was identified[11] as germacrane. Its NMR spectrum has been shown in Fig. 6.

The optical inactivity of the germacrane thus obtained is possibly due to the presence of a plane of symmetry in the molecule drawn across C_2 and C_7 , provided that the dispositionsof the methyl groups, at C_4 and C_{10} are cis-symmetrical with respect to the plane. The tosyl derivative of hexahydrogermacrol (16) was also prepared, which was then reduced with LAH to afford a mixture of unsaturated hydrocarbons of composition $C_{15}H_{28}$. The IR spectrum of this mixture resembled that of the $KHSO_4$-dehydration product 18 and 19 (Fig. 4). The saturated hydrocarbon germacrane was obtained by catalytic hydrogenation of this mixture. The germacranes thus obtained by two different paths were identical in all respects.



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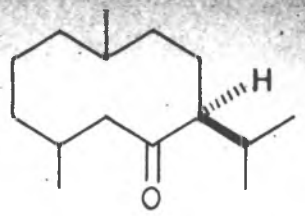

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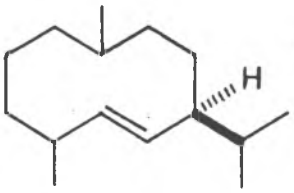

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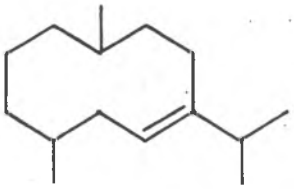

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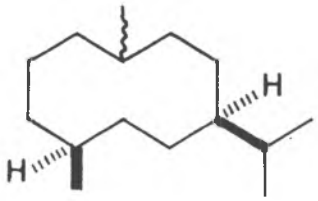

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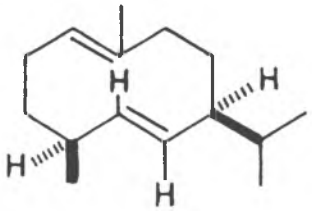

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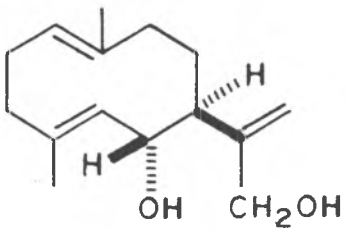

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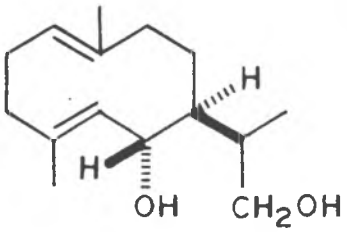

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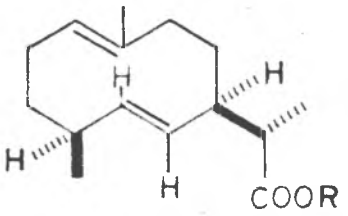

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R = CH_3

23

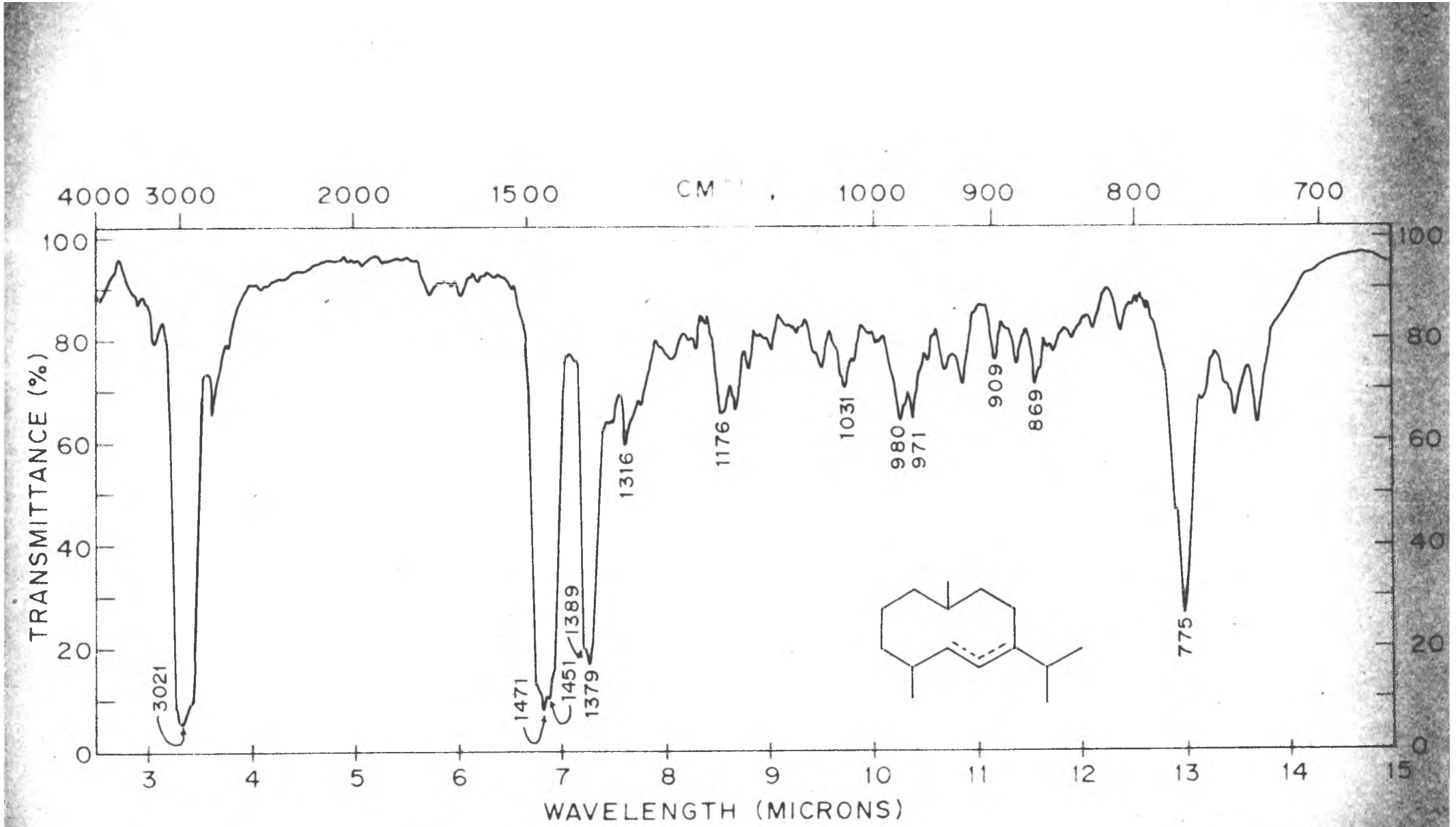

FIG. 4. DEHYDRATION PRODUCT OF HEXAHYDROGERMACROL (LIQ. CELL 0·05 mm)

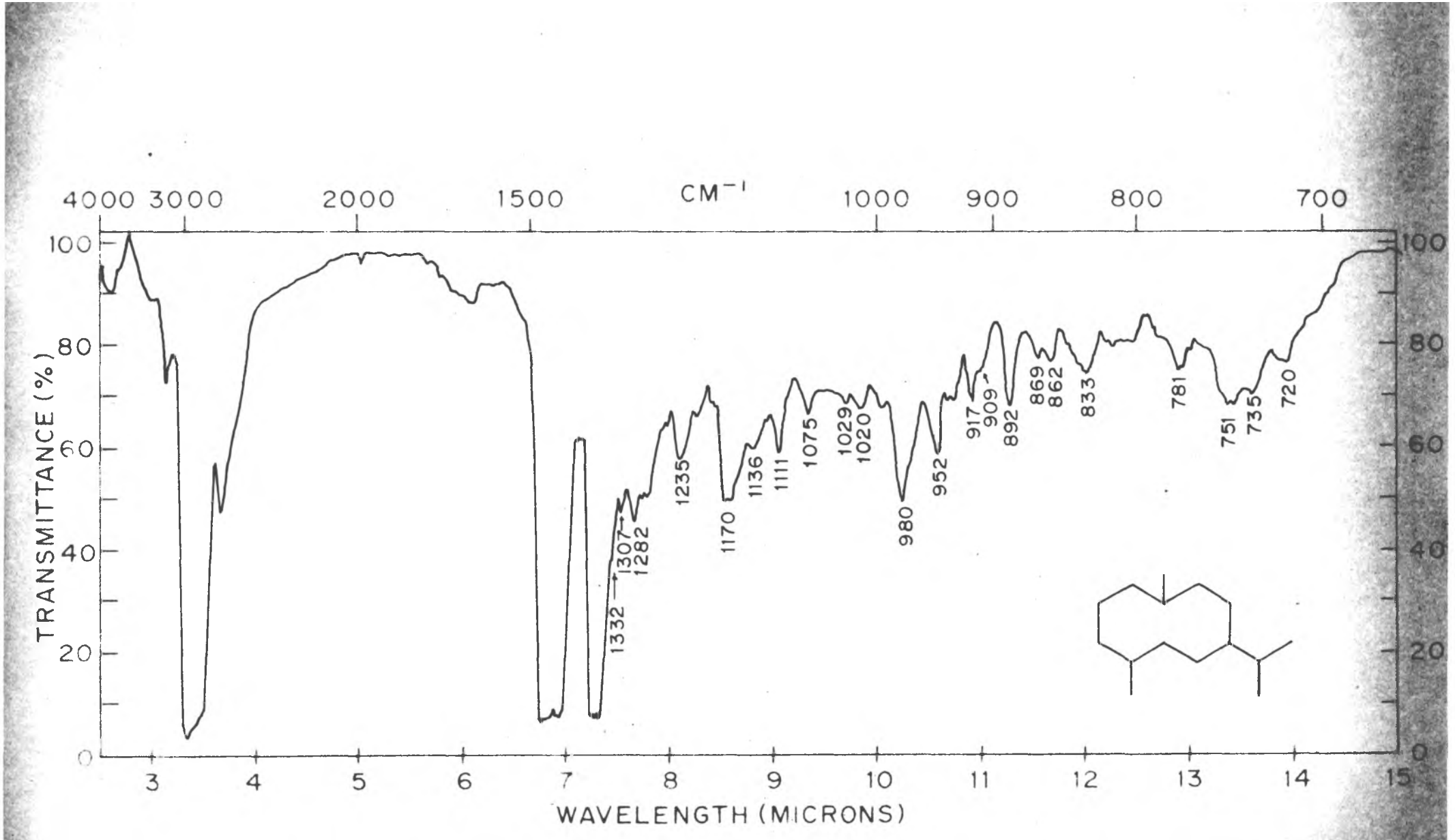

FIG. 5. IR SPECTRUM OF GERMACRANE (LIQ. CELL 0·1 mm.)

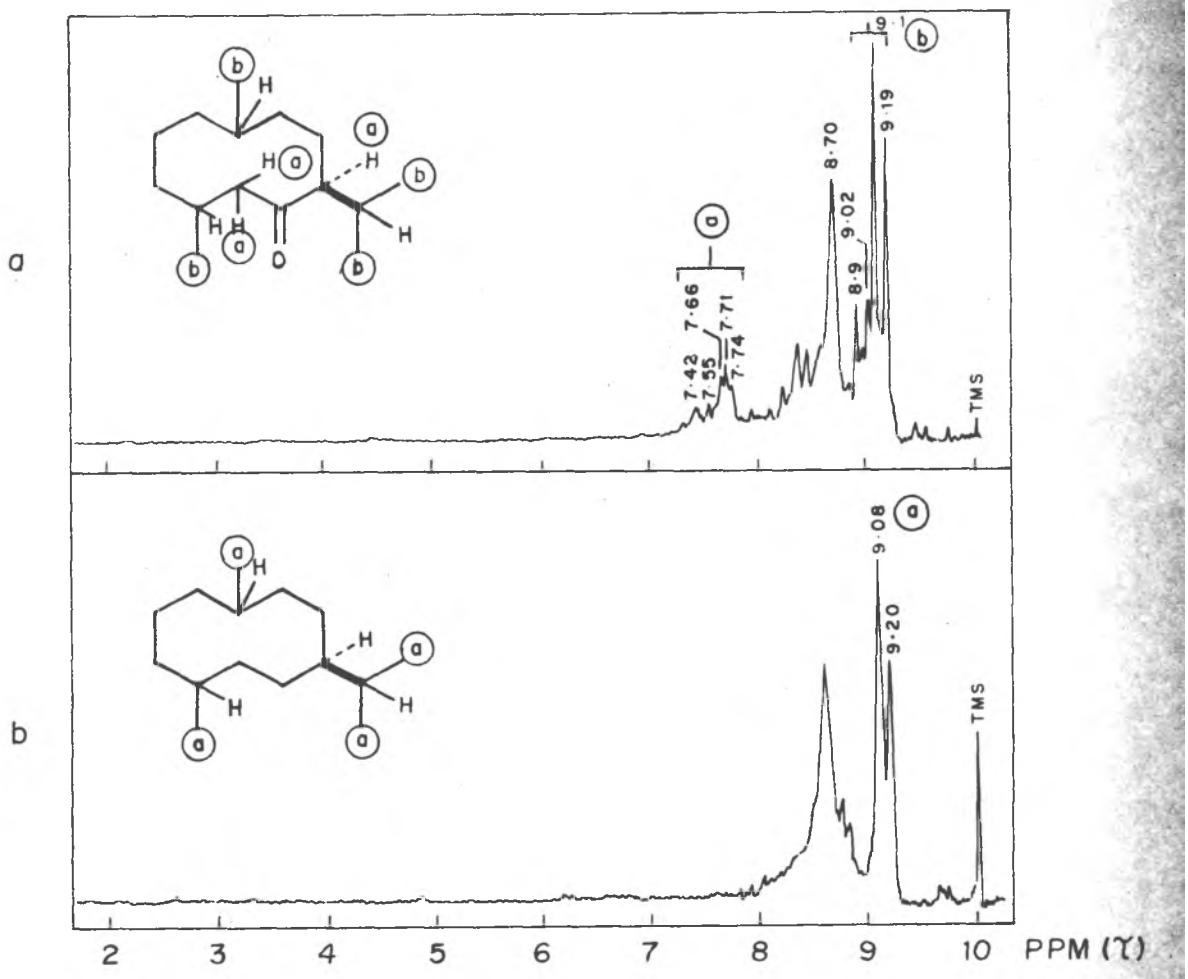

FIG. 6.

(a) NMR SPECTRUM OF HEXAHYDROGERMACRONE.

(b) NMR SPECTRUM OF GERMACRANE.

Subsequently, costunolide (9) was partially hydrogenated in alcohol medium to get the solid dihydrocostunolide (10) (IR spectrum shown in Fig. 7). This solid lactone was then further hydrogenated under pressure to get solid hexahydrocostunolide[12] (12) m.p. 62-63° (IR spectrum shown in Fig. 8). This was subjected to the same series of reactions as liquid hexahydrocostunolide to yield optically active hexahydrogermacrol (16), optically active hexahydrogermacrone (17) and optically inactive germacrane (8). The identity of these compounds obtained by two series of reactions was established by IR spectrum, physical constants and GLC analysis. The following table gives the comparative study of the physical constants of these compounds.

Starting material	Hexahydrogermacrol				Hexahydrogermacrone			
	n	$(\alpha)_D$	Carbon & Hydrogen Found	Calc.	n	$(\alpha)_D$	Carbon & Hydrogen Found	Cal
Liquid hexahydrocostunolide	n^{22} 1.4840	-6.7°	80.05	79.58	n^{24} 1.4726	+83.5°	80.95	80.2
			13.21	13.36			12.70	12.8
Solid hexahydrocostunolide	n^{28} 1.4825	-4.37°	79.80	79.58	n^{28} 1.4720	+83.81°	80.60	80.2
			13.40	13.36			12.63	12.8

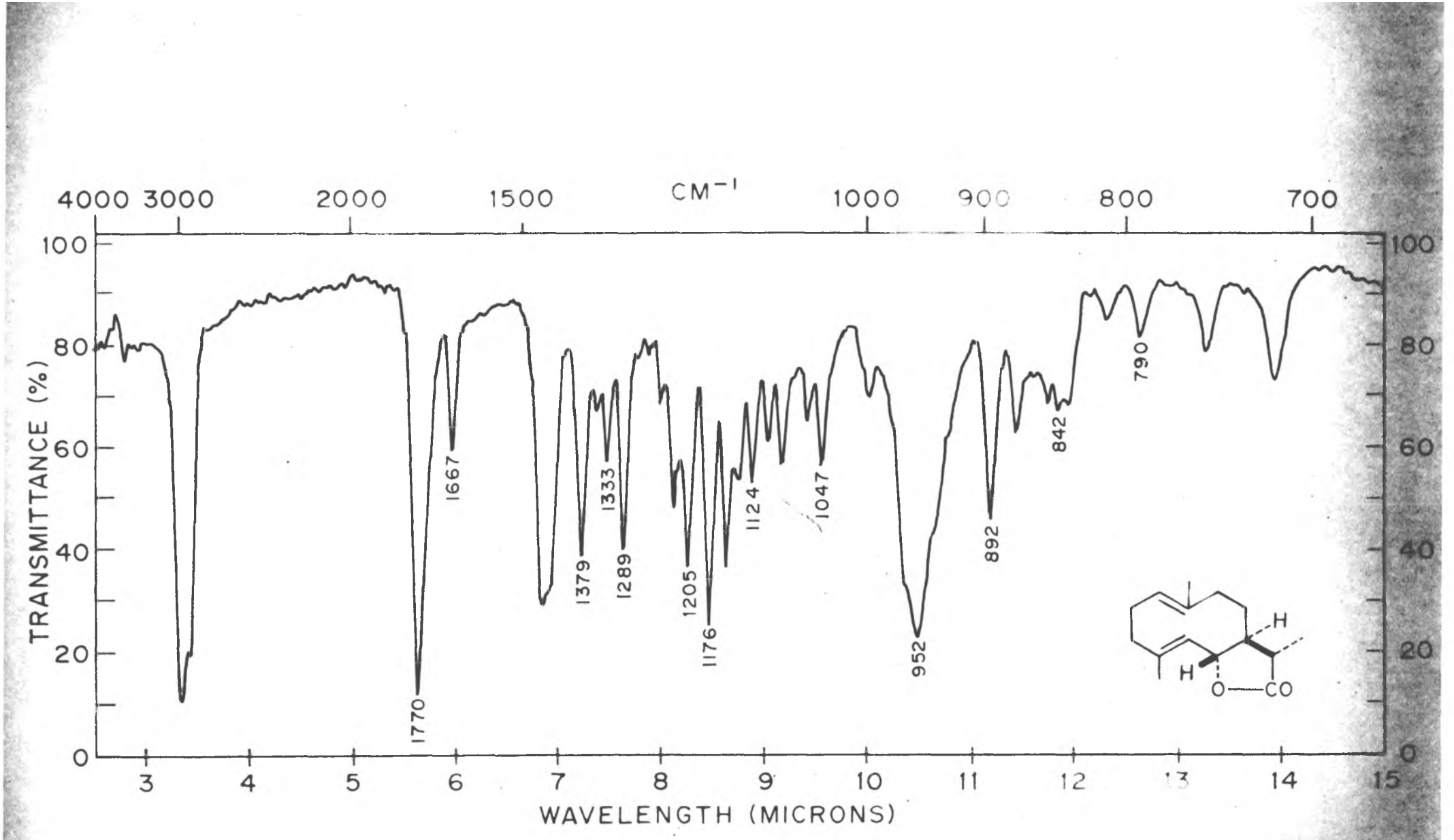

FIG. 7. IR SPECTRUM OF DIHYDROCOSTUNOLIDE (IN NUJOL).

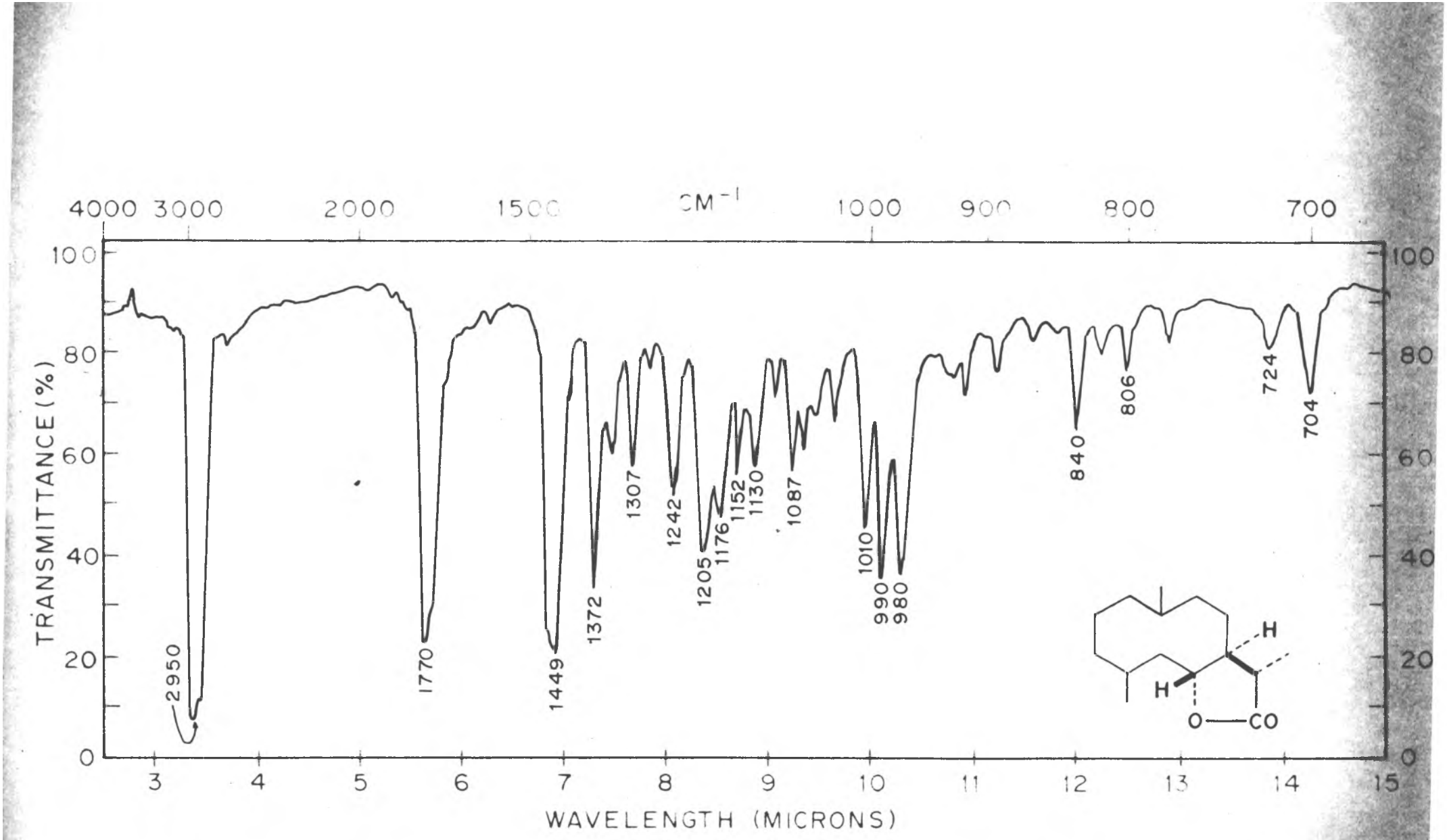

FIG. 8. IR SPECTRUM OF SOLID HEXAHYDROCOSTUNOLIDE (IN NUJOL).

The above conversion of solid hexahydrocostunolide and liquid hexahydrocostunolide to the identical alcohol, ketone and hydrocarbon, proves without any doubt that liquid hexahydrocostunolide is essentially a mixture of two C_{11} epimeric hexahydrocostunolides 12 and 13. Even though, any assumption regarding the disposition of the methyl groups at C_4 and C_{10} cannot be made at this stage, it is clear that the isopropyl group at C_7 in the compounds 16 and 17 is β-oriented.

An attempt was then made to prepare optically active germacrane starting from the hydrocarbon 20 of known stereochemistry.[13] This hydrocarbon can be prepared from costunolide (9) by two routes: (i) 9 was reduced by LAH to the mixture of two diols 21 and 22, which, as such, on metal amine reduction, using sodium and liquid ammonia gave a mixture of the hydrocarbon 20, a secondary alcohol and a primary alcohol, from which the hydrocarbon was separated by column chromatography; (ii) costunolide was partially hydrogenated to solid dihydrocostunolide, which on metal amine reduction using lithium in liquid ammonia furnished an acid 23, characterised through its methyl ester (IR spectrum is shown in Fig. 9). This was converted to the corresponding alcohol 24 by LAH, which in turn was converted to the hydrocarbon 20 by LAH reduction of its tosylate. The structure and stereochemistry of 23 and hence of 20 at C_4

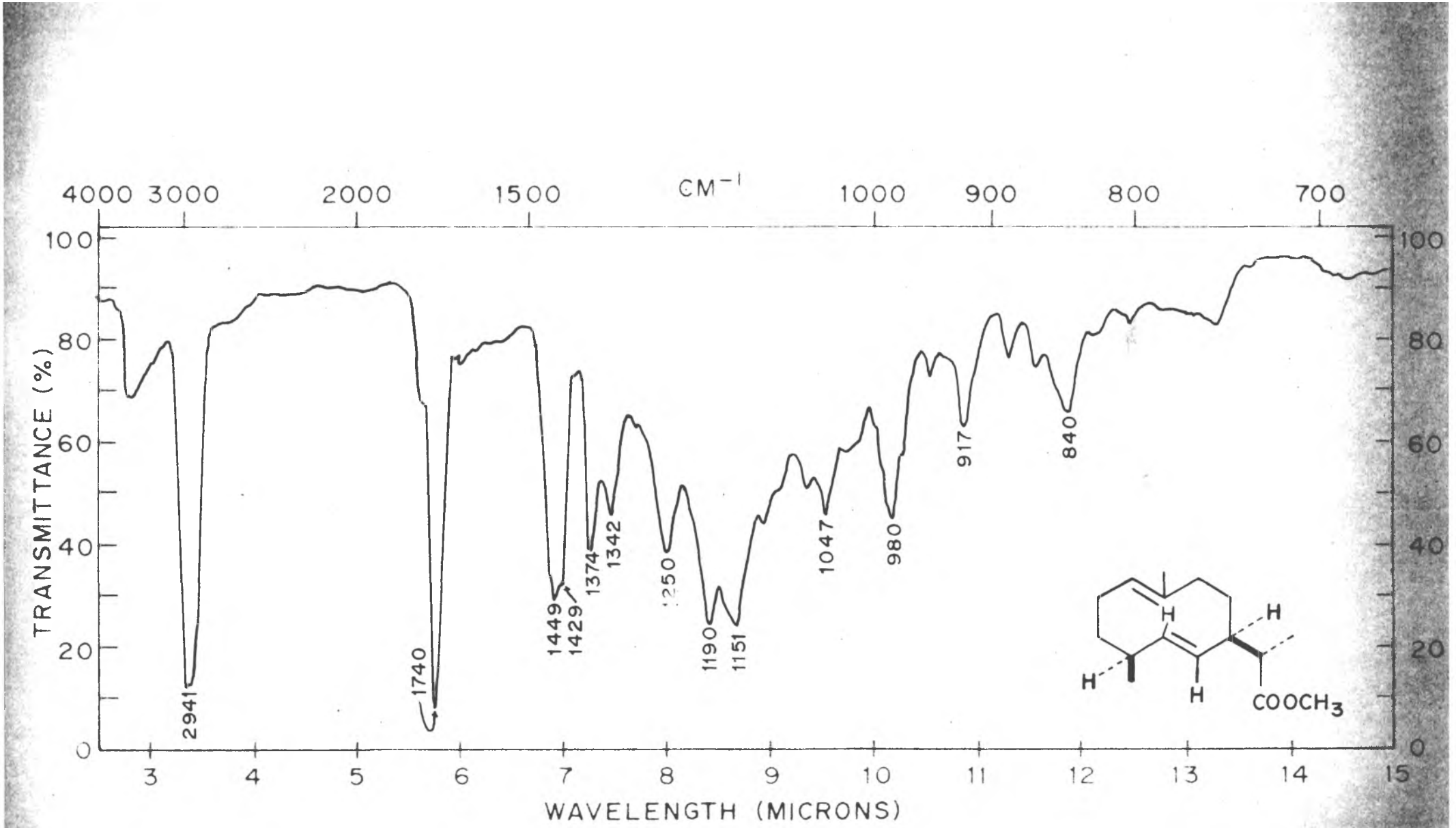

FIG. 9. IR SPECTRUM OF UNSATURATED ESTER (LIQ. FILM).

has been rigorously established by converting the acid 23 to santanolide 'c' (25) by acid catalysed cyclisation and also by isolating S (+)-α-methylglutaric acid from the products of its ozonolysis, which proves the stereochemistry of 23 at C_4.

In order to avoid the transannular acid catalysed cyclisation of the hydrocarbon 20, during hydrogenation, it was initially hydrogenated in ethyl acetate medium using platinum catalyst in order to reduce the trans disubstituted double bond. This, however, could be achieved satisfactorily only when the hydrogenation was carried out under pressure. The crude dihydropreduct(which still contains a little of the original hydrocarbon 20), as such, was then hydrogenated in acetic acid medium to get the completely saturated hydrocarbon $C_{15}H_{30}$, which was found to be 90% pure (GLC). This was identified by IR spectrum as germacrane. The small specific rotation shown by the compound was possibly due to the presence of an impurity, which might have been formed as a result of transannular cyclisation of a small amount of the hydrocarbon 20, occurring in the dihydro product.**

Since the hydrogenation of the hydrocarbon 20 did not proceed properly owing to the slow rate of hydrogenation in ethyl acetate medium, we wanted to prepare the saturated

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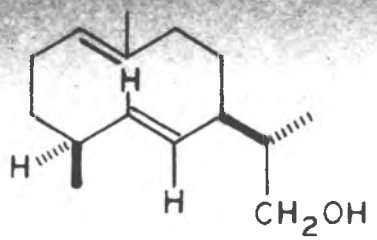
** This part of the work has been carried out by Dr. G.H. Kulkarni and Mr. R.S. Joshi of our laboratory.



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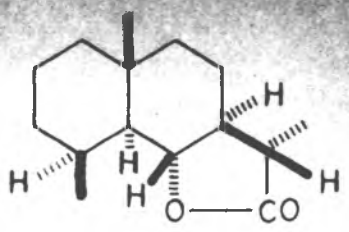

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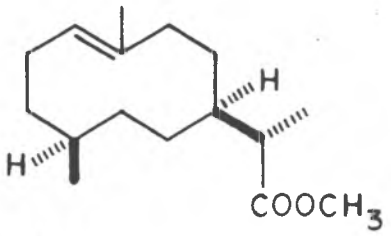

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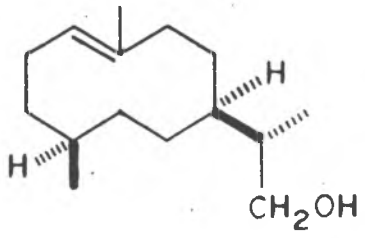

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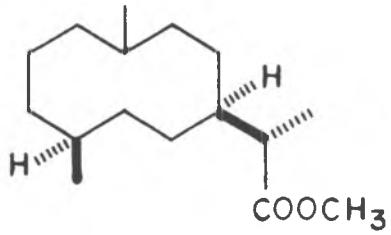

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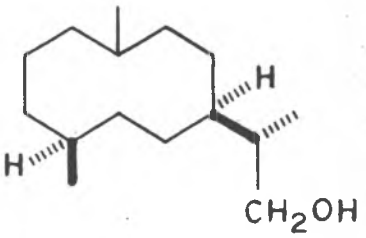

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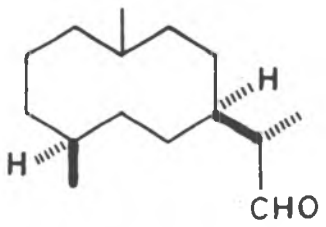

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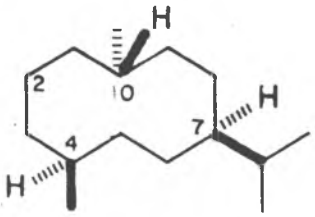

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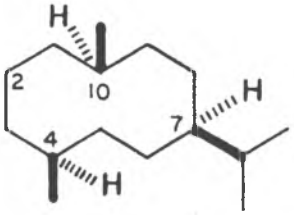

8c

hydrocarbon germacrane, starting from the ester 23, which unlike the hydrocarbon 20, is completely miscible in alcohol. The ester 23 whose stereochemistry at C_4, C_7 and C_{11} is definitely known[13] was hydrogenated under pressure in alcohol medium, initially at the room temperature and then at 50-60° to get a mixture of two esters, which were separated by chromatography. The earlier fraction of the chromatography contained a pure ester (GLC/TLC) (IR spectrum is shown in Fig. 10), which responds to tetranitromethane test for unsaturation, whose NMR spectrum (Fig.11) clearly indicated the presence of a trisubstituted double bond (signal at 4.60τ) and methyl group on a double bond (signal at 8.49τ). This indicates that during pressure hydrogenation only the disubstituted double bond has been hydrogenated and the compound that is obtained is therefore 26. This was converted to the corresponding primary alcohol 27 by LAH reduction. Since the compound 26 contains only one double bond now, the possibility of transannular cyclisation is ruled out during its hydrogenation in acetic acid medium. It was therefore hydrogenated in acetic acid medium using platinum catalyst to get the completely saturated monocyclic ester 28 (IR has been shown in Fig. 12), purified by chromatography and distillation. The ester could be obtained in pure condition (GLC/TLC) and showed rotation of + 23°. The saturated ester was then converted to the corresponding primary alcohol 29 by LAH reduction which

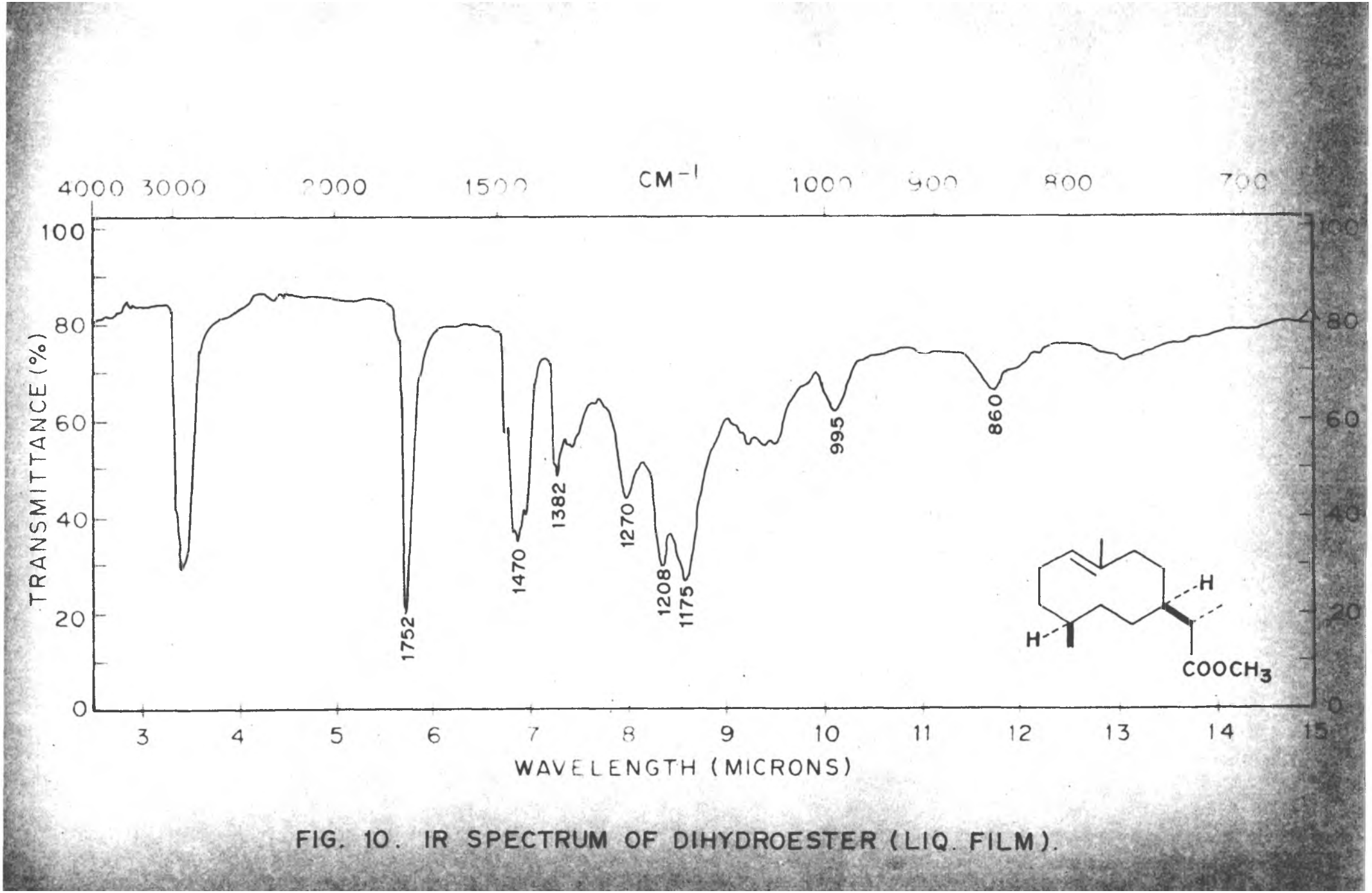

FIG. 10. IR SPECTRUM OF DIHYDROESTER (LIQ. FILM).

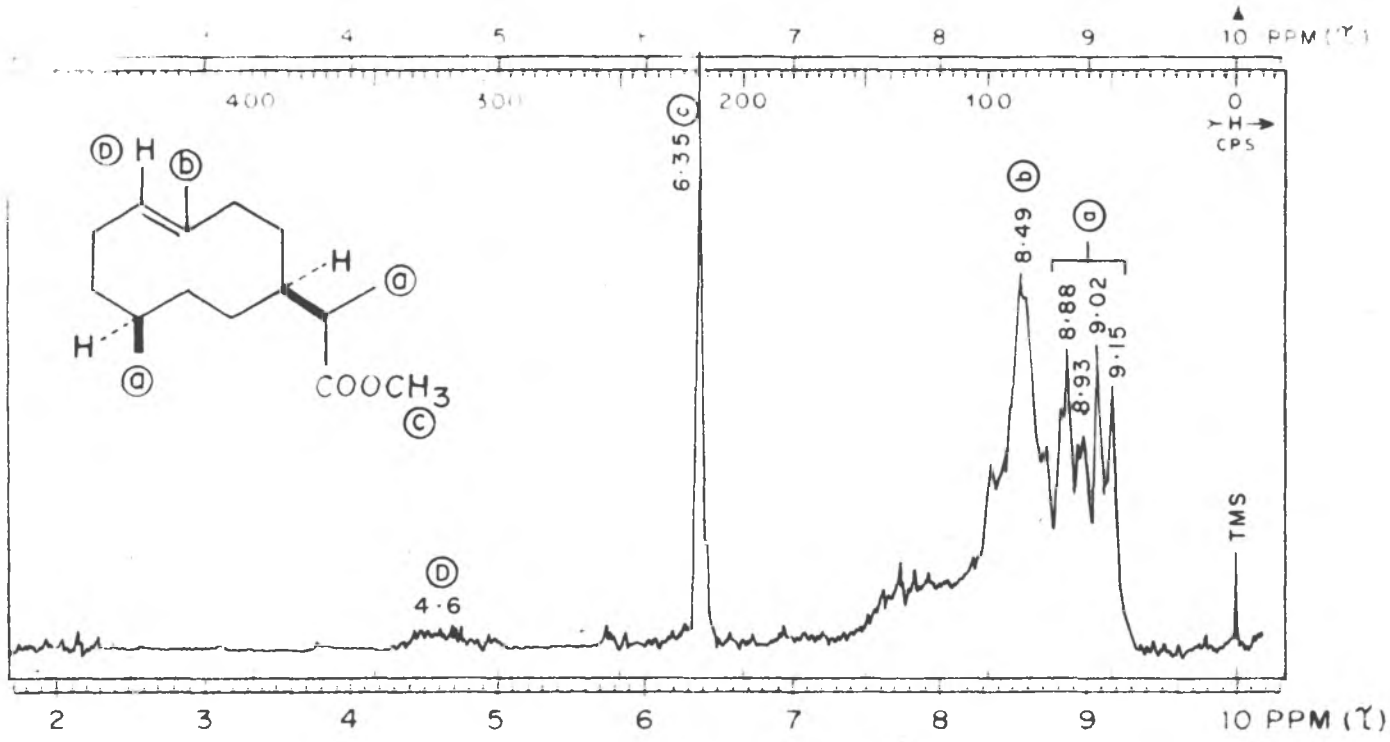

FIG. 11. NMR SPECTRUM OF DIHYDROESTER.

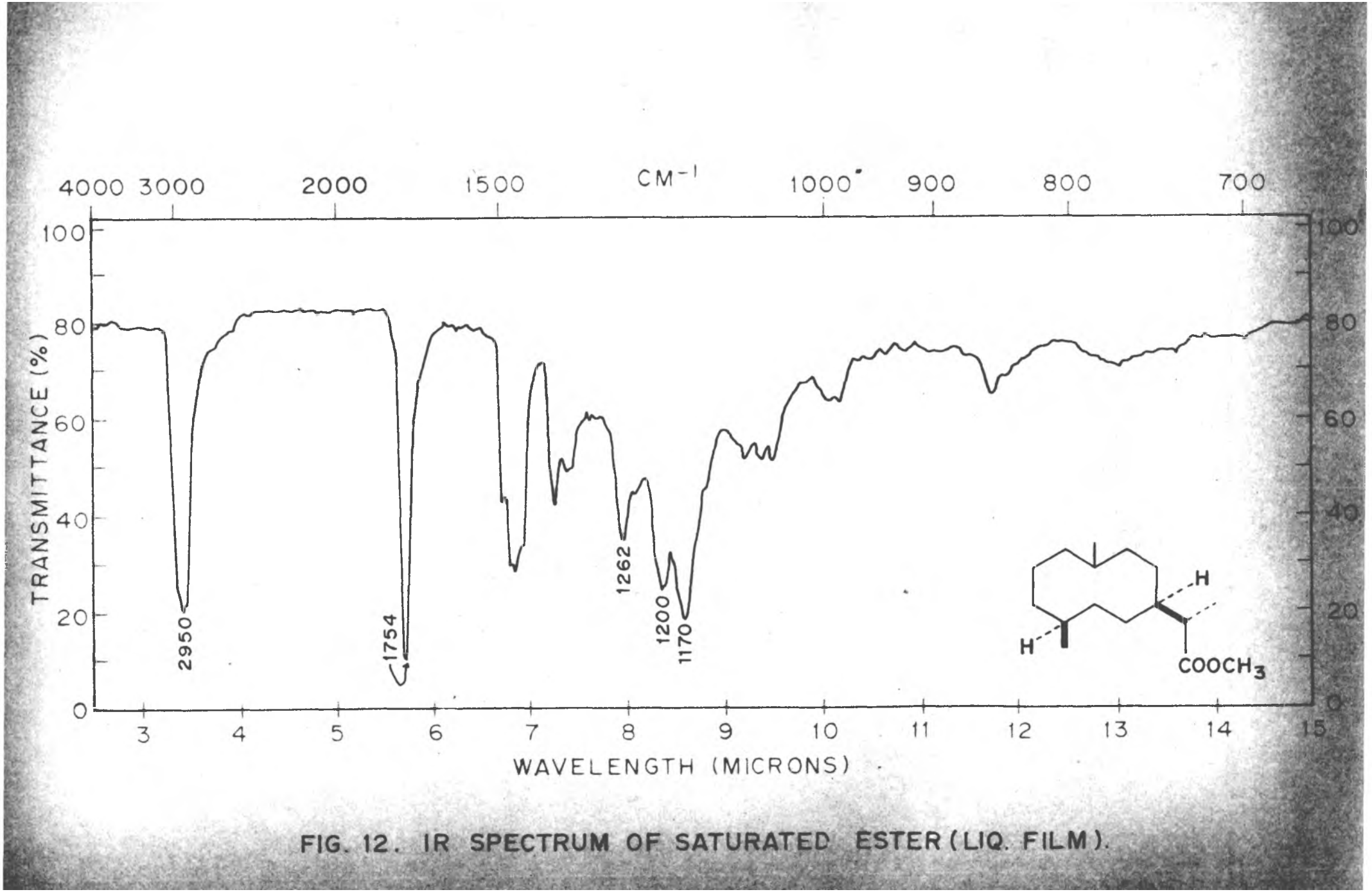

FIG. 12. IR SPECTRUM OF SATURATED ESTER (LIQ. FILM).

showed rotation of + 1.0°. Controlled oxidation of 29 using CrO_3- pyridine gave the saturated aldehyde (30), which was characterised through its semicarbazone, m.p. 149-50°. The alcohol 29 was then converted to the tosylate and the later reduced by LAH to give the hydrocarbon germacrane in pure condition (GLC/TLC). This hydrocarbon showed rotation of + 7.0°. The specific rotation of hydrocarbon can be explained on two different grounds as described below.

It has already been pointed out that the molecule of germacrane contains a plane of symmetry drawn across C_2 and C_7. The preparation of an optically active hydrocarbon is quite in contrast with the existence of such a plane of symmetry. It is quite possible that owing to the non-rigidity of the conformation of a ten-membered ring system, it may be difficult to construct such a plane of symmetry, so that the two portions obtained are exactly the mirror images, even assuming a cis-symmetrical relationship of the methyl groups at C_4 and C_{10}. Alternatively, if the construction of such a plane of symmetry is possible, the optical rotation of the hydrocarbon will definitely indicate that the methyl group at C_{10} is not cis-symmetrical with the methyl group at C_4, which has already been shown to be β-oriented. If the later view is taken as correct, then the methyl group at C_{10} is α-oriented, in which case the hydrocarbon should be represented by the stereoformula(8a).

EXPERIMENTAL

Liquid hexahydrocostunolide

Costunolide (9), m.p. 106-107°; $(\alpha)_D$ + 125° (20 g) dissolved in alcohol (300 ml) was hydrogenated (1000 lb/sq.in.) using platinum oxide catalyst at room temperature (6 hr) and then at 40-50° (6 hr). The alcohol was then removed under suction. The residue after dilution with water was extracted with ether. The ether layer was washed with aq. $NaHCO_3$ solution to remove acid (formed due to hydrogenolysis). The liquid hexahydrocostunolide obtained after removal of ether was purified by distillation (18 g), b.p. 130-35°(bath)/ 0.6 mm., d^{28} 1.007; $(\alpha)_D$ - 25° (c, 1.2); n_D^{28} 1.4880.

Analysis

Found: C, 75.50; H, 11.00.

$C_{16}H_{26}O_2$ requires: C, 75.58; H, 11.00%.

Hydroxy aldehyde (14)(from hexahydrocostunolide

Liquid hexahydrocostunolide (18.0 g) in dry ether (100 ml) was taken in a three necked, round bottom flask, fitted with a dropping funnel, a condenser and a mechanical stirrer. An ethereal solution of LAH (0.75 g in 250 ml) was gradually added under cooling at -10°. The reaction mixture was stirred for 3 hr. at the same temperature and

for another 3 hr. at the room temp. It was then decomposed by alcohol and water and worked up to give a mixture of 14 and 15, containing a little unreacted lactone.

Hexahydrogermacrol (16)

The mixture of 14 and 15 (17.0 g), dissolved in diethylene glycol (150 ml) was taken in a four necked flask fitted with a mercury sealed stirrer, a condenser, a thermometer pocket and an inlet for N_2. Nitrogen was allowed to bubble through and KOH (18.25 g) and hydrazine hydrate (13.0 ml) were introduced. The contents were heated to 200-220° and maintained at that temperature for 4 hr. with stirring. After cooling, the reaction product was diluted with water and extracted with ether. Removal of ether furnished a liquid (13.5 g) which was chromatographed on alumina (gr.II, 700 g) and eluted as follows:

Fr.No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	500	0.72
2	Pet.ether-benzene (2:1)	1000	7.35
3	Benzene	500	0.56
4	Ether	500	1.55

Fraction (2), which was essentially 16, was purified further by chromatography and distillation (GLC/TLC), b.p.120-140°(bath)/0.7 mm., n_D^{22} 1.4840, $(\alpha)_D$ - 6.2° (c, 7.4)*.

IR bands at: 3436, 2950, 1456, 1374, 1149, 1105, 1015, 961, 926 cm^{-1}.

Analysis

Found: C, 80.05; H, 13.21.

$C_{15}H_{30}O$ requires: C, 79.57; H, 13.36%.

The fraction (4) contained the diol 15, which was not critically examined.

Chromic acid oxidation of the monol 16

Hexahydrogermacrol (16) (2.1 g) was dissolved in acetone (20 ml) and Jones reagent was added dropwise until a brown colour persisted. It was kept at the room temperature for 30 min. and then worked up to give the crude ketone, which was purified by chromatography and distillation to give 17 in pure form (GLC/TLC), b.p. 115-120°(bath)/0.9 mm., n_D^{24} 1.4726; $(\alpha)_D$ + 83.5° (c, 3.61).++

IR bands at: 2950, 1695, 1464, 1408, 1370, 1269, 1163, 1122, 1042, 970 cm^{-1}.

Analysis

Found: C, 80.95; H, 12.70.

$C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%.

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* Literature records for hexahydrogermacrol $(\alpha)_D \pm 0°$

++ Literature records for hexahydrogermacrone, n_D^{24} 1.4770; $(\alpha)_D \pm 0°$.

KHSO4-dehydration of the monol 16

Hexahydrogermacrol (16) (1.3 g) was heated with $KHSO_4$ (2.1 g) in an atmosphere of nitrogen at 180° for 2 hr. The product after taking in pet.ether was filtered through a column of alumina (gr.I, 50 g). The pet.ether-eluted portion was purified by distillation to give mostly 19, b.p. 110-120°(bath)/1.0 mm., n_D^{30} 1.4711; $(\alpha)_D$ + 3.2° (c, 5.4).

IR bands at: 3021, 1471, 1451, 1389, 1379, 1316, 1176, 1031, 980, 909, 869 and 775 cm^{-1}.

Analysis

Found: C, 86.92; H, 13.40.

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

The hydrocarbon mixture containing mostly 19 (1.03 g) was hydrogenated in acetic acid medium using pt. catalyst. The volume of hydrogen absorbed (116 ml at NTP) corresponded to slightly over one double bond. The hydrogenated product was worked up and purified by chromatography and distillation to give in pure form (GLC/TLC), b.p. 110-120° (bath)/1.0 mm., n_D^{30} 1.4660, $(\alpha)_D \pm 0°$.

The IR spectrum (Fig. 5) taken in 0.1 mm cell was in agreement with that of 8 obtained from germacrone by Sorm et al.

IR bands at: 1332, 1307, 1282, 1235, 1170, 1111, 1075, 980, 952, 917, 869, 862, 833, 781, 751, 735 and 720 cm^{-1}.

The NMR spectrum was quite in agreement with the structure 8.

Analysis

Found: C, 85.70; H, 14.27.

$C_{15}H_{30}$ requires: C, 85.63; H, 14.37%.

Tosylation of hexahydrogermacrol and reduction of the tosylate

The alcohol (16) (1.0 g), dissolved in pyridine (15 ml) was mixed with tosyl chloride (2.1 g) and kept at the room temperature for 48 hr. The product was diluted with ice cold water and extracted with ether. The ether layer was washed with dilute HCl followed by water and dried (Na_2SO_4) Removal of ether furnished the tosylate (1.3 g) which was reduced with LAH (1.5 g) in ether solution in the usual way. The hydrocarbon obtained was purified by chromatography and distillation, b.p. 110-120°(bath)/0.7 mm., n_D^{25} 1.4710; $(\alpha)_D$ + 1.2° (c, 5.4).

Analysis

Found: C, 86.5; H, 13.87.

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

Its IR spectrum taken in 0.1 mm. cell was almost identical with that of the product obtained by $KHSO_4$-dehydration of hexahydrogermacrol 16, which by GLC analysis was shown to contain about 20% saturated hydrocarbon. The component occurring to the extent of 85% corresponded to the $KHSO_4$- dehydration product obtained from 16 (GLC).

The hydrocarbon mixture, $C_{15}H_{28}$, obtained above (0.49 g) absorbed (50.9 ml at NTP) hydrogen on hydrogenation in acetic acid medium using platinum catalyst, corresponding to 0.95 double bond. The hydrogenated product worked up in the usual way had the following properties: b.p. 110-120° (bath)/1.0 mm., n_D^{25} 1.4676; $(\alpha)_D \pm 0°$.

The IR spectrum was identical with that of germacrane.

Analysis

Found: C, 85.90; H, 14.40.

$C_{15}H_{30}$ requires: C, 85.63; H, 14.37%.

Solid hexahydrocostunolide (12)

Solid dihydrocostunolide (10) (28.34 g), m.p.76-77° $(\alpha)_D$ + 114°, was hydrogenated in alcohol solution, using platinum catalyst (1000 lb./sq.in.) in the manner described for liquid hexahydrocostunolide. The product was distilled, b.p. 130-140°/0.7 mm. and the distillate cooled in pet. ether solution at -18° for a day to yield the crude lactone, m.p. 55-56°, which after three crystallisation from pet.ether gave the pure lactone 12, m.p. 60-61°, $(\alpha)_D$- 5.2° (c, 4.4).

IR bands at: 2950, 1770, 1449, 1372, 1307, 1242, 1205, 1152, 1130, 1087, 1010, 990, 980, 840, 806, 724 and 704 cm^{-1}.

Analysis

Found: C, 75.50; H, 10.95.

$C_{15}H_{26}O_2$ requires: C, 75.58; H, 11.00%.

Solid hexahydrocostunolide was reduced with LAH under controlled conditions followed by Huang-Minlon reduction in the manner previously described to give hexahydrogermacrol (16) (GLC/TLC), b.p.120-130°(bath)/ 0.7 mm., n_D^{28} 1.4825; $(\alpha)_D$ - 4.4° (c, 2.5).

Analysis

Found: C, 79.80; H, 13.40.

$C_{15}H_{30}O$ requires: C, 79.57; H, 13.36%.

Hexahydrogermacrol (16) obtained above was oxidised by Jones' reagent in hexahydrogermacrone (17) (GLC/TLC), b.p. 110-120°(bath)/0.9 mm., n_D^{28} 1.4720; $(\alpha)_D$ + 83.8° (c, 1.5).

Analysis

Found: C, 80.60; H, 12.63.

$C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%.

The compounds (16 and 17) from both solid and liquid hexahydrocostunolides were identical in physical constants, IR spectrum and GLC retention period.

Liquid ammonia reduction of dihydrocostunolide (10)

Liquid ammonia (1500 ml) was drawn in a R.B. flask which was fitted with a mercury sealed stirrer, a dropping funnel and a condenser. Lithium pieces (2.87 g) were dropped during a period of 15 min. and the stirring continued for

another 15 min. The lactone 10 (10.0 g. in 300 ml dry ether) was added slowly through the dropping funnel. After the addition was over, the stirring was continued for another 5 hr. Excess of ammonia was allowed to evaporate and lithium was decomposed by adding alcohol. The product was then diluted with water and acidified. It was then extracted with ether several times, the ethereal layer washed free of mineral acid and extracted with sodium carbonate solution thoroughly. The carbonate solution was acidified carefully and again extracted with ether. The ether layer was then washed with water to remove free acids and dried over anhydrous sodium sulphate. The ether layer on evaporation gave the acid, which was esterified with diazomethane in the usual manner to give the ester 23 (2.67 g), b.p. 115-120°(bath)/ 0.1 mm., n_D^{28} 1.4892; $(\alpha)_D$ - 91.4° (c, 3.7).

Analysis

Found: C 75.95; H, 10.52.

$C_{16}H_{26}O_2$ requires: C, 76.75; H, 10.47%.

IR bands at: 2941, 1740, 1449, 1429, 1374, 1342, 1250, 1190, 1151, 1047, 980, 917 and 840 cm^{-1}.

Pressure hydrogenation of ester 23

The ester 23 (2.6 g) was dissolved in ethyl alcohol (150 ml) and platinum oxide (0.117 g) was added and the mixture hydrogenated under pressure (1000 lbs./sq.in.),

first at the room temperature and then at 50-60°. Working up was done in the usual way to get a mixture of esters (2.50 g). This was then chromatographed on alumina (gr.III, 120 g) and the pet.ether fraction gave pure (GLC/TLC) ester 26, b.p.140-145° (bath)/0.15 mm., n_D^{24} 1.4741; $(\alpha)_D$ + 32.08° (c, 2.5).

Analysis

Found: C, 75.75; H, 10.94.

$C_{16}H_{28}O_2$ requires: C, 76.14; H, 11.18%.

LAH reduction of the ester 26

The ethereal solution of the ester 26 (0.41 g in 50 ml ether) was added to a slurry of LAH (0.28 g in 50 ml ether) and stirred for 6 1/2 hr. Excess of LAH was decomposed by alcohol followed by water. It was then worked up in the usual way to give pure (GLC/TLC) alcohol 27 (0.43 g), b.p. 130-135° (bath)/0.2 mm., n_D^{22} 1.4940, $(\alpha)_D$ + 7.37° (c, 2.48).

IR bands at: 3040, 2900, 1460, 1380 and 1019 cm^{-1}.

Analysis

Found: C, 80.82; H, 12.18.

$C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%.

Hydrogenation of ester 26 in acetic acid medium

The ester 26 (1.13 g) was dissolved in acetic acid (50 ml) and Adam's catalyst (0.106 g) was added. The hydrogenation was continued for 24 hr., when the absorption was

complete for nearly one double bond. After filtration, the filtrate was then concentrated to remove most of the acetic acid. The residue was diluted with water, and extracted with ether. The ether layer washed free of acid by aqueous Na_2CO_3 and finally with water. After drying over Na_2SO_4, it was evaporated to give the ester 28, which was purified (GLC/TLC) by chromatography and distillation. Tetranitromethane test was negative, showing that the ester was completely saturated, b.p. 155-60° (bath)/2.0 mm., n_D^{22} 1.4720; $(\alpha)_D$ + 23.38° (c, 2.2).

IR bands at: 2950, 1754, 1262, 1200 and 1170 cm^{-1}.

Analysis

Found: C, 76.26; H, 11.76.

$C_{16}H_{30}O_2$ requires: C, 75.53; H, 11.89%.

LAH reduction of ester 28

The saturated ester 28 (0.762 g) in dry ether (50 ml) was added dropwise to a slurry of LAH (1.0 g in 50 ml of dry ether) during a period of 15 min. The stirring was continued for 7 hr. and the excess of LAH was decomposed by alcohol followed by water and the product worked up in the usual manner to give the corresponding saturated alcohol 29, which was purified by chromatography and distillation, b.p. 125-130° (bath)/1.6 mm., n_D^{22} 1.4874; $(\alpha)_D$ + 1.00° (c, 2.13).

Analysis

Found: C, 79.98; H, 13.00.

$C_{15}H_{30}O$ requires: C, 79.57; H, 13.36%.

CrO_3-Pyridine oxidation of the alcohol 29

The alcohol 29 (0.12 g) was dissolved in dry pyridine (5 ml) and was added to a CrO_3-pyridine complex (0.42 g of CrO_3 in 10 ml of dry pyridine) and kept at the room temperature for 48 hr. It was worked up in the usual way to give a liquid product (0.10 g). It was passed through alumina (gr.II, 3.0 g) and eluted with pet.ether, followed by benzene. The pet.ether fraction (0.022 g) gave the aldehyde (30), which formed a semicarbazone, m.p. 149-150°, but could not be characterised further due to paucity of material.

Tosylation of 29 and LAH reduction of the tosylate

The alcohol 29 (0.353 g) was dissolved in dry pyridine (18 ml) and freshly crystallised p-toluene sulphonyl chloride (0.42 g) was added. The mixture was kept for 36 hr. at the room temperature and then worked up in the usual way to give the tosylate (0.572 g).

The ethereal solution of the tosylate was added to a slurry of LAH (0.89 g in 50 ml dry ether) and stirred for 6 hr. Excess of LAH was decomposed by alcohol followed by water and worked up in usual way to give the crude hydrocarbon 81(0.336 g) which was passed through alumina (gr.I; 6.0 g) and eluted with pet.ether. It was further

purified by distillation over sodium, b.p. 120-125° (bath)/ 6.0 mm., n_D^{22} 1.4700; $(\alpha)_D$ + 7.69° (c, 4.0).

Analysis

Found: C, 86.10; H, 14.63.

$C_{15}H_{30}$ requires: C, 85.63; H, 14.37%.

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PART III

STRUCTURE OF DEHYDROCOSTUS LACTONE

ABSTRACT

The structure 3 previously assigned to dehydrocostus lactone (one of the major constituents of costus root oil), has been confirmed by converting it into (i) guaiane (1) $C_{15}H_{28}$, (ii) s-guaiazulene (21) and S-chamazulene (24) in fairly high yields from some of its derivatives. The lactol (13) obtainable from dehydrocostus lactone was found to be temperature-sensitive. When subjected to Huang-Minlon reduction at 135°, it gave among other products, a mixture of two alcohols (16) and (17). When subjected to the same reaction at 170°, it gave S-guaiazulene and another alcohol (18), in which both the originally occurring exocyclic double bonds have migrated inside to tetra-alkylated position, so as to give a conjugated system. This alcohol (18), the structure of which was supported by IR, UV and NMR spectra furnished a hydrocarbon (32) when subjected to metal-amine reduction, indicating the presence of allylic hydroxyl group in it. This proves that the lactone attachment in dehydrocostus lactone is at C_6-C_7. The saturated alcohol (22) prepared from hexahydrodehydrocostus lactone (6), on chromic acid oxidation gave the corresponding ketone (25) and the keto-carboxylic acid (27). The structure of the latter has been established by bromination and dehydrobromination, when it afforded a cross-conjugated dienone ester (30), the structure of which was supported by IR, UV and NMR data.

The presence of sesquiterpenic lactones in costus root oil was first reported by Semmler and Feldstein[1] who examined costus root oil obtained through Schemmel & Co. They subjected the oil to vacuum distillation at 11 mm. and collected various fractions boiling within a range of about 10°. In addition to α- and β- costenes, aplotaxene, costol and costus acid, they also reported that the higher boiling fractions, viz. (i) 190-200°/11 mm. and (ii) 200-210°/11 mm., consist of two sesquiterpenic lactones, which they called as costus lactone and dihydrocostus lactone respectively. But they were unable to isolate any solid crystalline lactone from the oil. The presence of solid crystalline lactone in costus root oil was first reported by Ukita[2] in 1939 who extracted the roots with pet.ether, distilled the oil under vacuum to get a fraction boiling at 175-190°/6 mm. and from this isolated a crystalline lactone, $C_{15}H_{18}O_2$, m.p. 60.5°, in low yield. Crabalona[3] later on isolated probably the same lactone from the oil after several years standing. This lactone reported by these two workers is presumably identical with dehydrocostus lactone isolated by Naves by vacuum distillation of oil at 2 mm. Though Naves records that the lactone isomerises at 66°, the method of isolation adopted by him involved drastic temperature treatment and the yield of the lactones was only 0.125% of the oil.

The constitution of dehydrocostus lactone was initially studied by Naves,[4] who on the basis of the formation of a mixture of two azulenes (S- and Se-guaiazulenes) by Se-dehydrogenation of the saturated lactone and also on the results of its quantitative ozonolysis, concluded that it contains two exocyclic double bonds and possesses a guaiane skeleton (1).

During the course of the investigation of the constituents of costus root oil Sorm[5] et al have examined some of the constituents systematically and elucidated their structures. They were able to isolate in 5-8% yield, the same dehydrocostus lactone of Naves by vacuum distillation of oil at one stage (140-143°/0.5 mm.) followed by subsequent crystallisation of the particular fraction. They repeated the experiments of Naves and came to the conclusion that quantitative ozonolysis shows the presence of three exocyclic double bonds and not two as previously reported. Examination of the IR spectrum[5] of dehydrocostus lactone in chloroform showed the presence of only exomethylene double bonds (890, 1640 cm^{-1}) and clearly indicated the absence of any methyl groups in the molecule as revealed by the absence of the $-CH_3$ bending vibrations around 1380 cm^{-1} (IR spectrum of dehydrocostus lactone is shown in Fig. 1). This clearly indicated that dehydrocostus lactone contains three double bonds, all of which are present as $>C=CH_2$

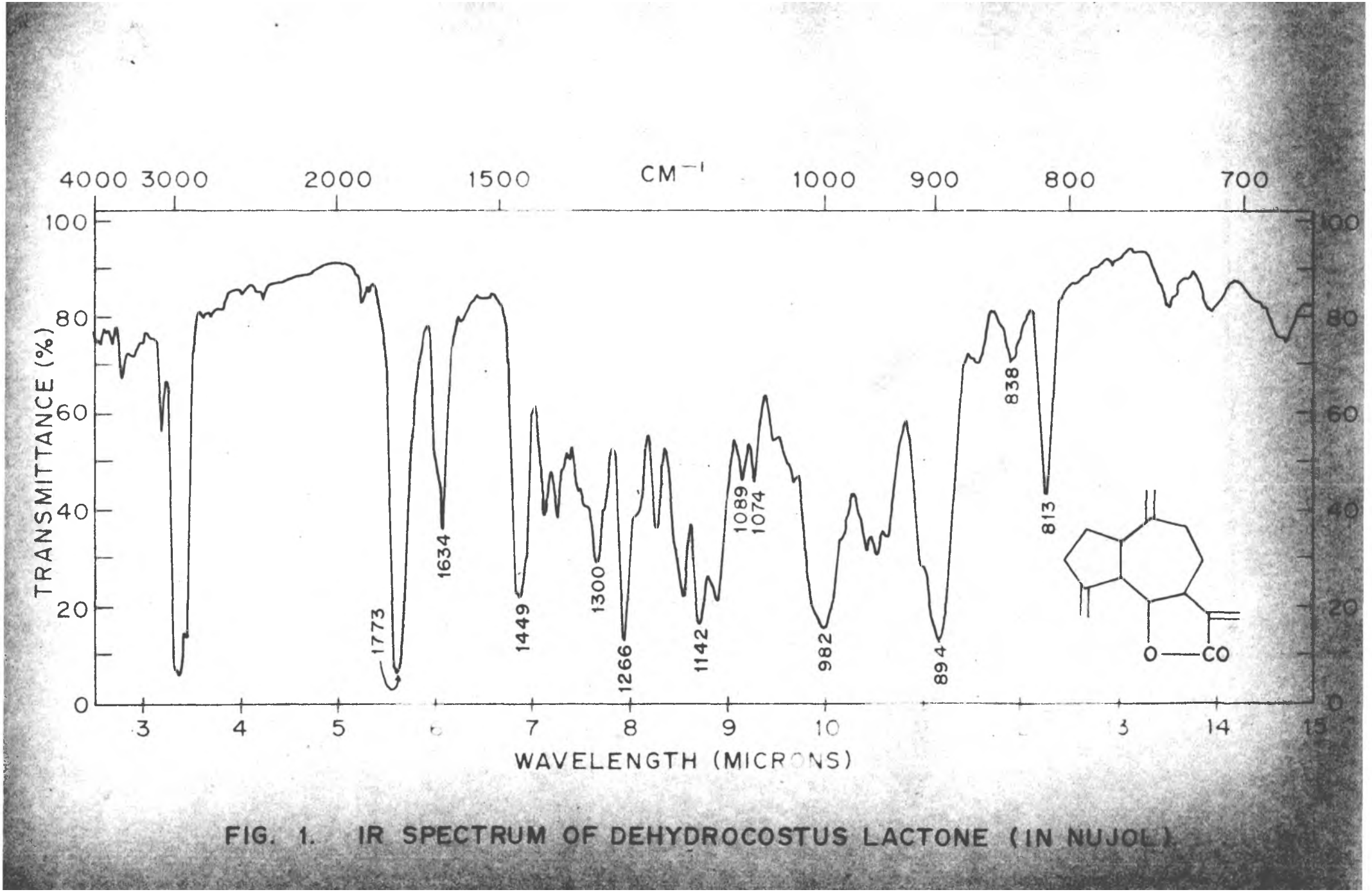
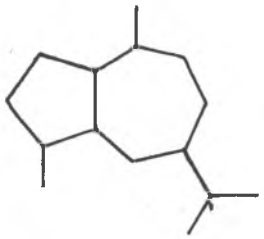


FIG. 1. IR SPECTRUM OF DEHYDROCOSTUS LACTONE (IN NUJOL).

grouping. The presence of an α,β- unsaturated α-methylene-γ-lactone, was indicated by the IR (peaks at 1760, 1400[21] and 815[22] cm^{-1}) and UV spectrum (high end absorption at 210 mμ). A comparison of the IR spectrum of the completely saturated lactone viz. hexahydrodehydrocostus lactone with that of dihydrodeoxycarpesia lactone obtained from carpesia lactone (2), indicated that the two compounds are identical in their carbon frame work; and hence they suggested structure 3 for dehydrocostus lactone. Selenium dehydrogenation of hexahydrodehydrocostus lactone was reported to give a mixture of four azulenes in very low yields. These results were also consistant with the structure 3 for dehydrocostus lactone.

Among the lactones reported to occur in costus root oil, dehydrocostus lactone seemed to be the only crystalline product, the isolation of which has been reported by several independent workers, though always in poor yields. During the course of our[6] investigation on the costus root oil (prepared from both Kashmir and Punjab costus roots) extracted by using a modified low temperature solvent extraction procedure, we could isolate a mixture of two crystalline lactones which constitute about 50% of the total extractive. The lactone mixture was shown by us to consist of (i) costunolide (4), a monocyclic sesquiterpenic lactone with a unique distribution of double bonds in a ten-membered carbocyclic



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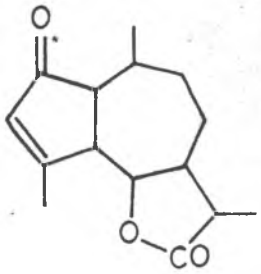

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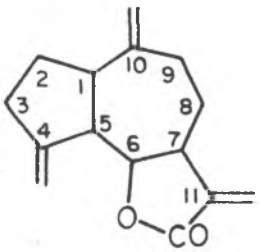

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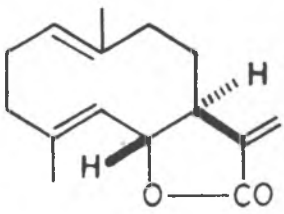

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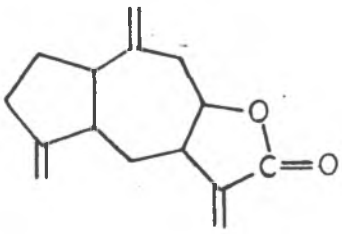

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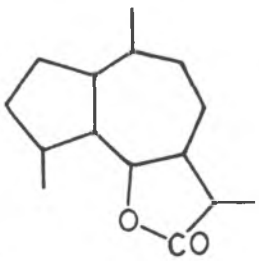

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ring, occurring to the extent of 15% and (ii) dehydrocostus lactone occurring to the extent of 35%. Some interesting transformations of costunolide have already been reported[7] and some of them have been described in Chapters II and IV. Since we had a substantial quantity of dehydrocostus lactone at our disposal, we naturally felt interested in undertaking a detailed examination of this lactone with a view to elucidate its structure beyond doubt. We carried out some interesting transformations of it with a view to obtain some useful products. The conversion of this lactone into azulenes in good yields was one of our objectives. Attempts were also made to elucidate its stereochemistry; but we have only partly succeeded.

Since the azulenes were obtained by earlier workers only in low yields, this does not lend sufficient support to the structure 3 especially when many non-azulenic terpenoids are known to yield comparable amounts of azulenes on dehydrogenation. Further an alternative structure 5 for dehydrocostus lactone also could not be completely ruled out, as most of the deductions, which have led to the structure 3 are based on the IR spectrum of saturated liquid materials (possibly mixture of epimers) of doubtful homogeneity. It was therefore felt desirable to furnish further confirmatory evidences based on reactions involving homogeneous and crystalline materials for the structure 3.

For the sake of convenience it is assumed that 3 represents the correct structure of dehydrocostus lactone. In our initial experiments 3 was fully hydrogenated in alcohol medium using platinum catalyst under pressure to give hexahydrodehydrocostus lactone (6), which, from the results of chromatography, GLC analysis, fractionation through spinning band column etc. was found to be a mixture of epimers, mostly around C_{11}.

With the ultimate object of getting a product related to nepetalinic acid (7), via the intermediate ketocarboxylic acid (8), hexahydrodehydrocostus lactone (6), obtained by direct hydrogenation was oxidised with chromic acid in acetic acid, but instead of the desired ketocarboxylic acid, the product obtained was a lactone carboxylic acid, possibly represented by 9 or 10, or a mixture of both. Most of the 6 was recovered unchanged. Subsequently 6 was reacted with phenylmagnesium bromide and the product dehydrated to give [redacted], presumably, the diene (11). But contrary to expectation, its oxidation did not lead to any conclusive results.*

We then directed our attention to the preparation of pure dihydrodehydrocostus lactone (12) by stereospecific reduction of the methylene group in conjugation with the

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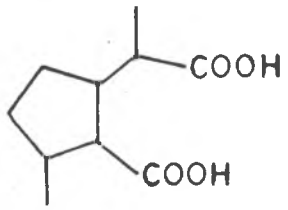
* This part of the work was done by Dr. D. Simonovic in the laboratory and included in his thesis.



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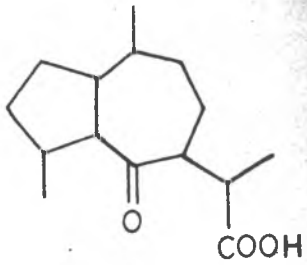

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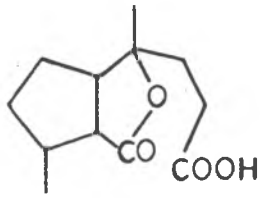

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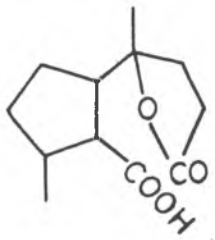

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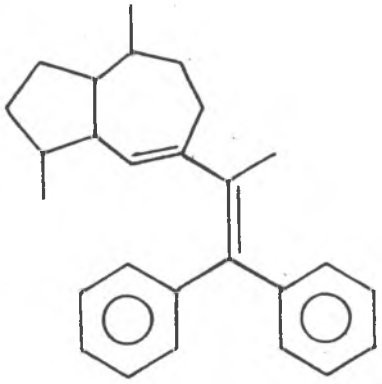

11

lactone carbonyl to give a single epimer at C_{11}. Reduction of dehydrocostus lactone to dihydrodehydrocostus lactone using sodium and alcohol has been described earlier by Naves[4] et al. We, however, did not find it possible to obtain an epimerically pure product by this method, as apart from a mixture of the epimers at C_{11}, some tetrahydroproducts were also produced unexpectedly. A small amount of the parent lactone also remained unreacted and could be isolated as the solid picrate of ammonia adduct. Reduction experiments using sodium and liquor ammonia[20] also failed to give pure 12. Finally the recent method reported by Japanese workers[9] to get the dihydro lactone via oxidation of the pure crystalline lactol (13) (IR spectrum is shown in Fig. 3) which is obtained by prolonged reduction of dehydrocostus lactone by sodium borohydride, was found to give pure dihydro dehydrocostus lactone containing one single epimer (GLC/TLC) (IR spectrum is shown in Fig. 2). Direct reduction of 3 by sodium borohydride gave 80% pure (GLC) dihydrodehydrocostus lactone. It was further observed that the lactol 13 could be obtained more conveniently on a preparative scale by controlled reduction of 12 with LAH. The complete reduction of dihydrodehydrocostus lactone with LAH gave a crystalline diol (14), m.p. 53-54°, IR spectrum has been shown in Fig. 4.

In this connection, it may be worthwhile mentioning that dihydrodehydrocostus lactone is one of the main

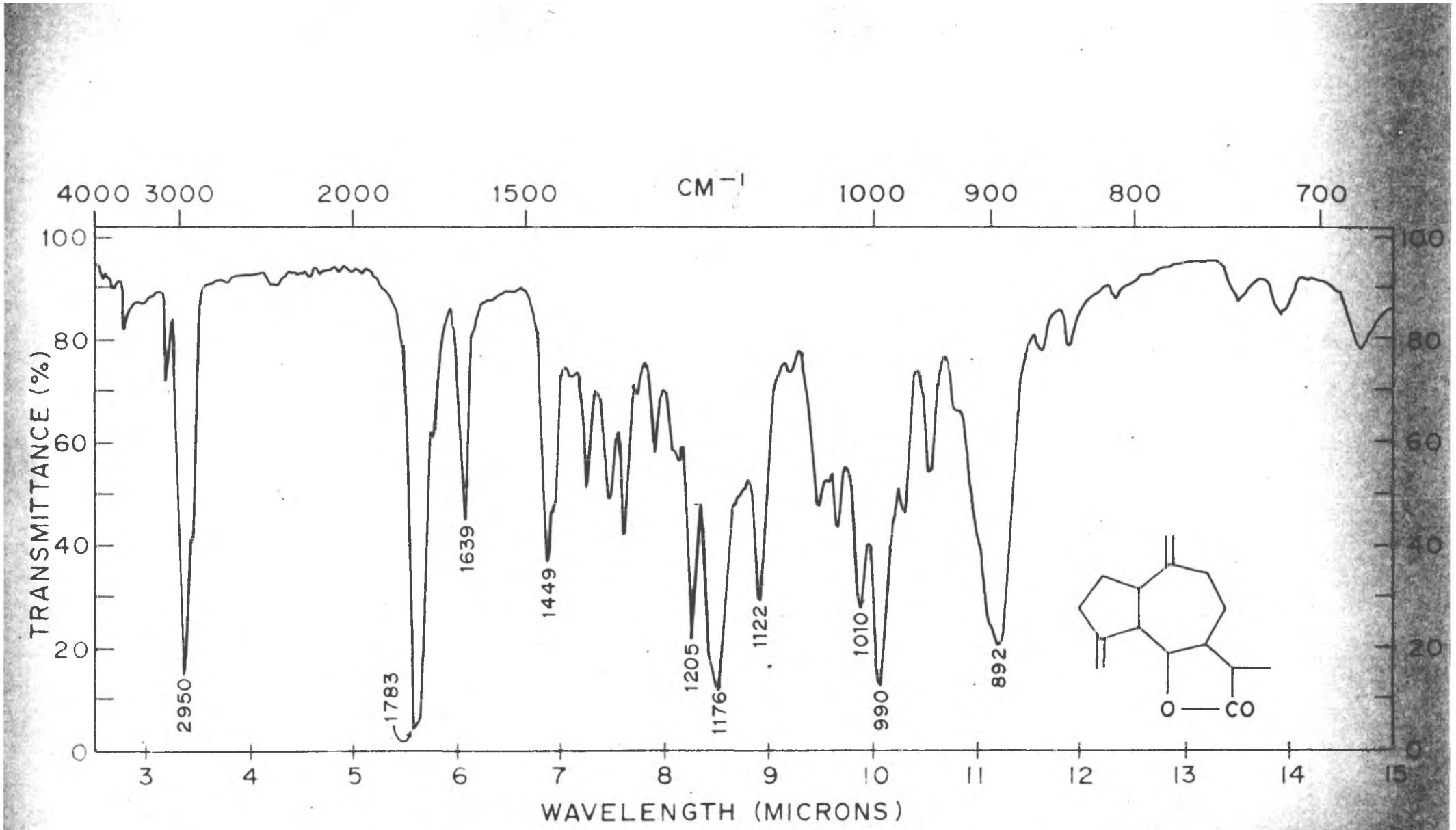

FIG. 2. IR SPECTRUM OF DIHYDRO DEHYDROCOSTUS LACTONE (LIQ. FILM).

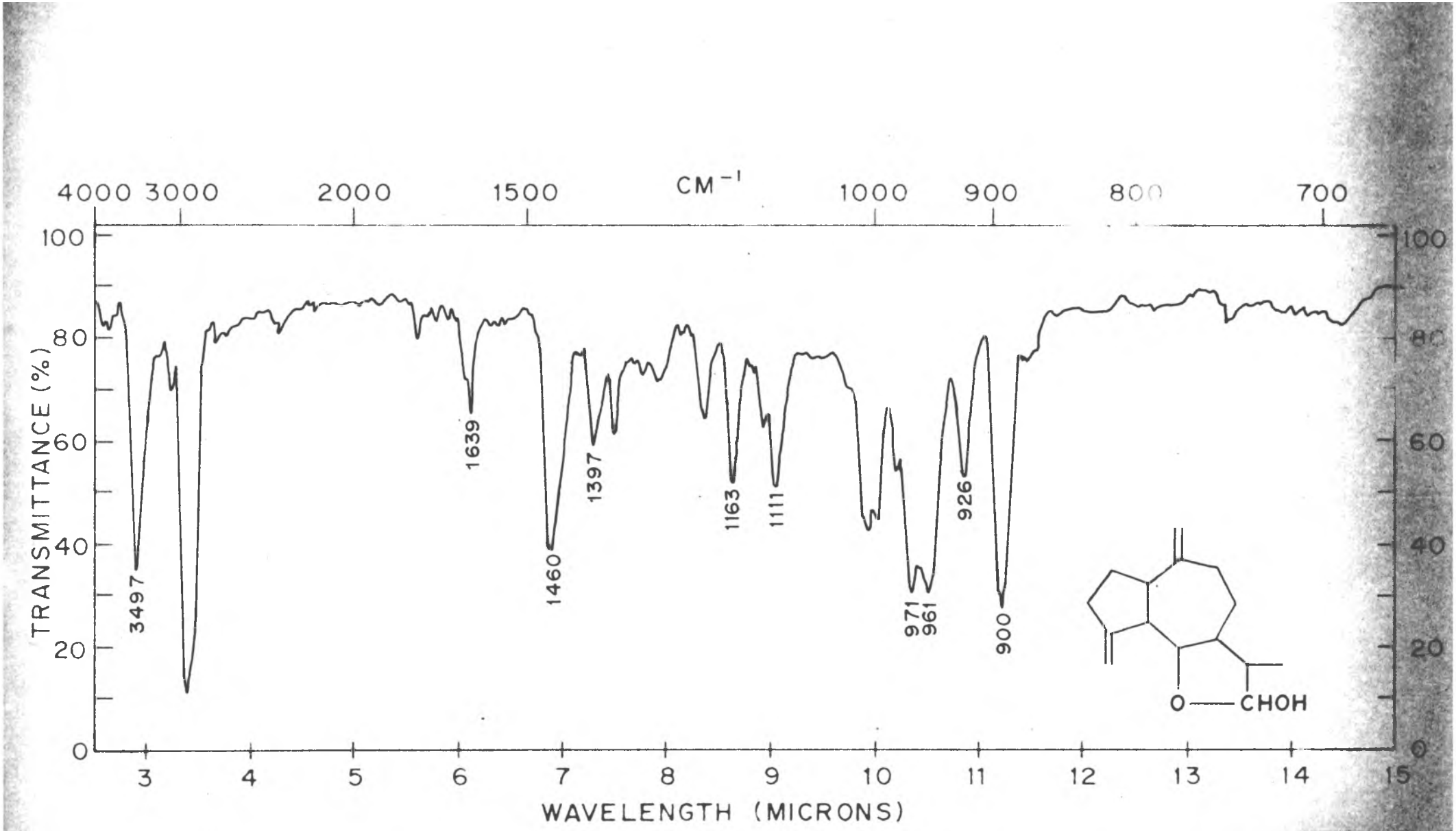

FIG. 3. IR SPECTRUM OF LACTOL (IN NUJOL).

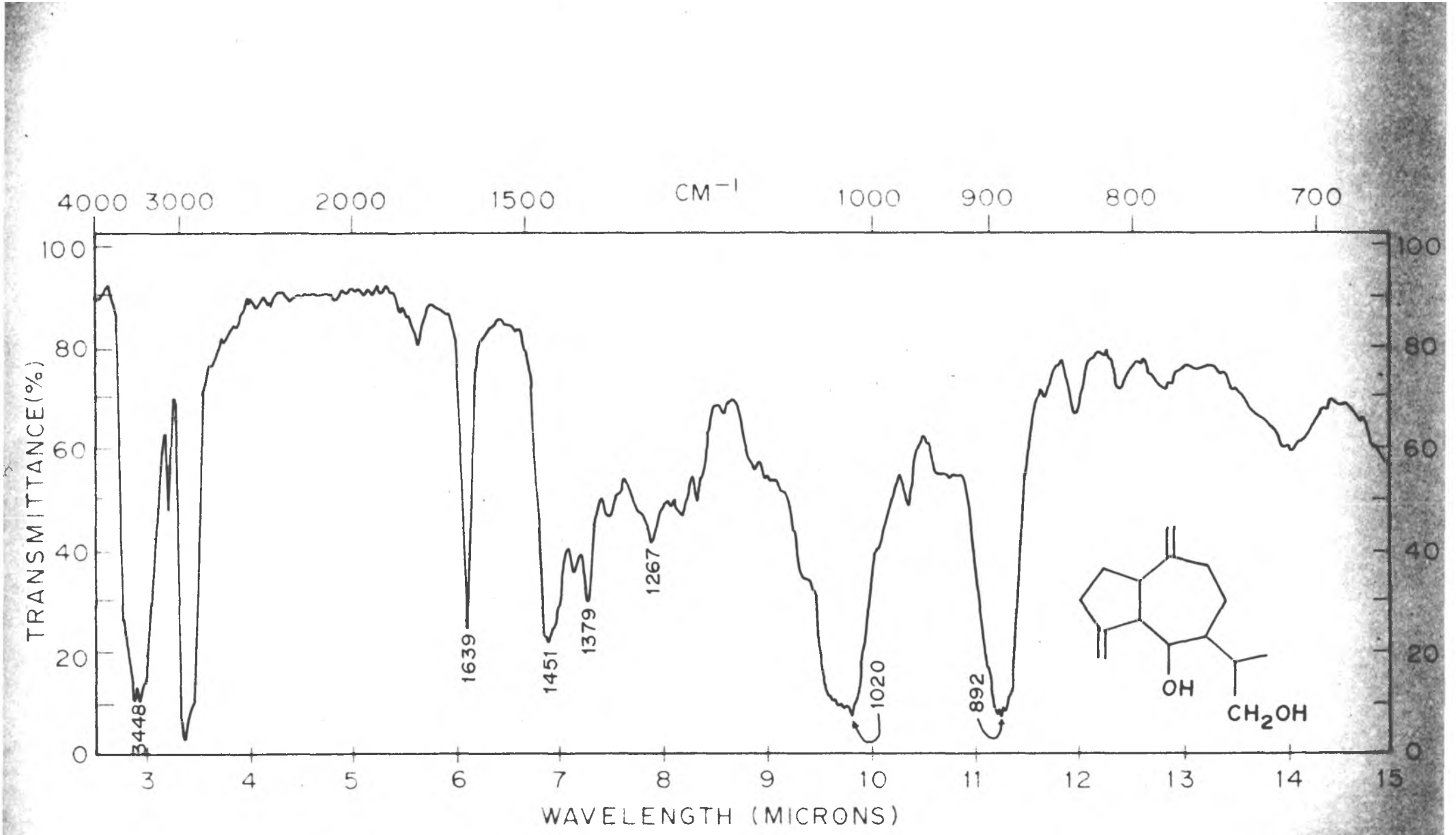

FIG. 4. IR SPECTRUM OF DIOL (IN NUJOL).

constituents of liquid lactones isolated from costus root oil. Its structure was determined in our laboratory[6] on the basis of its catalytic hydrogenation, quantitative ozonolysis, dehydrogenation to give a mixture of azulenes and also the comparison of the IR spectrum with the dihydro-derivative of dehydrocostus lactone obtained by sodium and alcohol reduction. But the natural lactone was consisting of epimers probably at C_{11} and it has been possible only to isolate one of the epimers in 85% purity. The structure has been further confirmed by the NMR spectrum and also by comparison of physical constants and IR spectrum with an authentic sample prepared as indicated above. The Japanese workers isolated a lactone, $C_{15}H_{20}O_2$, from the medicinal plant Sen mokko, which was identical with dihydrodehydro-costus lactone. They named it as mokko lactone. They obtained dihydrodehydrocostus lactone, as mentioned above, in the form of a low melting solid (m.p. 38°), but in our hands the pure (GLC/TLC) dihydrodehydrocostus lactone obtained via the crystalline lactol is a liquid.

The crystalline lactol (13) has been found to give different products when subjected to Huang-Minlon reduction at different temperatures. When the reaction is carried out at 135°,* a mixture of two monols was obtained which were separated by chromatography over alumina. The

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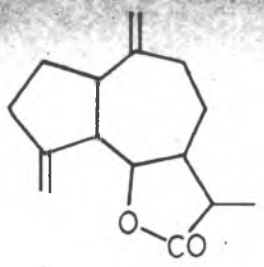
* The Huang-Minlon reduction at temperature 135° was carried out by a colleague, Mr. S.V. Hiremath.



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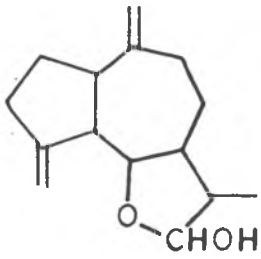

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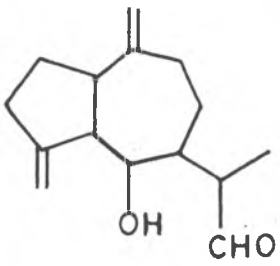

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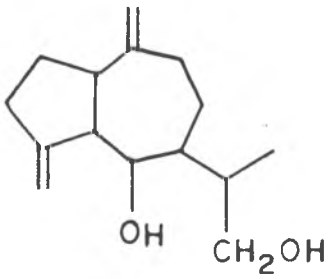

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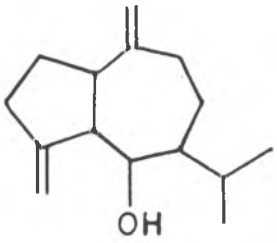

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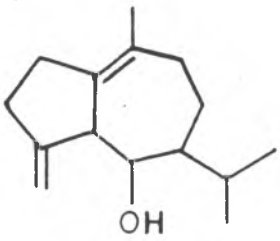

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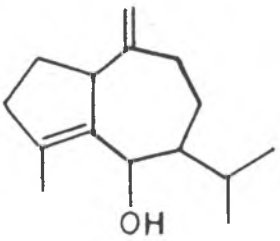

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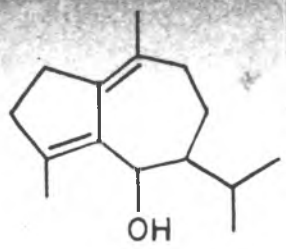
monol (15), $C_{15}H_{24}O$ (GLC/TLC pure) eluted in the earlier fractions, showed the presence of exomethylene double bonds in its IR spectrum. The NMR spectrum showed signals at 9.3, 9.13, 9.04τ(6H) due to the methyls of the isopropyl group at C_7. In the olefinic region, it showed signals at 5.34, 5.23, 4.82τ (4H) due to four protons on exomethylene double bonds at C_4 and C_{10}. A triplet at 7.11, 6.95, 6.82τ (1H) was also observed which is due to proton at C_6. The NMR spectrum is in complete agreement with the structure (15).

From the middle fractions of chromatography, another pure (GLC/TLC) isomeric ~~a~~ monol (16), $C_{15}H_{24}O$, whose IR spectrum clearly indicated the presence of exomethylene group was obtained. Its NMR spectrum, however, indicated the presence of only one exomethylene group (signals at 5.45, 5.35τ(2H) and a methyl group on a double bond (signals at 8.35τ(3H) but did not show signal due to a proton on trialkylated double bond. It is therefore obvious that one of the two original exomethylene double bonds have migrated inside the ring. The spectral data would suggest that the alcohol could be represented by either of the structures(15 or17) and was shown by further experiments to be 16.

When the Huang-Minlon reduction of the lactol was carried out at higher temperatures (170°), along with other

products, the pure (TLC) heteroannular dienic alcohol (18) was obtained as the main component, in which both the original methylenic double bonds have migrated inside the ring. Its IR spectrum (Fig. 5) did not show the presence of any exomethylene group. The NMR spectrum (Fig. 6) indicated the presence of two methyl groups on double bonds, signals at 8.38τ(3H) and 8.18τ(3H) and did not show any signals due to trialkylated protons. In conformity with this, it showed the characteristic UV absorption at 252 mμ (ε_{max}9000). Oxidation of the compound with Jones' reagent, as well as other oxidising agents such as CrO_3-pyridine and activated MnO_2, however, failed to give the expected conjugated dienone (19) in pure condition, although indication as to its formation as one of the products was observed in the UV spectrum of the oxidised product (λ_{max}. 290 mμ, ε_{max}. 3000). Much of the monol remained unaffected, even when the reaction is carried out for longer time, suggesting that the alcoholic -OH group is in a hindered position. During the formation of 18 from 13, S-guaiazulene (21) (characterised through its TNB adduct) was also obtained as a by-product in 15% yield. It was also observed that the proportion of S-guaiazulene is raised to 30% when the same reaction is carried out at 220°.

In another series of experiments 6 as obtained by direct catalytic hydrogenation of 3 or hydrogenation of epimerically pure 12, was reduced by LAH under controlled



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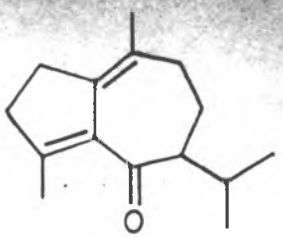

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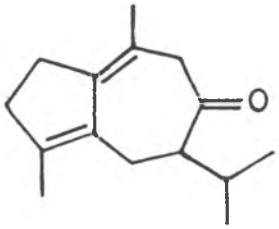

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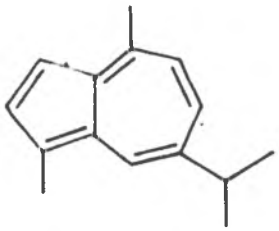

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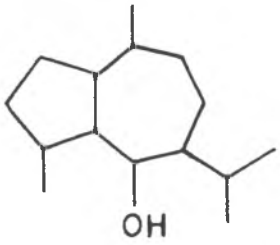

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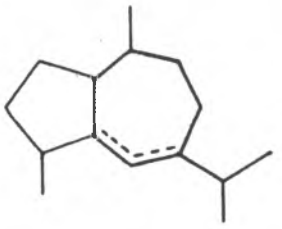

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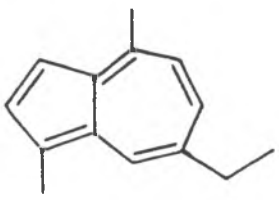

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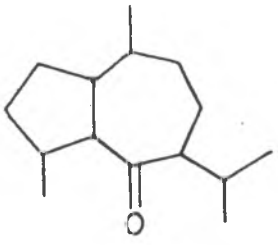

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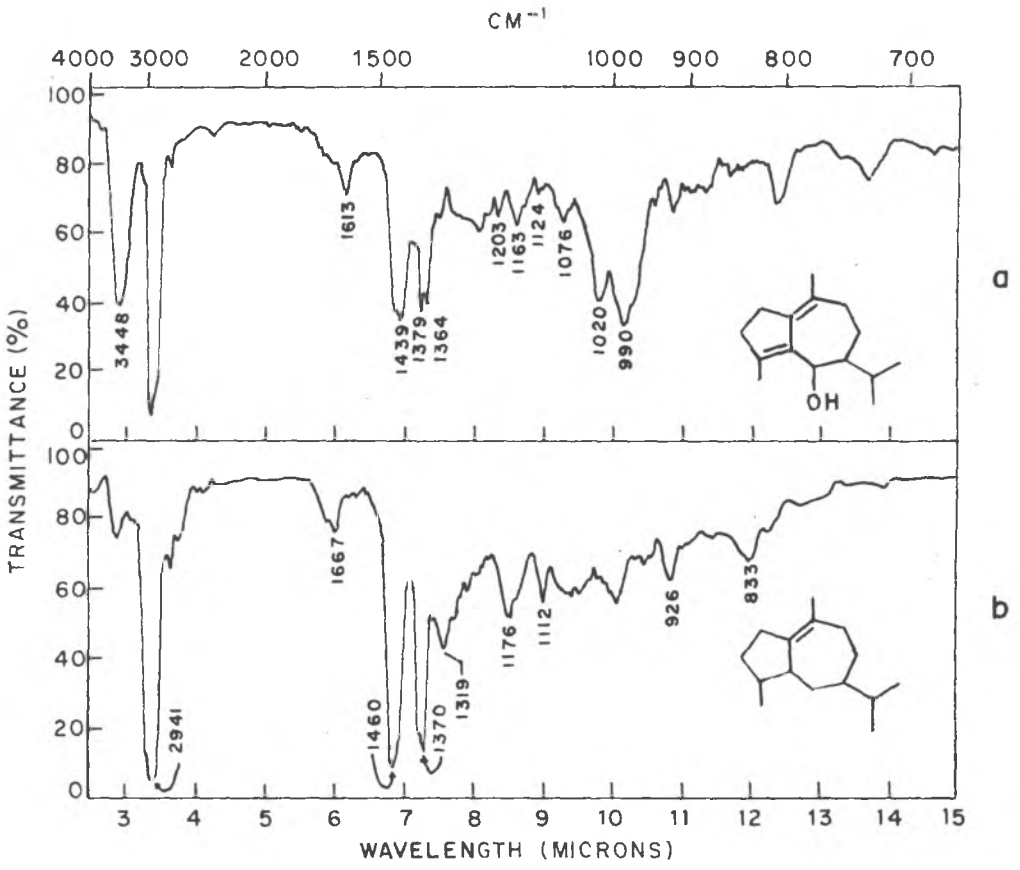


FIG. 5.

(a) IR SPECTRUM OF DIENIC MONOL (LIQ. FILM).

(b) IR SPECTRUM OF THE HYDROCARBON (LIQ. CELL. 0·1 mm.)

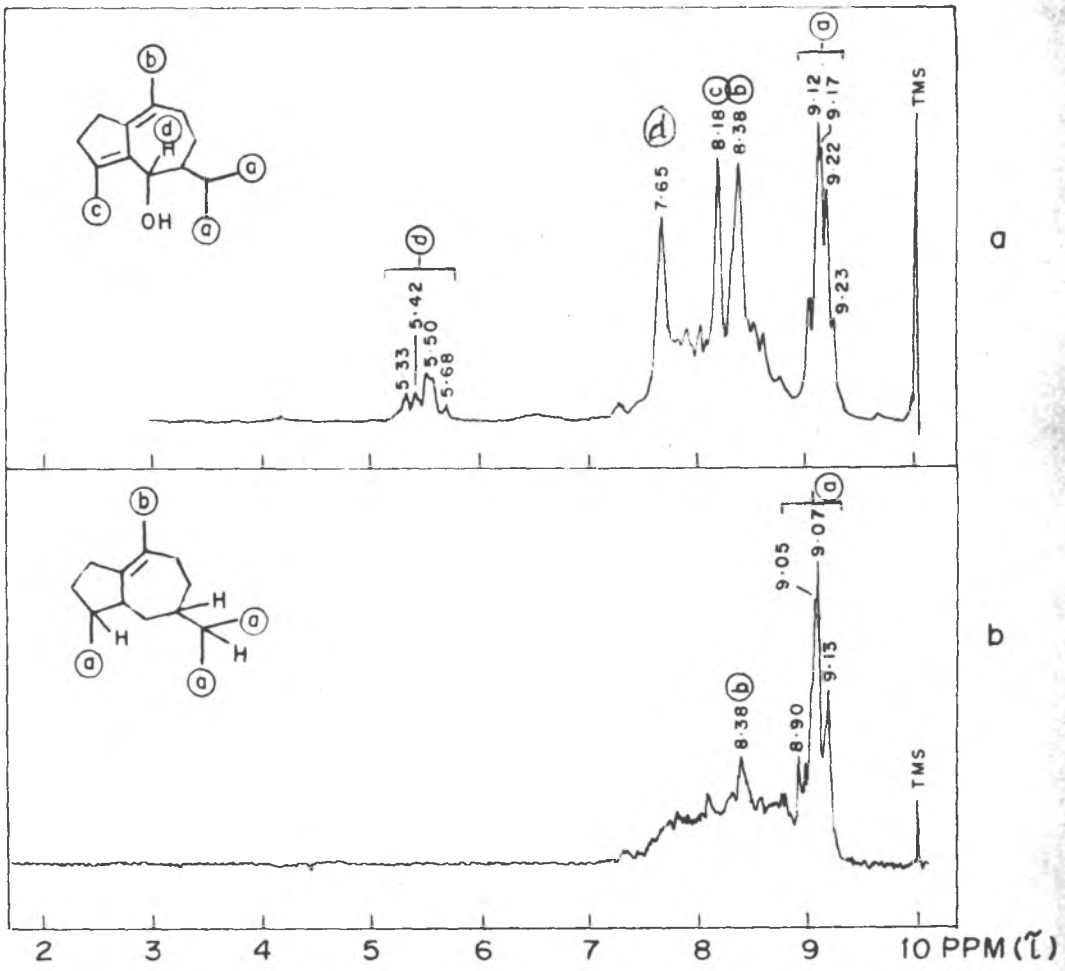


FIG. 6.

(a) NMR SPECTRUM OF DIENIC MONOL.

(b) NMR SPECTRUM OF THE HYDROCARBON.

conditions,[10] and the resulting hydroxy aldehyde (lactol) was subjected to Huang-Minlon reduction to furnish in good yield the pure (GLC/TLC) crystalline monol (22), $C_{15}H_{28}O$ (IR spectrum is shown in Fig. 7). Dehydration of the monol with potassium hydrogen sulphate gave a mixture (GLC) of two unsaturated hydrocarbons, $C_{15}H_{26}$, both containing trialkylated double bonds (IR and NMR evidences). Hydrogenation of this mixture (23) gave a saturated hydrocarbon, $C_{15}H_{28}$ (GLC two isomers 80:20), identified as guaiane (1) by IR spectrum (Fig. 7) and physical properties. The formation of guaiane from 3 would therefore support that the latter possesses a guaiane skeleton. Sulphur dehydrogenation of the monol 22 gave S-guaiazulene (21) in 8% yield, identified by IR spectrum (Fig. 9), GLC and characterised through its TNB adduct. When 12 was heated with alkali in diethylene glycol in an atmosphere of nitrogen at 180 -200° for 5 hr., along with other products, chamazulene (24) could be isolated in 8% yield, identified by IR spectrum[11] (Fig.10), GLC analysis and characterised through its TNB adduct.

In our further examination of the monol 22, it was oxidised with chromic acid to yield the pure (GLC/TLC) crystalline ketone, $C_{15}H_{26}O$ (25) (IR spectrum is shown in Fig. 7), along with a keto carboxylic acid, $C_{15}H_{26}O_3$, characterised through its methyl ester, $C_{16}H_{28}O_3$. The ketocarboxylic ester showed a broad band at 1724 cm^{-1},

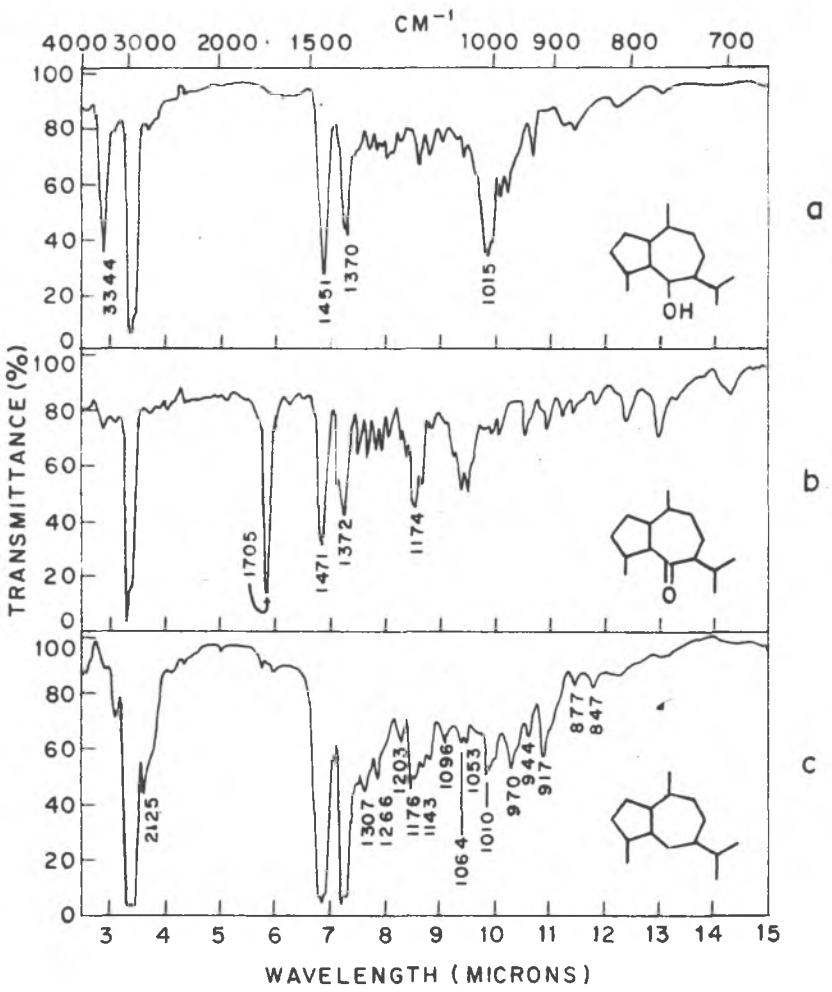


FIG. 7.

(a) IR SPECTRUM OF SATURATED MONOL (IN NUJOL).

(b) IR SPECTRUM OF SATURATED KETONE (IN NUJOL).

(c) IR SPECTRUM OF GUAIANE (IN LIQ. CELL 0·1 mm.)

in its IR spectrum (Fig. 11) from which, however, the presence of both the keto and the ester groups could not be definitely inferred. Sodium borohydride reduction of the product followed by re-esterification with diazomethane afforded a hydroxy ester whose IR spectrum (Fig. 12) clearly indicated the presence of a hydroxy and an ester group from which it was concluded that the original molecule contains both the keto and ester groups. The sodium borohydride reduction of the ketocarboxylic acid was found to be a slow process and the complete reduction could be achieved only after prolonged treatment with excess of the reagent under refluxing conditions. In support of this, the NMR spectrum (Fig. 8) of the above product did not show any olefinic proton or vinyl methyl groups, but showed signals due to an ester methyl at 6.32 τ (3H). In addition it indicated the presence of four methyl groups (9.12, 9.10, 9.05, 8.90, 8.86 τ (12H) all of which are attached to saturated carbon atoms. The protons adjacent to the carbonyl group were also observed at 8.05, 8.00, 7.90 and 7.82 τ. It was, however, not possible to say definitely the exact number of such protons - whether two or three. These spectroscopic data could be satisfactorily explained by two formulae 26 or 27. This product was considered to be suitable material for degradation to nepetelinic acid, if it were represented by the structure 26, as it was obtained from crystalline alcohol 22. The

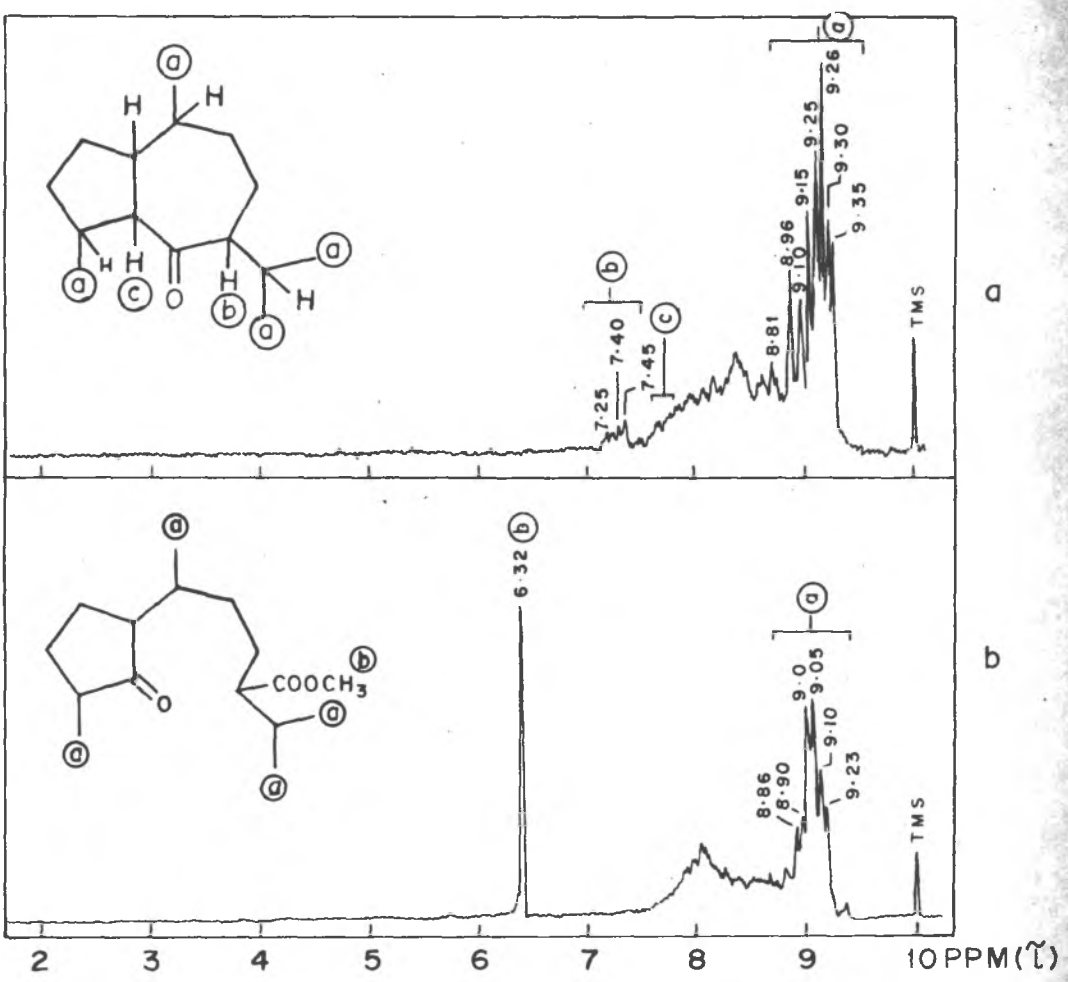


FIG. 8.

(a) NMR SPECTRUM OF SATURATED KETONE.

(b) NMR SPECTRUM OF KETOCARBOXYLIC ESTER.

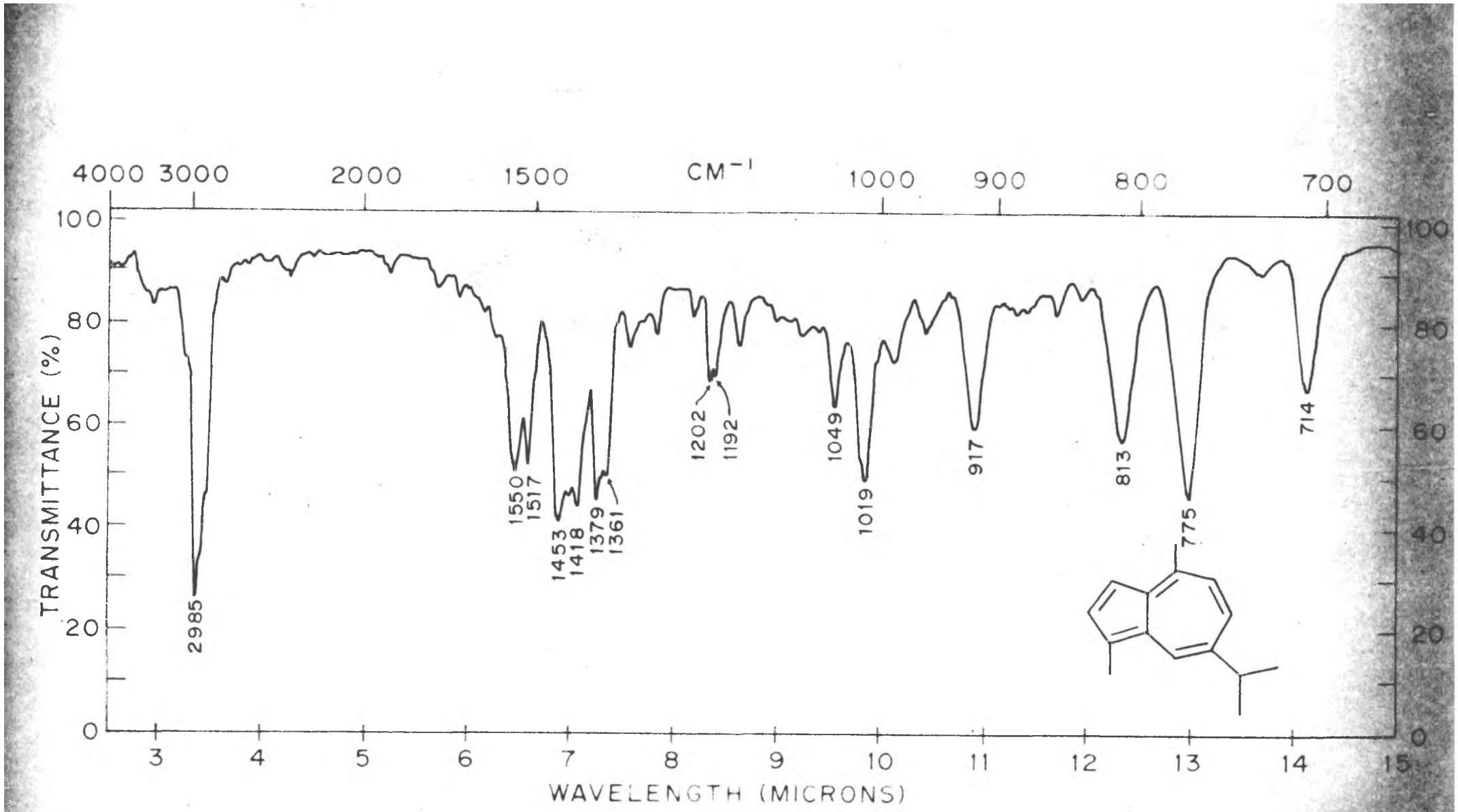


FIG. 9. IR SPECTRUM OF S-GUAIAZULENE (LIQ. FILM).

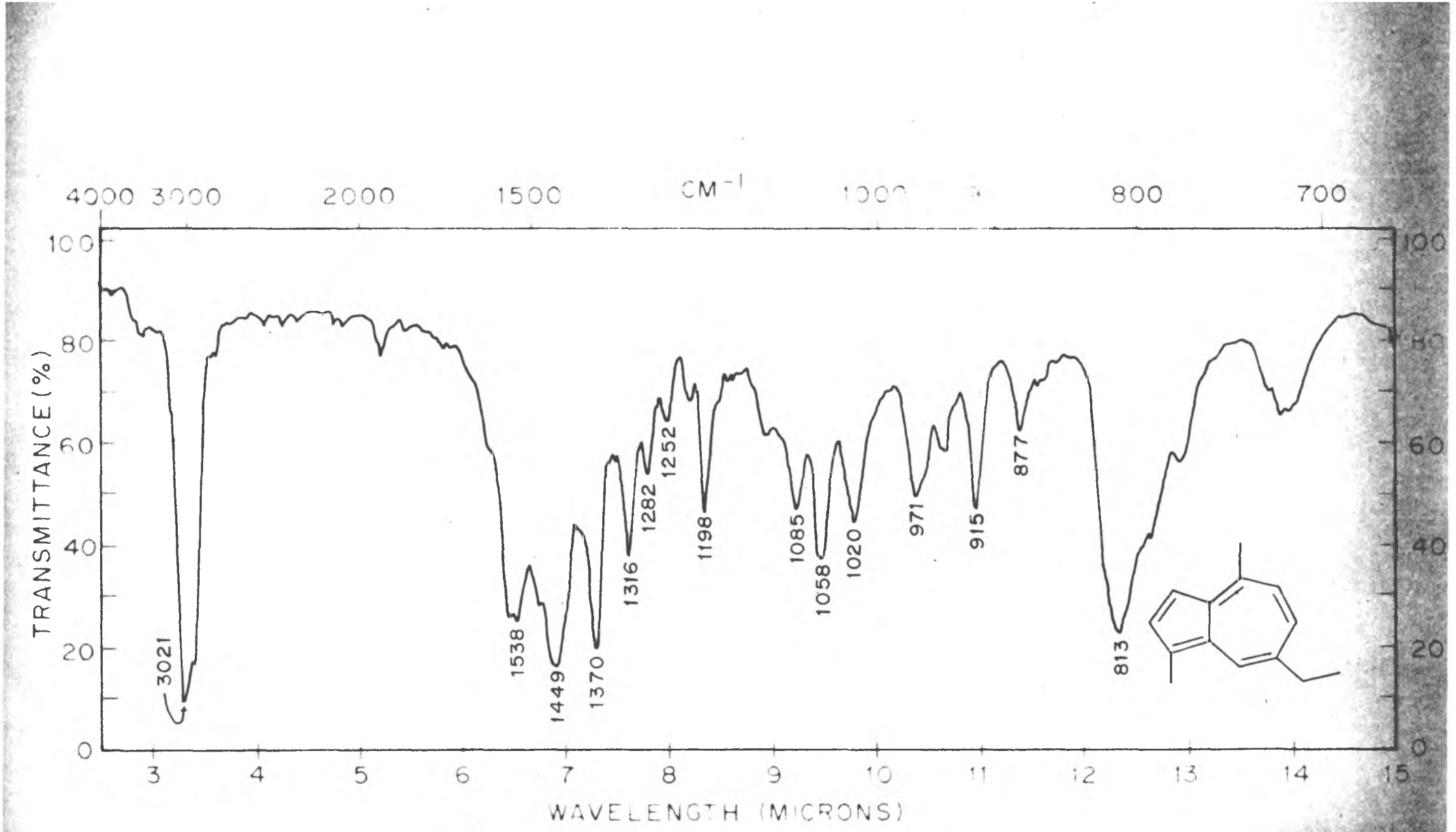
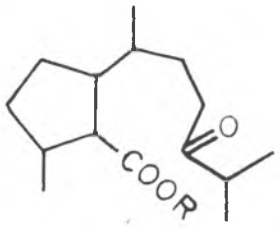


FIG. 10. IR SPECTRUM OF CHAMAZULENE (LIQ. FILM).

slow reduction of the keto group by $NaBH_4$, taken together with the fact that no semicarbazone is formed, would suggest that the structure would be 27. Dehydration of the hydroxy ester by thionyl chloride-pyridine afforded a mixture of two unsaturated esters, IR spectrum of which showed presence of a trisubstituted double bond.

In order to decide between structures 26 and 27, the compound was brominated with excess of N-bromosuccinimide,[12] when a bromo compound (29) was obtained. The estimation of the bromine indicated that three bromine atoms have been incorporated in the molecule. Dehydrobromination of the above compound by boiling with pyridine in nitrogen atmosphere afforded a mixture of compounds from which a crystalline solid, m.p. 95-96° (30) has been isolated by column chromatography followed by crystallisation. The IR spectrum (Fig. 13) of this product showed peaks at 1725 cm^{-1} due to ester carbonyl, 1695 and 1660 cm^{-1} due to α,β-unsaturated carbonyl and 1645 cm^{-1} due to the -C=C- stretching of a conjugated double bond. Since the peaks at 1695, 1660 and 1645 cm^{-1} were almost equal in intensities, it is quite probable that the chromophore present in the molecule is the cissoid α,β-unsaturated ketone. The UV spectrum of this product showed λmax. at 258 mμ, εmax.12,900. No definite conclusion, however, could be drawn from these data as to the exact location of the keto group.



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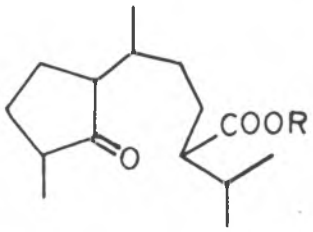

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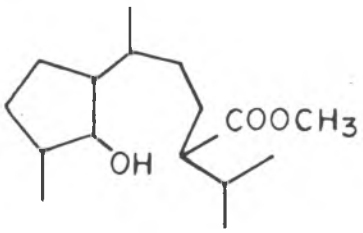

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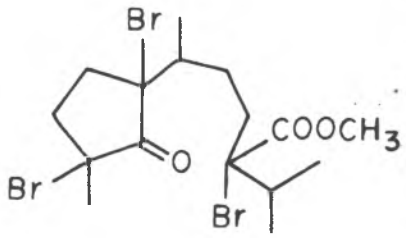

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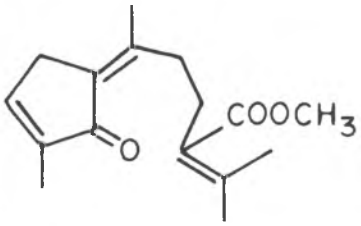

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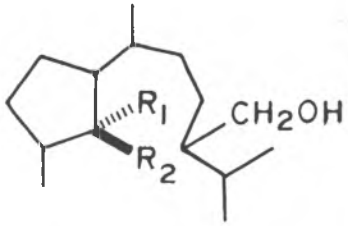


31 R_1 = OH ; R_2 = H

31a R_1 = H ; R_2 = OH

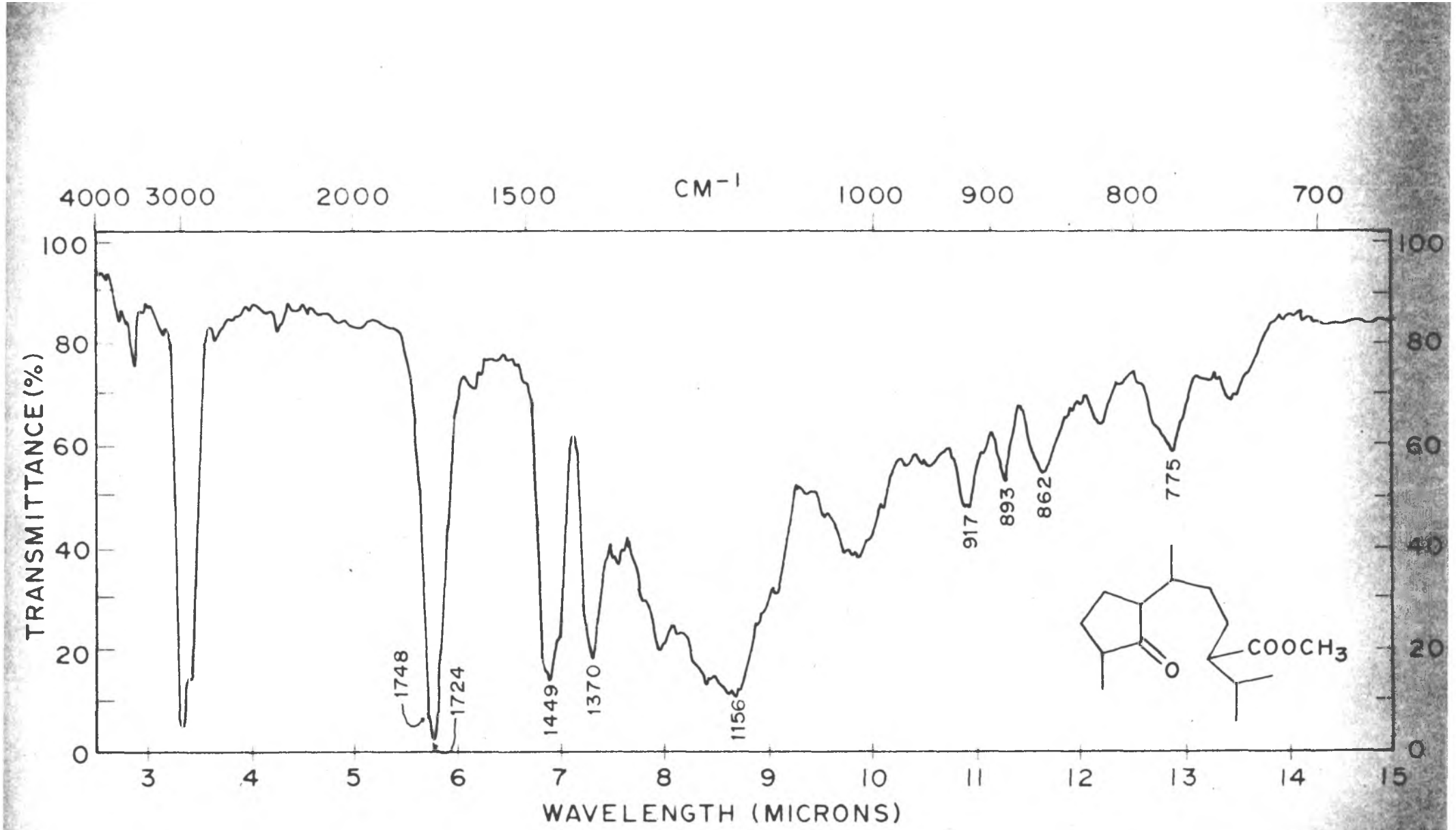


FIG. 11. IR SPECTRUM OF KETOCARBOXYLIC ESTER (LIQ. FILM).

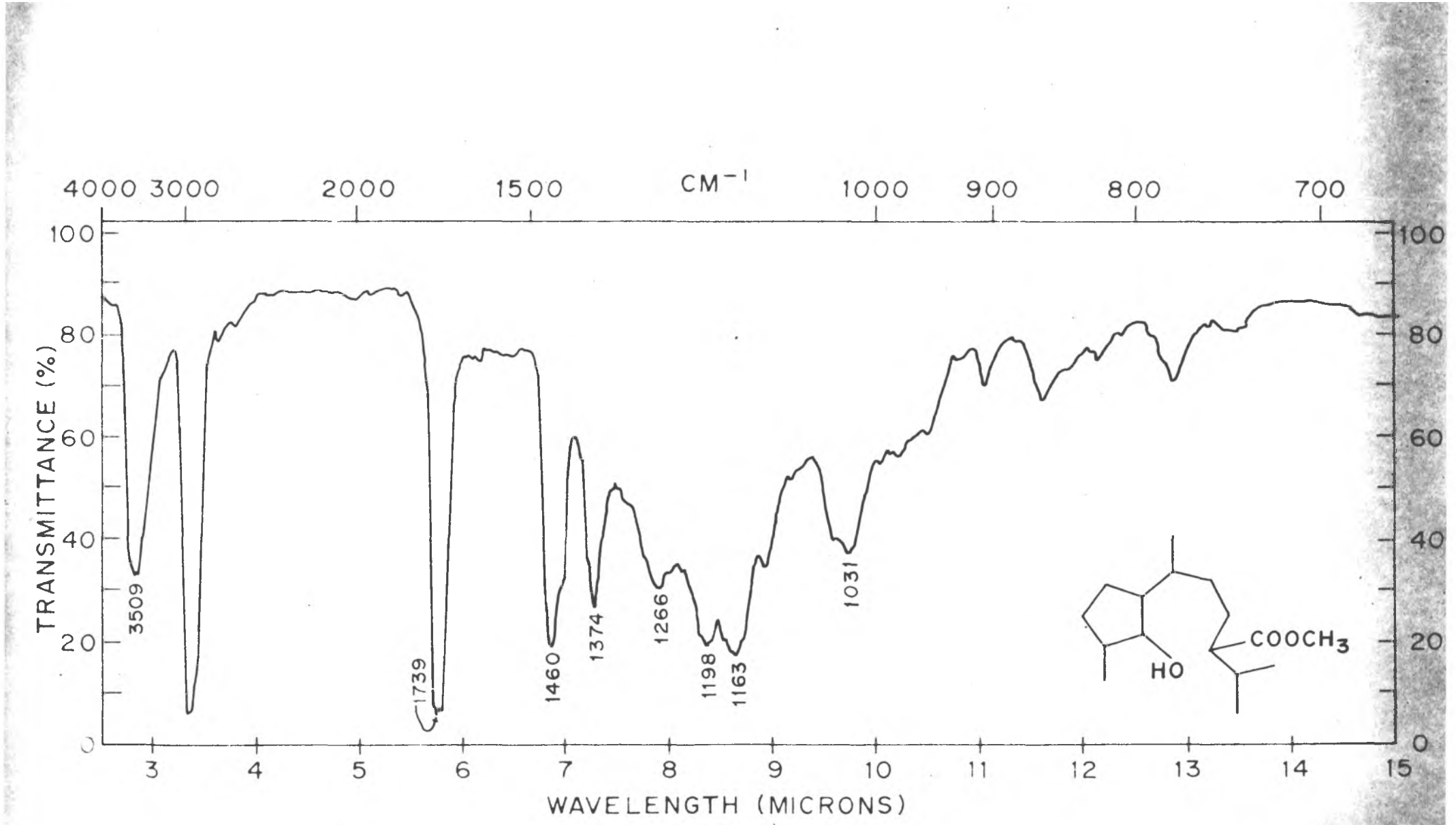


FIG. 12. IR SPECTRUM OF HYDROXYESTER (LIQ. FILM).

The NMR spectrum (Fig. 14) of the product 30 clearly indicated that all the methyl groups present in the molecule are situated on carbon carrying double bonds (signals at 8.29, 8.24, 8.03 and 7.92 τ (12H) ; a signal at 6.32 τ (3H) indicated estermethyl group and at 3.02τ(1H) due to a single olefinic proton on a conjugated trisubstituted double bond. The signal observed at 6.32τ was quite consistant with the ester methyl of an α,β- unsaturated conjugated acid.[13] These data, however, could not be explained by the structure 26 of the original molecule, which would have given a compound containing at least one saturated methyl group and more than one olefinic proton. Although the presence of an α,β- unsaturated cyclopentenone could be inferred from the IR spectrum of the compound 30 by presence of a peak at 1695 cm^{-1}, it was felt desirable to furnish further proof to show the presence of cyclopentenone in the molecule 27. Preferential reduction of the ester did not take place in the above reaction even with calculated quantity of LAH. Controlled LAH reduction using reversed addition procedure[10] failed to yield the keto primary alcohol but gave instead a hydroxy ester (28) by preferential reduction of the keto group. The hydroxy ester was identical in its IR spectrum, with the one (Fig. 12) obtained by sodium borohydride reduction of 27. Some diol mixture (31 & 31a) was also formed due to complete reduction of both the

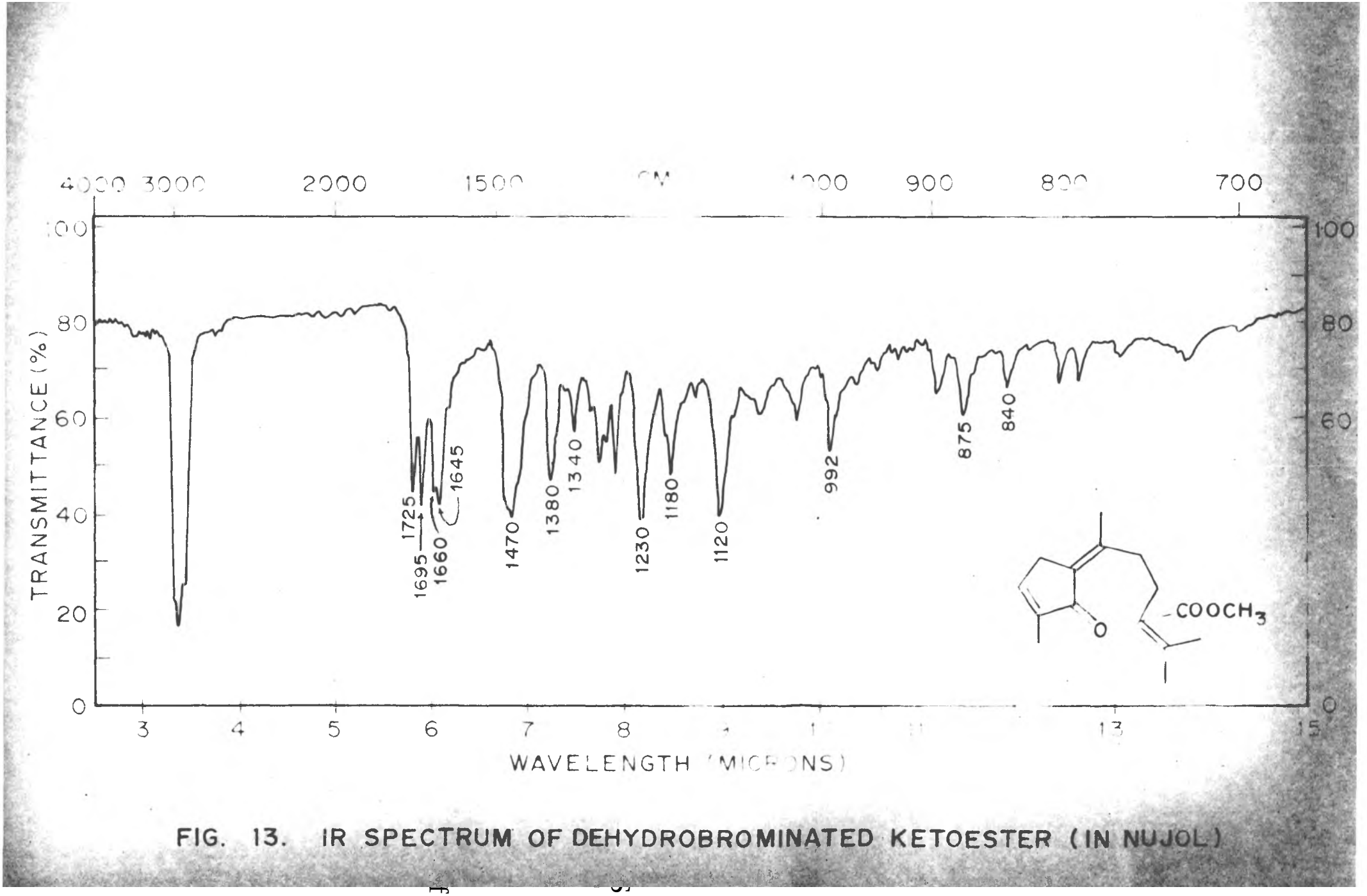


FIG. 13. IR SPECTRUM OF DEHYDROBROMINATED KETOESTER (IN NUJOL)

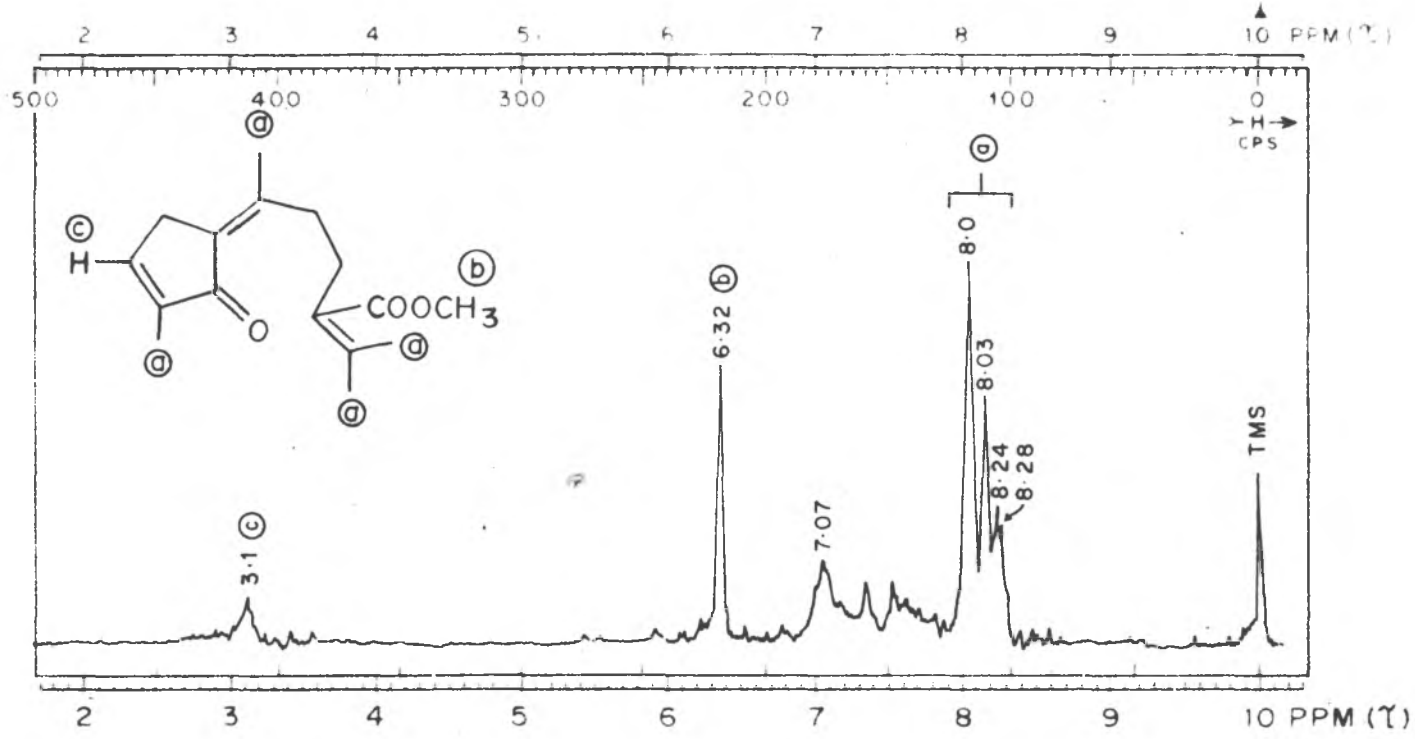


FIG. 14. NMR SPECTRUM OF DEHYDROBROMINATED KETOESTER.

functional groups. Attempts to prepare monotosylate by preferential tosylation of primary hydroxy group followed by LAH reduction of the same to furnish a mixture of epimeric secondary alcohols were also unsuccessful.

Although the above experiments would strongly support the guaianolide nature of the lactone, the point of attachment of the lactone ring had to be unequivocally established. The absolute stereochemistry of dehydrocostus lactone at the ring juncture as well as that of the lactonic moiety had also to be decided. As such the NMR spectrum (Fig. 15) of dehydrocostus lactone would suggest that the point of attachment of the hydroxyl oxygen atom of the lactone moiety is at C6 and not at C8, as the spectrum shows a triplet at 6.17, 6.04 and 5.88τ (1H) and not a quadruplet, as would have been expected if the attachment were at C8. Similar triplets were also observed in the NMR spectrum (Fig. 15) of dihydrodehydrocostus lactone (12) and the dienic monols (15) and (16). This is also chemically supported by the formation of the conjugated dienone (19) as one of the products of oxidation of the conjugated dienic monol (18). The alternative structure 5 would have resulted in the formation of a product 20 with anisolated keto function.

When the dienic monol (18) was subjected to metal amine reduction,[14] using either sodium or lithium

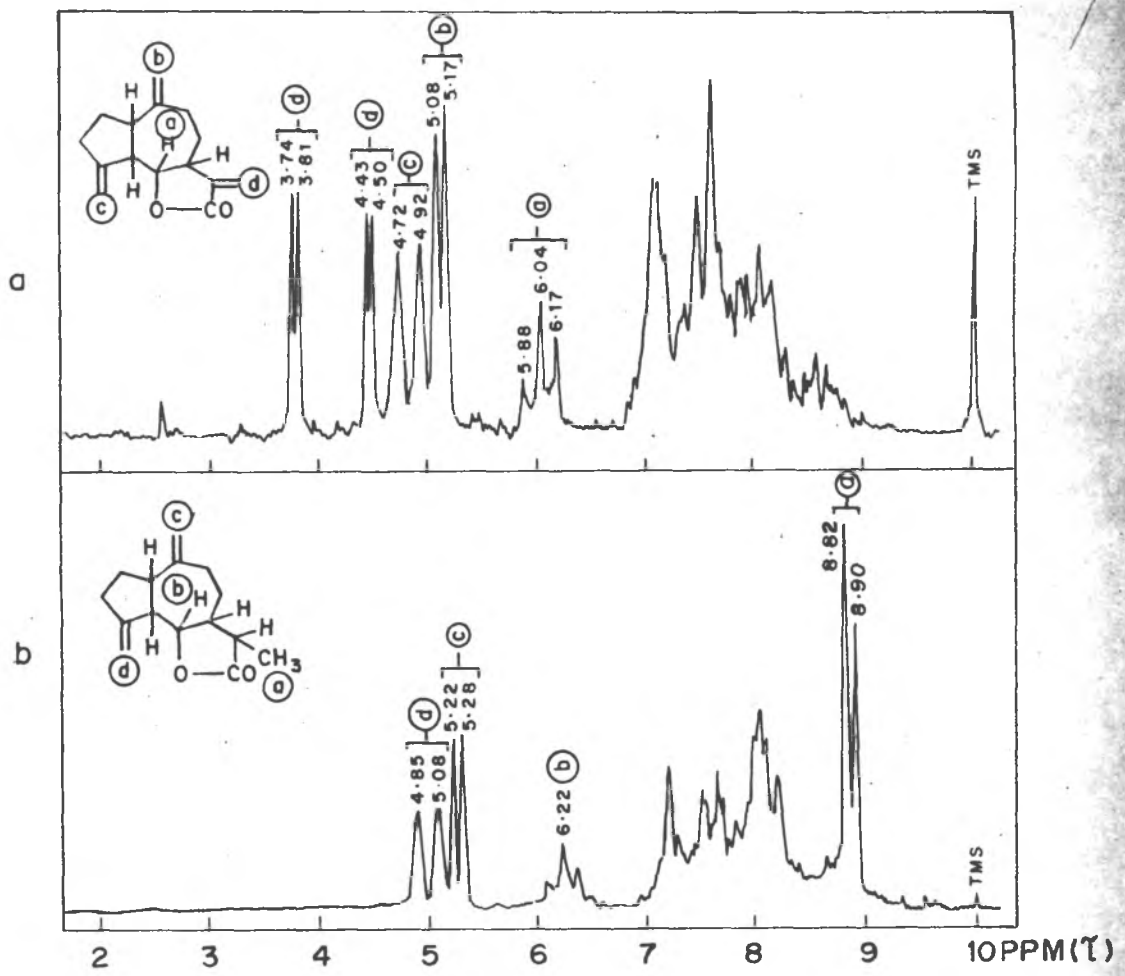


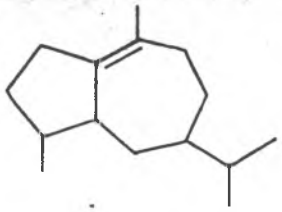
FIG. 15.

(a) NMR SPECTRUM OF DEHYDROCOSTUS LACTONE.

(b) NMR SPECTRUM OF DIHYDRO DEHYDROCOSTUS LACTONE.

in liquid ammonia, a hydrocarbon (32) (IR spectrum has been shown in Fig. 5) was formed as the main product, along with traces of a conjugated hydrocarbon (UV). The structure 32 for the major hydrocarbon is supported by its NMR spectrum (Fig. 6) which showed the presence of three saturated methyl groups (signals at 9.13, 9.07, 8.97, 8.90 τ (9H) and one methyl group on a double bond (signal at 8.38 τ (3H) . In the olefinic region no signals were observed, indicating that the double bond must be tetra-alkylated. The hydrocarbon 32 must have been obtained by further reduction of the conjugated hydrocarbon (33) obtained from the dienic monol (18) as the primary product of hydrogenolysis. The possibility of any allylic migration of the double bond leading to the formation of homoannular diene (34) during metal amine reduction is not likely, as a heteroannular dienic system is expected to be more stable than the homoannular one. The formation of hydro-carbon (32) via the intermediate 33, clearly establishes the position of hydroxyl group in dehydrocostus lactone at C6.

With a view to determine the stereochemistry of dehydrocostus lactone at C_6 and C_7, viz. the stereochemistry of lactone moiety, we were interested in converting the dihydrodehydrocostus lactone to a compound in which both the exomethylene double bonds have migrated inside the ring to tetraalkylated positions in conjugation with one



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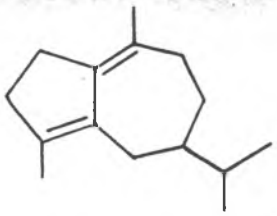

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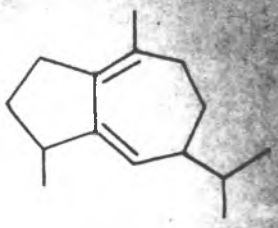

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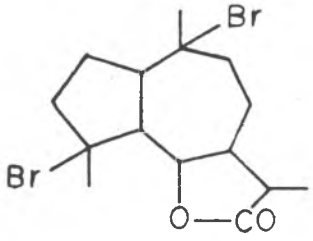

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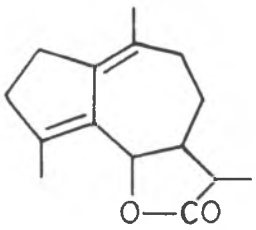

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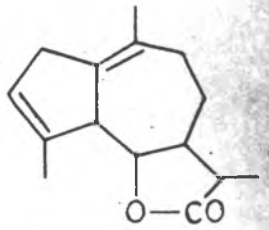

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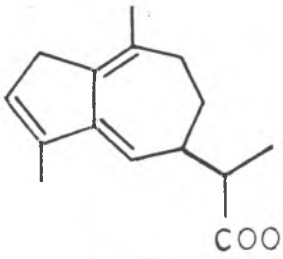
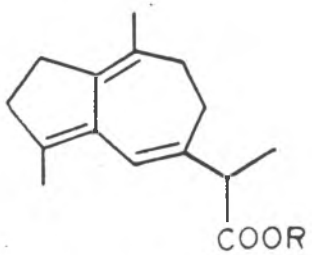


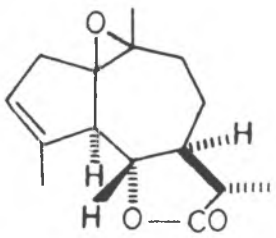
$R = CH_3$

38



$R = CH_3$

39



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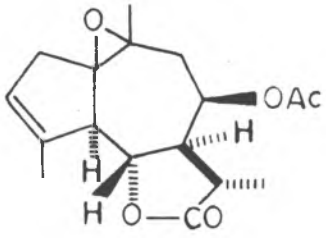

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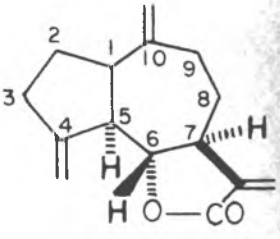

3a

another. This compound would be expected to cleave on ozonolysis to furnish a keto lactonic carboxylic acid from which valuable information about the stereochemistry of the lactone moiety could be obtained. As already the formation of chamazulene was observed by treating dihydrodehydrocostus lactone with alkali at higher temperatures (180-200°), attempts were made to carry on this reaction at lower temperatures, i.e. between 120-160°, so that the two double bonds could migrate inside the ring. But attempts to prepare such a compound were not successful, as the main reaction products were acids and only a small portion of a complex mixture of lactones was obtained during the reaction.

Dihydrodehydrocostus lactone (12) was then hydrobrominated to give a dibromocompound (35), dehydrobromination of which was tried under different conditions using different dehydrobrominating agents, with a view to get the desired compound, but in all these cases the major product was again a mixture of acids along with small quantities of a lactone mixture. When the dehydrobromination was done with alcoholic KOH for 4 hrs. on a steam bath, it was possible to isolate from the reaction products a lactone mixture in about 15% yield. This was chromatographed on gr.II alumina and one fraction was isolated which showed a maxima at 254 mμ with $\varepsilon_{max.}$ 2500.

This indicated that the expected lactone 36 was formed only in small proportions, the major product being a lactone 37 in which the presence of one olefinic proton was indicated in its NMR spectrum. The acids formed during the reaction were esterified with CH_2N_2 and the mixture of esters obtained chromatographed on alumina to get a fraction which showed in UV spectrum peaks at 254 mμ and 288 mμ respectively. Although the peak at 254 mμ could not be satisfactorily explained on the basis of structure 38, the peak at 285 mμ indicated the presence of a conjugated triene system in the molecule which is possible only if the β-elimination of C_6 hydroxyl group takes place. From these reactions it can be only inferred that though the dehydrobromination reaction proceeds mostly in the expected manner, so as to furnish a compound containing a conjugated system of two tetra-alkylated double bonds , it is accompanied by simultaneous β-elimination of the hydroxyl group at C_6 under the alkaline conditions of the reaction. The lactone that could be isolated from this reaction was mainly the one in which the dehydrobromination has taken place in a way different from the desired way, i.e. possibly as is represented in the structure 37.*

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* Further experiments in this regard have been carried out by a colleague, Mr. A.S. Bawdekar, in the laboratory with somewhat better success, though it has not been possible so far to obtain the conjugated dienic lactone 36 in the pure state.

The ORD curve of the saturated ketone (25) (Fig.16) showed a positive cotton effect (a, + 64 units) which did not alter much on addition of HCl, indicating thereby that the ketone group is in a hindered position.[15] This fact supports the attachment of the lactone moiety at C_6-C_7 as the other possibility viz. C7-C8 would have resulted in a non-hindered ketone. Since the reference compounds in the (3:5:0) bicyclodecane system of known absolute configuration are not available, no definite conclusions regarding the stereochemistry of the ring juncture at C_5 and C_{10} could be drawn from the ORD measurements. Since the conformation of a cycloheptanone is not yet definitely known, the octant rule also could not be applied without ambiguity. If the Hudson and Klyne lactone rule[16] which has so far been applied only to saturated γ- and δ -lactones attached to a six-membered ring, can be extended further to γ- and δ -lactones attached to a seven-membered ring also, some idea as regards the stereochemistry at the asymmetric centre C_6 in dehydrocostus lactone, can be inferred. The molecular rotation difference between the saturated lactone hexahydrodehydrocostus lactone (6), prepared from epimerically pure dihydrodehydrocostus lactone (12), and the saturated monol (22) prepared from 6 gives a positive Δm value (+ 22.4) from which it can be assumed that the C_6 asymmetric centre is having the D-configuration. This means that when the formula of hexahydrodehydrocostus lactone is written in the

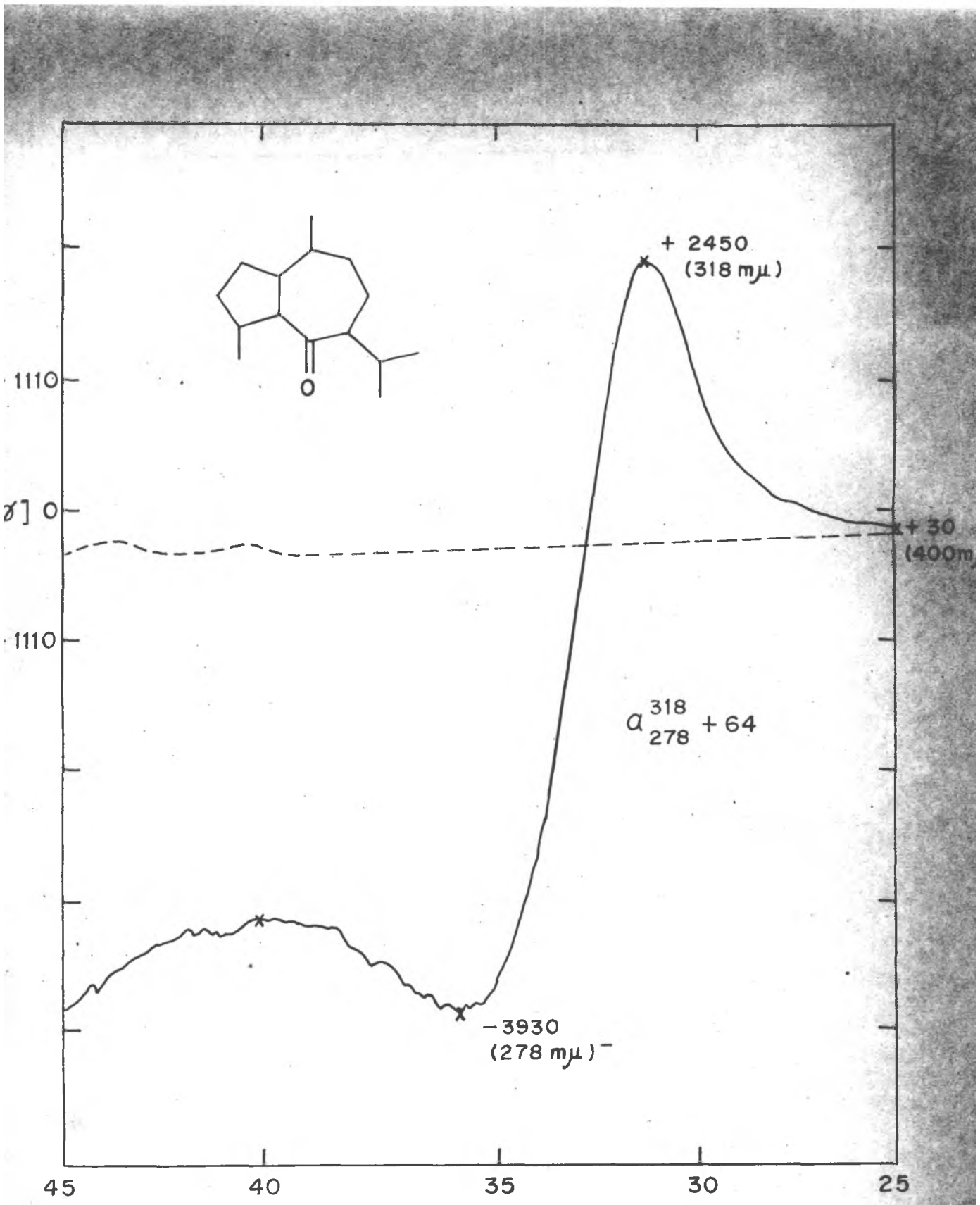

FIG. 16. ORD CURVE OF SATURATED KETONE.

conventional form, the hydrogen at C_6 is β-oriented and the C_6 oxygen is α-oriented.

In the NMR spectrum of dehydrocostus lactone 3 and dihydrodehydrocostus lactone 12 (Fig. 15), cleanly resolved triplets are observed, centred at 6.04 τ and 6.22 τ respectively giving a clear indication of strong coupling between the C_6 proton with C_5 and C_7 protons respectively, with J values of about 8 and 9.6 cps respectively.

It has already been reported[17] that in the case of lactones attached to a seven-membered ring, as in the case of some guaianolides, small differences are observed between the splittings of the H_6 with H_7 in the trans and cis fused lactones, where $J_{6,7}$ is 9.0 c.p.s. and 7.8 c.p.s. respectively. It has also been mentioned that three contiguous protons present in a seven membered ring and strongly coupled to each other, cannot be assigned to an eclipsed configuration, because of prohibitive strain and thus must be due to a trans-trans-arrangement.

An argument of this type has been advanced in the elucidation of the stereochemistry of lactone ring fusion in globicin[18] (41) and arborescin[19] (40). In both the lactones, $J_{5,6}$, $J_{6,7}$ are 10 c.p.s. (taken on a 100 Mc/s instrument), indicating a trans-trans-arrangement of H_5, H_6 and H_7 and hence a trans-lactone fusion as shown.

Since the NMR spectra of 3 and 12 were taken on 60 Mc/S instrument, a direct comparison of the $J_{5,6}$, $J_{6,7}$ values, with those obtained in globicin and arborescin could not be made. Although the NMR spectra of dehydrocostus lactone and dihydrodehydrocostus lactone were taken on a 60 Mc/s instrument only, indications as regards the strong coupling between C_5, C_6 and C_6, C_7 was observed by means of clean triplets centred at 6.04 and 6.22 respectively. Although the J values are not exactly identical, as in the case of globicin and arborescin, they are reasonably close to the observed values, and it is quite probable that an all trans arrangement may be present in dehydrocostus lactone also, as indicated in the structure (3a).

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

Hexahydrodehydrocostus lactone (6)

Dehydrocostus lactone (50 g) (m.p.60-61°; $(\alpha)_D$ - 13° (c, 2.5) dissolved in alcohol (250 ml) was hydrogenated under pressure (1000 lbs/sq.in.) using platinum catalyst (0.25 g) initially at room temperature (6 hr) and then at 50° (6 hr). Alcohol was removed under suction, the residue diluted with water and extracted with ether. Removal of ether furnished a liquid (48 g), purified by distillation, b.p. 130-35°/0.5 mm., n_D^{32} 1.4980; $(\alpha)_D$ + 36° (c, 4.2) (Found: C, 76.68; H, 9.89. $C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.24%).

$NaBH_4$ reduction of dehydrocostus lactone

Dehydrocostus lactone (23.0 g) was dissolved in MeOH (70 ml) and $NaBH_4$ (1.0 g) added in instalments during one hour. The reaction mixture was kept at the room temp. for 24 hr., diluted with water containing HCl and extracted with ether. The ether layer was washed with water and dried. Removal of ether gave a liquid (22.3 g) which was chromatographed on alumina (gr.III; 440 g) and eluted with pet.ether and ether. The pet.ether-eluted fraction(13.0 g) contained mostly the lactone 12, $(\alpha)_D$ + 10° (c, 1.5). The

ether-eluted portion (4 g) on cooling in pet.ether solution gave the solid lactol 13 (1.01 g), crystallised from pet. ether, m.p. 110-111°; $(\alpha)_D$ -36° (c, 2.5).

Controlled LAH- reduction of dihydrodehydrocostus lactone(12)

The lactone (22.0 g) was reduced by gradual addition of an ethereal solution of LAH (1.6 g of 75% purity in 200 ml) under cooling (-10°). The reaction mixture was stirred for 3 hr. at -10° and for another 3 hr. at room temperature. It was then worked up to give a solid (21 g), m.p. 90-95°, which was crystallised from pet.ether to give pure lactol (15 g), m.p. 110-111°, $(\alpha)_D$ - 32° (c, 3.5).

IR bands at: 3497, 1639, 1460, 1377, 1163, 1111, 971, 961, 926 and 900 cm^{-1}.

Analysis

Found: C, 77.46; H, 9.77.

$C_{15}H_{22}O_2$ requires: C, 76.88; H, 9.46%.*

Chromic acid oxidation of lactol (13)

The lactol 13 (1.14 g) was dissolved in acetone (30 ml) and Jones reagent was added dropwise till a brown colour persisted. It was kept at room temperature for 1 hr. and worked up to give a liquid, purified by chromatography

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* Japanese workers report m.p. 110-111°; $(\alpha)_D$ -40° for the lactol.

and distillation to give pure 12 (GLC), b.p. 155-160° (bath)/0.25 mm., n_D^{26} 1.5211; $(\alpha)_D$ + 19° (c, 2.28).

IR bands at: 2950, 1783, 1639, 1449, 1205, 1176, 1122, 1010, 990 and 892 cm^{-1}.

Analysis

Found: C, 77.30; H, 8.58.

$C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.68%.**

Hexahydrodehydrocostus lactone (6) from dihydrodehydrocostus lactone (12)

The epimerically pure dihydrodehydrocostus lactone 12 (7.56 g) dissolved in alcohol (150 ml) was hydrogenated under pressure (1000lbs./sq.in.) using platinum catalyst (0.088 g), initially at room temperature (6 hr) and then at 50° (6 hr). Alcohol was removed under suction, the residue diluted with water and extracted with ether. Removal of ether furnished a liquid (7.32 g), purified by distillation, b.p. 130-135°/0.45 mm., n_D^{26} 1.4965; $(\alpha)_D$ + 18.0° (c, 2.5).

IR bands at: 1783, 1451, 1176 and 985 cm^{-1}.

Analysis

Found: C, 76.42; H, 10.13.

$C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.24%.

** Japanese workers report m.p. 35-37°, $(\alpha)_D$ + 18.2° for 12.

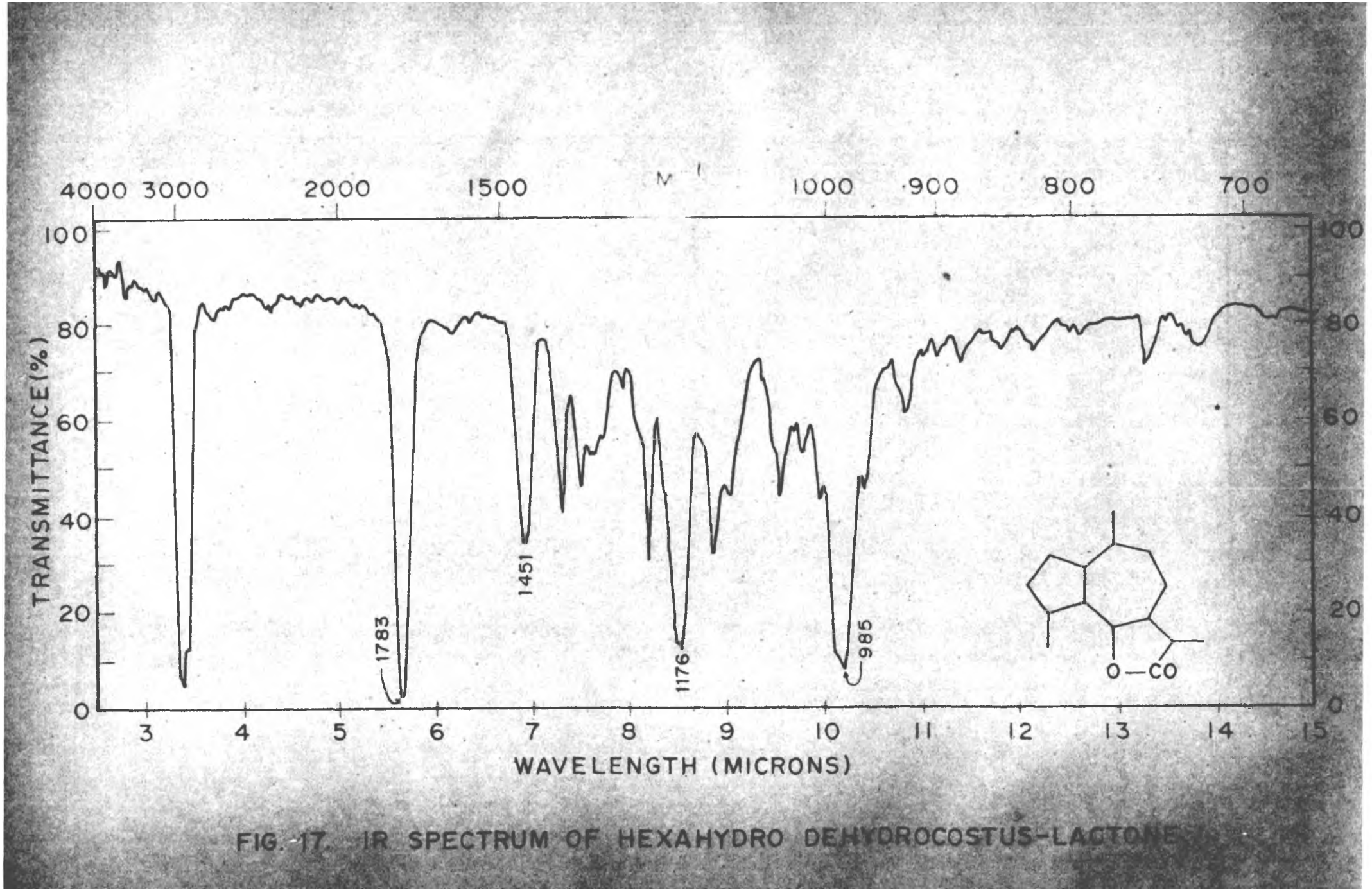

FIG. 17. IR SPECTRUM OF HEXAHYDRO DEHYDROCOSTUS-LACTONE

LAH - reduction of lactone (12) to the diol (14)

The lactone 12 (2.89 g) in dry ether (100 ml) was added to a solution of LAH (1.70 g in 75 ml dry ether) at 0° during a period of 30 min. The reaction mixture was refluxed for 5 hr. The excess of LAH was decomposed by moist ether followed by water and then worked up in the usual way to give a semisolid material (2.49 g) which was then cooled at 0° in pet.ether when crystalline material separated out. This was crystallised from pet.ether to a constant m.p. 53-54°; $(\alpha)_D$ + 36.8° (c, 1.6).

IR bands at: 3448, 1639, 1451, 1379, 1267, 1021 and 892 cm^{-1}.

Analysis

Found: C, 76.15; H, 10.21.

$C_{15}H_{24}O_2$ requires: C, 76.22; H, 10.22%.

Huang-Minlon reduction of lactol (13) at 170-175°

The lactol 13 (18.0 g), dissolved in diethylene glycol (130 ml), was taken in a three-necked flask fitted with a condenser, a thermometer pocket and an inlet for nitrogen. Nitrogen was allowed to bubble through and KOH (18.0 g) and hydrazine hydrate (25 ml; 85%) were introduced. The contents were heated to 170-175° and maintained at that temperature for 4 hr. After cooling, the reaction product was diluted with water and extracted with ether. Removal of ether furnished a dark liquid (15.8 g) which was

chromatographed on alumina (gr.II; 600 g) and eluted as follows:

Fr.	Solvent	Volume (ml)	Weight (g)
1	Pet. ether	1500	4.20
2	Pet.ether+benzene(1:1)	1800	0.6
3	Benzene	1000	0.375
4	Ether	1500	4.20
5	Methanol	1000	5.5

The fraction (4) was rechromatographed on alumina (gr.III; 84 g) and eluted with pet.ether and ether. The fraction eluted with pet.ether gave the dienic monol 18 (1.1 g) (TLC pure) which showed λmax. 252 mμ (εmax. 9000); $(\alpha)_D$ + 45° (c, 1.5).

IR bands at: 3448, 1613, 1439, 1379, 1364, 1203, 1076, 1020 and 990 cm^{-1}.

Analysis

Found: C, 81.28; H, 10.77.

$C_{15}H_{24}O$ requires: C, 81.76; H, 10.98%.

From fraction (1), S-guaiazulene was isolated and characterised through its TNB adduct, m.p. and mixed m.p. 149°.

Controlled LAH-reduction of hexahydrodehydrocostus lactone (6)

The lactone 6 (27.0 g) was reduced by gradual addition of an ethereal solution of LAH (2.60 g; 75% purity in 200 ml) under cooling at -10°. The reaction mixture was stirred for 3 hr. at -10° and for another 3 hr. at the room temperature. It was decomposed with alcohol and water and worked up to give crude hydroxy aldehyde (26.0 g) containing some unreacted lactone and the diol.

Huang-Minlon reduction of above hydroxy aldehyde

A mixture of hydroxy aldehyde mentioned above (26.0 g), diethylene glycol (150 ml), KOH (27.0 g) and hydrazine hydrate (26 ml) was heated at 200-220° for 5 hr. in an atmosphere of N_2 and the product worked up as described earlier to give a liquid (21 g) which was chromatographed on alumina (gr.II; 100 g) and eluted as follows.

Fr.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether	600	6.15
2	Pet.ether+benzene (2:1)	4500	10.8
3	Pet.ether+benzene (1:1)	2000	0.8
4	Ether	500	1.5

The fraction (2) which solidified was crystallised from dilute alcohol to give the pure monol 22 (GLC/TLC), m.p. 53-54°, $(\alpha)_D$ + 6.3° (c,2.1).

IR bands at: 3344, 1451, 1370 and 1015 cm^{-1}.

Analysis

Found: C, 80.37; H, 12.58.

$C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%.

Chromic acid oxidation of the monol (22)

The monol 22 (1.0 g) was dissolved in acetone (20 ml) and Jones' reagent was added drop by drop till a brown colour persisted (about 5 ml). The product was diluted with water and extracted with ether. The ether layer was washed with water, and extracted with Na_2CO_3 aq. to remove the acid. The neutral portion (0.5 g) obtained after removal of ether was chromatographed on alumina (gr.III; 15 g) and eluted with pet.ether to give the corresponding ketone purified by distillation and crystallisation from pet.ether to give the pure (GLC/TLC) ketone 25, m.p. 54-55°, $(\alpha)_D$ - 50°(c, 1.3);

IR bands at: 1705, 1471, 1372 and 1174 cm^{-1}.

Analysis

Found: C, 80.81; H, 11.70.

$C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%.

The Na_2CO_3 extract was acidified with dilute H_2SO_4 and extracted with ether. The acid obtained after the removal of ether was esterified with diazomethane to give

26 or 27 (GLC pure), b.p. 150-55°(bath)/0.6 mm., n_D^{25} 1.5200; $(\alpha)_D$ + 20° (c, 2.0). The NMR spectrum (Fig. 8) indicated the presence of four saturated methyl groups (signals at. 9.12, 9.10, 9.05, 8.90, 8.86 τ(12H).

Analysis

Found: C, 71.60; H, 10.93.

$C_{16}H_{28}O_3$ requires: C, 71.60; H, 10.52%.

$KHSO_4$- dehydration of monol (22)

The monol 22 (2.38 g) and $KHSO_4$ (3.58 g) were taken in a flask fitted with a reflux condenser in an atmosphere of N_2 at 180-190° for 3 hr. After cooling the product was dissolved in pet.ether and chromatographed on alumina (gr.I, 100 g) and eluted with pet.ether to give a mixture (GLC) of two hydrocarbons 23 (2.12 g), b.p. 110-115°(bath)/9.6 mm., n_D^{25} 1.4860 $(\alpha)_D$ - 3.7° (c, 6.4) (F

Analysis

Found: C, 87.00; H, 13.50.

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

Sulphur dehydrogenation of monol (22)

The monol 22 (0.5 g) was heated with sulphur (1.0 g) for 5 hr. at 200-220° in an atmosphere of N_2. The reaction product dissolved in pet.ether was chromatographed on alumina (gr.I, 20 g) and eluted with pet.ether.

The blue coloured fraction was separated into azulenic and non-azulenic parts by 80% phosphoric acid. The azulene part (40 mg) gave the TNB adduct of S-guaiazulene, m.p. 149-150°.

Analysis

Found: C, 61.67; H, 4.80; N,10.23.

$C_{21}H_{21}N_3O_6$ requires: C,61.36; H, 5.13; N,10.21%.

Chamazulene (24) from dihydrodehydrocostus lactone (12)

The lactone 12 (3 g) dissolved in diethylene glycol (50 ml) was heated with KOH (3.0 g) in an atmosphere of N_2 at 180-200° for 5 hr. Water was added and the mixture was extracted with ether. The product (1.5 g) obtained after removal of ether was chromatographed on alumina (50 g., gr.I) and the violet coloured fraction eluted with pet. ether was separated into azulenic and non-azulenic portions by 80% phosphoric acid. The azulene obtained was further purified by chromatography and identified as chamazulene 24 by its IR spectrum (Fig. 10) and the TNB adduct, m.p. 131-32°.

IR bands at: 3021, 1538, 1449, 1370, 1316, 1292, 1252, 1198, 1085, 1058, 1020, 871, 915, 877 and 813 cm^{-1}.

Analysis

Found: C, 61.55; H, 5.02; N, 10.20.

$C_{20}H_{19}N_3O_6$ requires: C,60.45; H, 4.82; N, 10.58%.

Metal-amine reduction of dienic monol (18)

In a 500 ml. three-necked flask, equipped with a mercury seal stirrer, liquid ammonia (150 ml) was drawn. Lithium (0.35 g) was added, with stirring during 15-20 min. A solution of the monol 18 (0.73 g) in dry ether (60 ml) was added gradually and the mixture stirred for 3 hr. Ammonia was allowed to evaporate and the residue decomposed by dropwise addition of alcohol followed by water. After further dilution with water, it was extracted with ether, washed repeatedly with water and dried (Na_2SO_4). After removal of ether, the product was chromatographed on neutral alumina (gr.I; 20 g) and the fraction eluted with pet.ether was distilled to give the hydrocarbon 32 (0.23 g), b.p. 120-125°(bath)/4.5 mm., n_D^{25} 1.4930; $(\alpha)_D$ + 15° (c, 1.36). UV end absorption: ε_{210} 4,350.

IR bands at: 2941, 1667, 1460, 1370, 1319, 1176, 1112, 926 and 833 cm^{-1}.

Analysis

Found: C, 87.10; H, 12.37.

$C_{15}H_{26}$ requires: C, 87.30; H, 12.70%.

Sodium borohydride-reduction of the ketoester (27)

The keto ester 27 (3.1 g) was dissolved in methanol (30 ml) and sodium borohydride (0.63 g) was added during 15 min. with shaking. The reaction mixture refluxed for 30 hr. It was worked up in the usual way to give the

mostly the crude hydroxy acid. This was esterified with diazomethane and purified by chromatography and distillation to give pure (GLC/TLC) ester 28, b.p. 149-150° (bath)/0.15 mm., n_D^{28} 1.4500; $(\alpha)_D^{28}$ - 10.25° (c, 1.56).

IR bands at: 3509, 1739, 1460, 1374, 1268, 1198, 1163 and 1031 cm^{-1}.

Analysis

Found: C, 71.14; H, 11.00.

$C_{16}H_{30}O_3$ requires: C, 71.07; H, 11.18%.

Bromination and dehydrobromination of the ketoester (27)

The ester 27 (2.08 g) was dissolved in carbon tetrachloride (20 ml) and NBS (3.5 g) was added and the mixture was refluxed for 20 hr. After cooling, it was filtered and excess of bromine was removed by washing with sodium thiosulphate solution, dried over Na_2SO_4 and evaporated to give the crude bromo compound (3.50 g), b.p. 125-30°/0.1 mm., n^{23} 1.522; $(\alpha)_D$ - 91.64° (c, 1.41).

Analysis

Found: Br, 50.42.

$C_{16}H_{25}O_3Br_3$ requires: Br, 47.42%.

The bromo compound 29 (3.5 g) was dissolved in pyridine (25 ml) and refluxed for 6 hr. in an atmosphere of N_2. Most of the volume of pyridine was then distilled off and the remaining material after cooling was worked up in the usual way to give a crude product (1.71 g). This was chromatographed on alumina (gr.II; 70 g) and eluted as follows:

Fr.	Solvent	Volume (ml)	Weight (g)
1	Pet. ether	500	-
2	Pet.ether + benzene (2:1)	400	0.721
3	Benzene	200	0.501
4	Ether	150	0.175

The pet.ether-benzene fraction on cooling gave some solid, which was filtered off and crystallised from pet.ether to constant m.p. 95-96°,

UV spectrum: $\lambda_{max.}$ 258 mμ; $\varepsilon_{max.}$ 12,800.

IR bands: 1725, 1695, 1660, 1645, 1470, 1380, 1342, 1230, 1180, 1120, 992, 875 and 840 cm^{-1}.

Analysis

Found: C, 72.88; H, 8.44.

$C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%.

LAH reduction of the keto ester (27)

The ester 27 (0.89 g) was dissolved in dry ether (30 ml) and added to a slurry of LAH (0.48 g. in 30 ml. ether) dropwise at 0°. The mixture was stirred for 4 hr. at the room temperature and refluxed for 2 hr. Excess of LAH was decomposed by alcohol, followed by water. It was then worked up in the usual way to get the mixture of diols 31 and 31a (0.96 g), b.p. 165-70°(bath)/0.8 mm., n_D^{24} 1.4690; $(\alpha)_D$ - 19.41° (c, 2.7).

Analysis

Found: C, 74.20; H, 12.63.

$C_{15}H_{30}O_2$ requires: C, 74.32; H, 12.41%.

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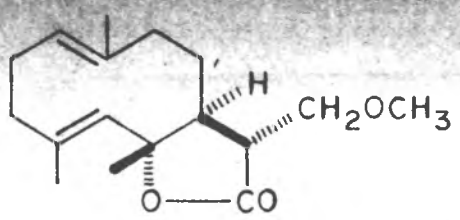
PART IV

STEREOCHEMISTRY OF 12 - METHOXY DIHYDROCOSTUNOLIDE AT THE C_{11} - POSITION

A B S T R A C T

The stereochemistry of 12-methoxydihydrocostunolide (1a) at the position C_{11} has been established by converting both 12-methoxydihydrocostunolide and solid dihydrocostunolide into the same methyl ether (22) by a series of reactions carried out under mild conditions. Since the stereochemistry of solid dihydrocostunolide (3) at the position C_{11} has been rigorously established, the formation of same methyl ether indicates that methoxymethylene group at C_{11} in 12-methoxydihydrocostunolide is β- oriented and not α-oriented as previously inferred on the basis of molecular rotation data and analogy.

In a previous communication[1] from our laboratory the isolation of a methoxy lactone, $C_{16}H_{24}O_3$, from costus root oil has been reported. From degradative experiments and comparison with analogous compounds, it was concluded that the compound is actually 12-methoxy dihydrocostunolide represented by the stereoformula (1a). (The IR and NMR spectra are shown in Figs. 1 & 2 respectively). In the same communication, it was also reported that the methoxy lactone can be very easily obtained from costunolide (2) by the action of methanol in the presence of a trace of base. From the results of experiments published in a series of papers from our laboratory and elsewhere, the absolute configuration of costunolide has been conclusively established[2] as (2). From this it can be safely concluded that the stereochemistry of 12-methoxydihydrocostunolide at the centres C_6 and C_7 would be the same as in costunolide. There was, however, no experimental proof regarding the stereochemistry of the methoxy methylene group at the C_{11} position. It was considered to be β-oriented from analogy with dihydrocostunolide in which the methyl group at C_{11} was taken at that time to be β-oriented[3] as in (3a). In view of the recent crystallographic observations[4], that in α-santonin (4) the methyl group at C_{11} is infact α-oriented and not β-oriented, as was previously assumed, the methoxy methylene group at C_{11} in 12-methoxydihydro-costunolide, should also be α-oriented and that it should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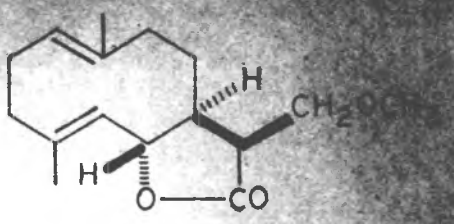
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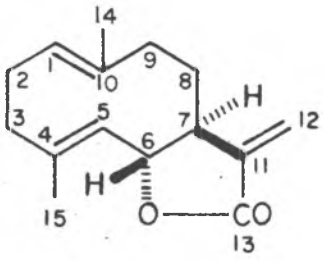
1 a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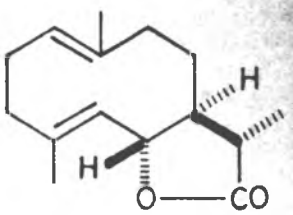
2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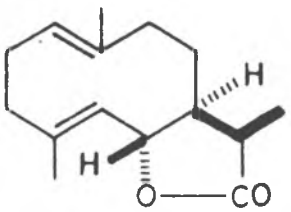
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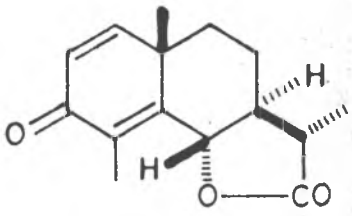
3 a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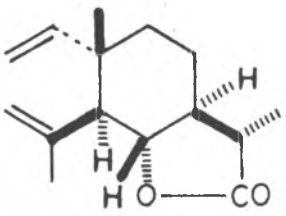
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5

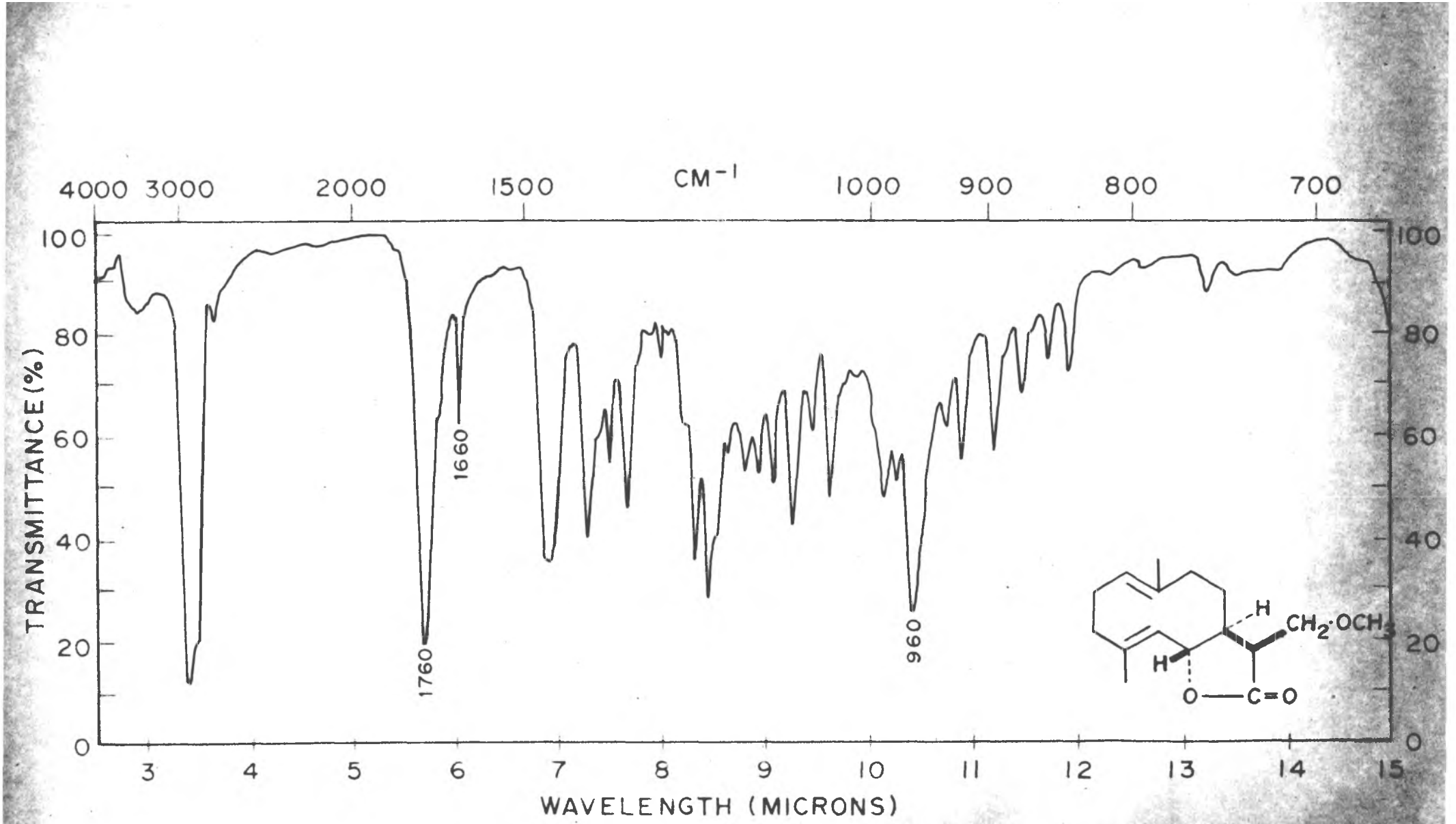


FIG. 1. IR SPECTRUM OF-12-METHOXYDIHYDRO COSTUNOLIDE (IN NUJOL).

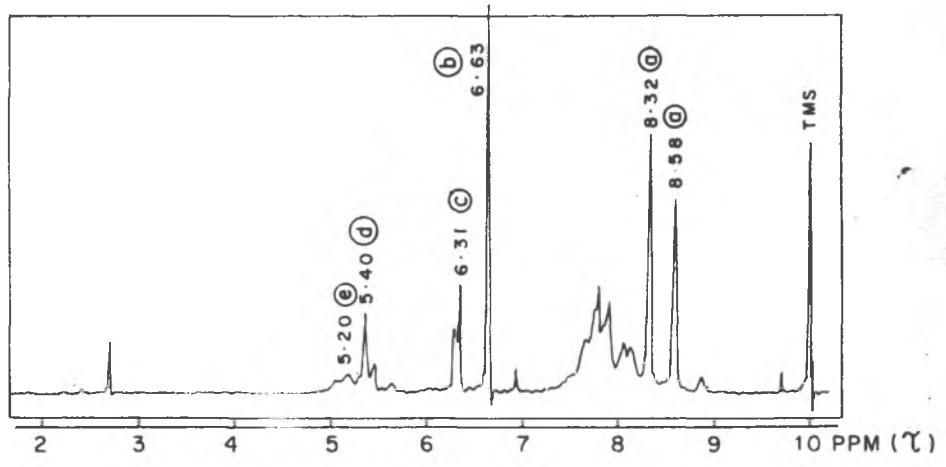
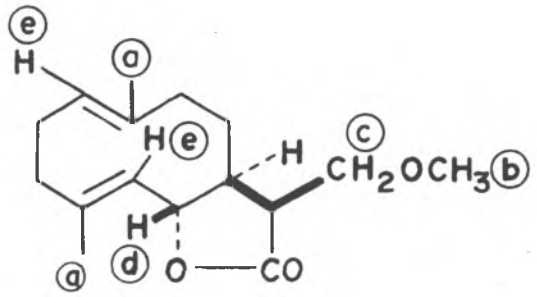
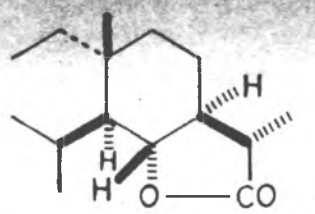


FIG. 2. NMR SPECTRUM OF 12-METHOXYDIHYDROCOSTUNOLIDE.



be represented by stereoformula (1). This contention is supported by the close similarity between dihydrocostunolide (4) and 12-methoxydihydrocostunolide in their spectral and rotation data.

During the course of our study on the stereo-specific pyrolytic rearrangement[5,6] and the acid-catalysed transannular cyclisation[7] of solid dihydrocostunolide (4) and 12-methoxydihydrocostunolide[8] (assuming it to be represented by the structure 1), we have been able to prepare two series of lactones, viz. (i) derived from dihydrocostunolide (4) and hence which contains an α-oriented methyl group at C_{11}, and (ii) derived from 12-methoxydihydrocostunolide, in which the stereochemistry of methoxymethylene group at C_{11} is not definitely known, (but assumed to be α-oriented from analogy). It has been already shown,[5-8] that the stereochemistry at C_5, C_6, C_7 and C_{10} in the two series of lactones derived from dihydro-costunolide and 12-methoxydihydrocostunolide is identical, the only difference in stereochemistry, if there be any, should be at C_{11} in the two series. A comparison of the rotation contribution of the various mono- and bicyclic lactones (5, 6, 9, 11 and 13) obtained from dihydrocostunolide, with those of the corresponding compounds (7, 8, 10, 12 & 14) prepared from 12-methoxydihydrocostunolide, would also suggest that the stereochemistry of the methoxymethylene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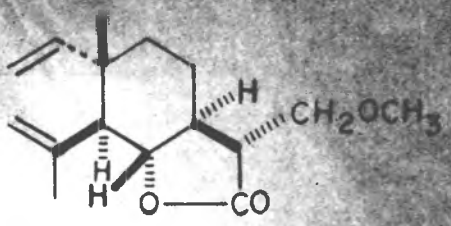
CO

6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H
O
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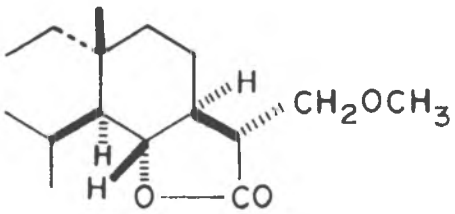
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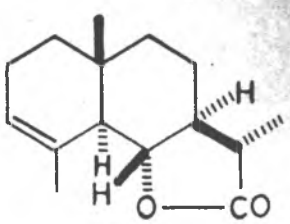
H
O
CO

8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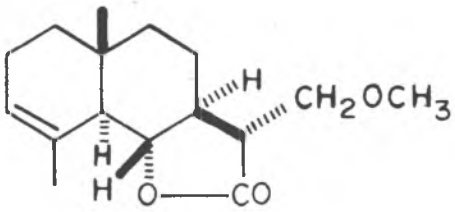
9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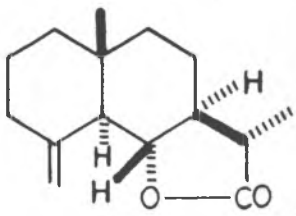
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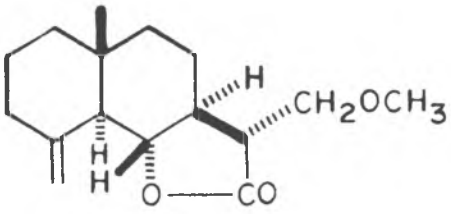
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12

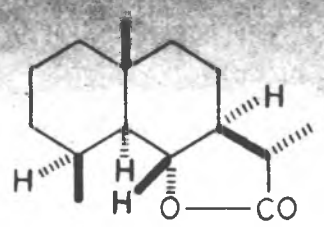
group at C_{11} in 12-methoxydihydrocostunolide and the related compounds should be the same, as in the lactones derived from dihydrocostunolide (4), i.e. the methoxy methylene group at C_{11} should be α-oriented (as shown in the formulae).

The table below gives the molecular rotation and the optical rotations of the lactones obtained in the two series.

The comparison would strongly support the α-orientation of the methoxymethylene group in the lactones derived from 12-methoxy dihydrocostunolide. It, however, does not unambiguously prove the stereochemistry at C_{11}. Some convincing chemical evidences in support of the α-orientation were considered necessary. This prompted us to undertake a series of reactions, results of which are recorded in this part. Astonishingly enough these results have given convincing proof that the methoxymethylene group in 12-methoxydihydrocostunolide is in fact β-oriented and not α-oriented as has been assumed to be during the last several years, and that it is represented by the stereoformula 1a. The experiments employed for this purpose are described in the subsequent pages.

In one of our recent publications,[9] we have described the metal amine reduction of dihydrocostunolide (4)

	Lactones from dihydrocostunolide			Lactones from 12-methoxydihydrocostunolide		
No.	Name of the lactone	$(\alpha)_D$	M_D	Name of the lactone	$(\alpha)_D$	M_D
1	Dihydrocostunolide (4)	+113°	+264.2°	12-Methoxydihydro-costunolide (1)	+115°	+303.6°
2	Saussurea lactone (5)	+ 66°	+154.5°	12-Methoxysaussurea lactone (7)	+ 78°	+205.3°
3	Tetrahydrosaussurea lactone (6)	+ 42°	+99.58°	Tetrahydro-12-methoxy saussurea lactone(8)	+ 35°	+117.9°
4	α-Cyclodihydro-costunolide (9)	+ 84°	+195.6°	α-Cyclo-12-methoxy dihydrocostunolide(10)	+ 83°	+218.4°
5	β-Cyclodihydro-costunolide (11)	+140°	+327.1°	β-Cyclo-12-methoxy-dihydrocostunolide(12)	+146°	+385.5°
6	Santanolide 'c'	+56°	+132.1°	12-Methoxy-santanolide 'c' (14)	+69°	+182.5°



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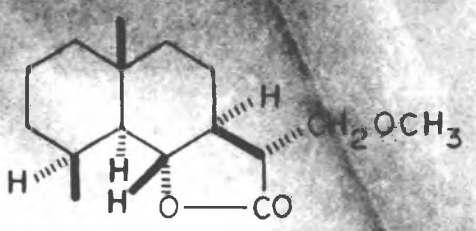

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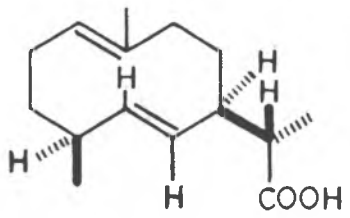

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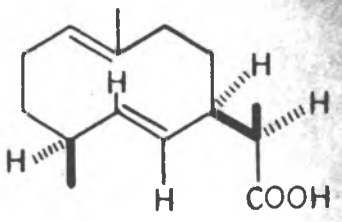

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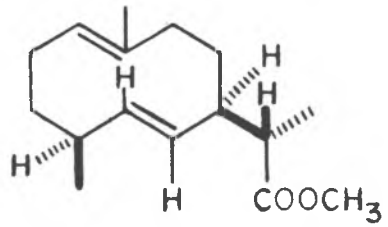

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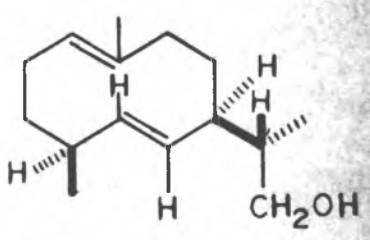
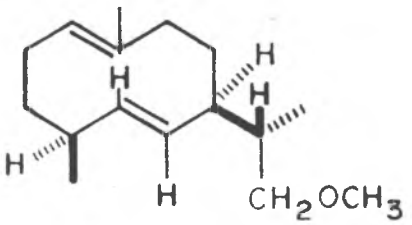


17



18 a

to give an acid, $C_{15}H_{24}O_2$ (15), characterised through its methyl ester, $C_{16}H_{26}O_2$ (16) (IR spectrum shown in Fig. 3). The structure and stereochemistry of 16 have been determined by its ozonolysis. The ester 16 was reduced (LAH) to the known alcohol 17, $C_{15}H_{26}O$, which was methylated by dimethyl sulphate and alkali to give a mixture (TLC) of two C_{11}- epimeric methyl ethers, presumably (18a & 18b), which showed $(\alpha)_D$- 19°. The reactions from dihydrocostunolide (4) to methyl ether 18, have been shown as a matter of convenience separately in scheme 1. Since the above method gave a mixture of ethers, the alcohol was methylated by diazomethane[10] and traces of BF_3-ethereate at 0° to give only one C_{11}-epimeric ether with $(\alpha)_D$ -133°.*

When 12-methoxydihydrocostunolide (1a) was subjected to metalamine reduction by using lithium in liquid ammonia, a methoxycarboxylic acid (19) was obtained as the desired major product, along with two other demethoxylated products (presumably 15 and 16a) as the minor constituents. These acids were esterified by diazomethane to give a mixture of esters, which were separated by

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* The formation of a methyl ether from the corresponding alcohol by this method, takes place with the retention of configuration as already illustrated by many examples,[10] hence the original stereochemistry of the alcohol (17) at C_{11} is also retained in methyl ether (18a).

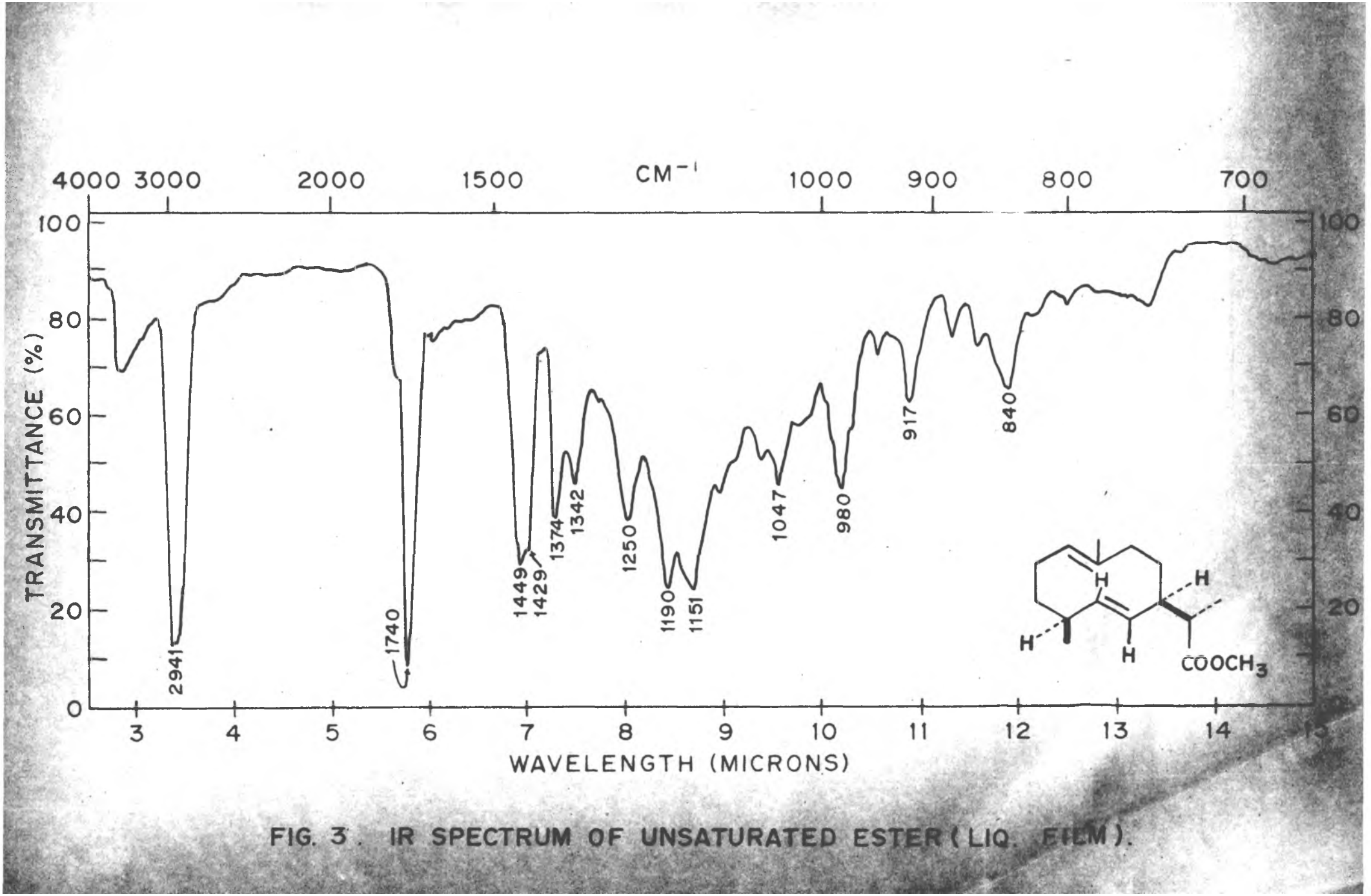
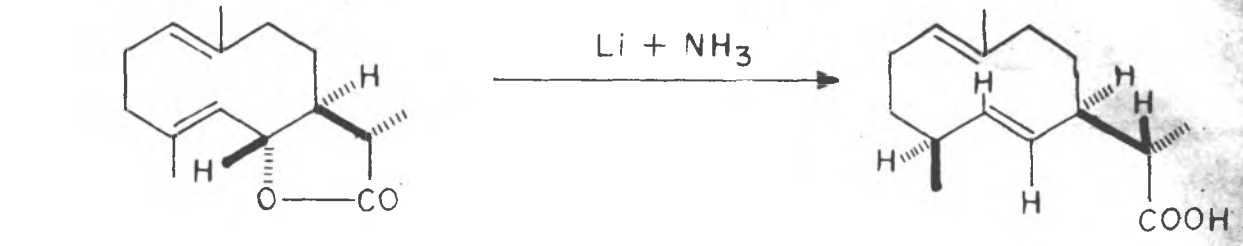
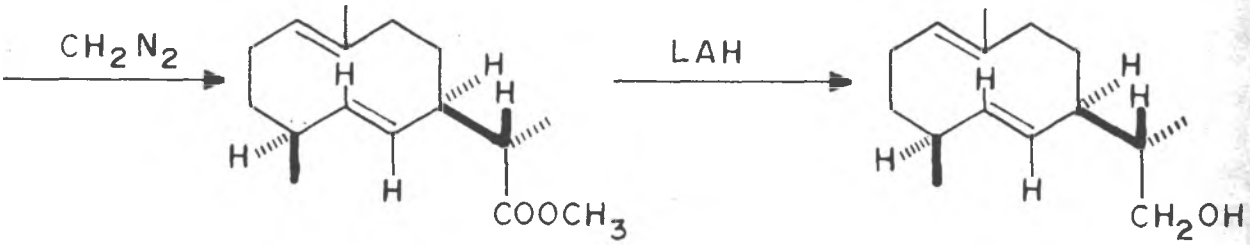
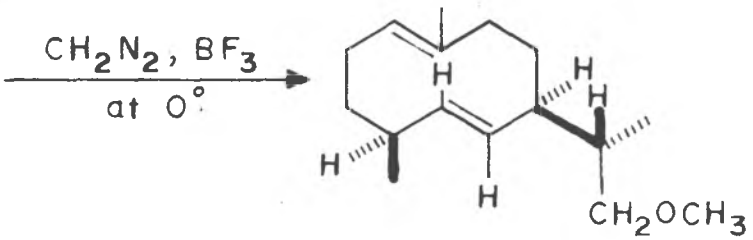


FIG. 3. IR SPECTRUM OF UNSATURATED ESTER (LIQ. FILM).

SCHEME I


Li + NH3
H
H
O—CO
H
H
H
H
COOH


CH2N2
H
H
H
H
COOCH3
LAH
H
H
H
H
CH2OH


CH2N2, BF3
at 0°
H
H
H
H
CH2OCH3

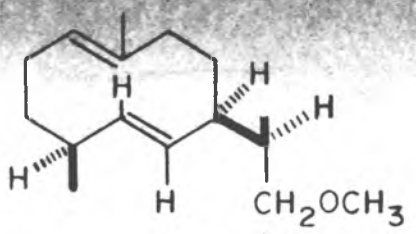
chromatography over alumina. The earlier fractions of chromatography contained a mixture of 15 and 15a, analysing for $C_{16}H_{26}O_2$, the IR spectrum of which resembled that of 16, obtained from dihydrocostunolide(4). The formation of 15 or 15a from 12-methoxydihydrocostunolide can be explained in two ways:

(i) Initial removal of elements of methanol from 12-methoxydihydrocostunolide under reaction conditions to give costunolide (2), which then undergoes hydrogenolysis in the usual manner (as in dihydrocostunolide) followed by the reduction of the conjugated double bond leading to the formation of two epimers, and

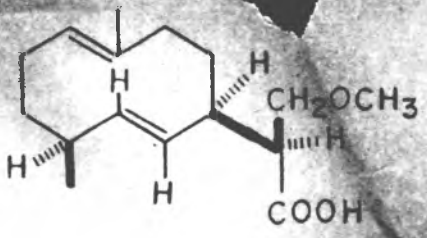
(ii) Initial hydrogenolysis of 12-methoxy [redacted] dihydrocostunolide to give the acid 19, which then loses elements of methanol to give a conjugated acid (derived from costunolide) followed by the reduction of the conjugated double bond.

This is in keeping with the assumption that the addition of methanol to conjugated lactones (esters, ketones etc.) is a reversible reaction.

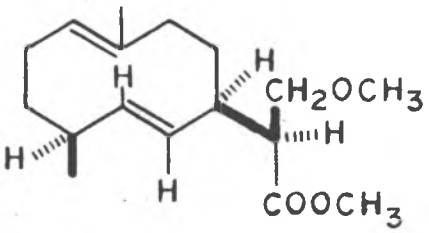
The later fractions of the chromatography contained mostly the desired methoxy ester 20, which was further purified by chromatography and distillation to give pure (GLC/TLC) 20, $C_{17}H_{28}O_3$. Its IR spectrum (Fig. 4) showed



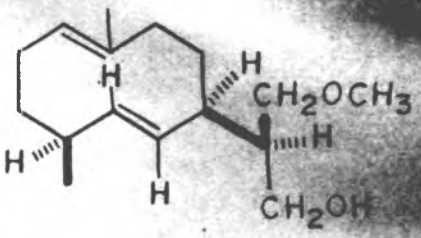
18 b



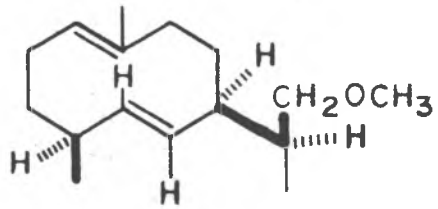
19



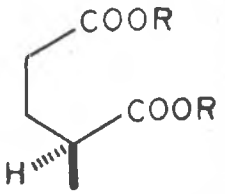
20



21

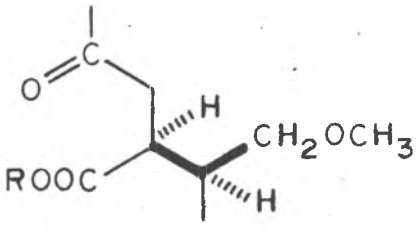


22



23 a R=H

23 b R=CH_3



24 a R=H

24 b R=CH_3

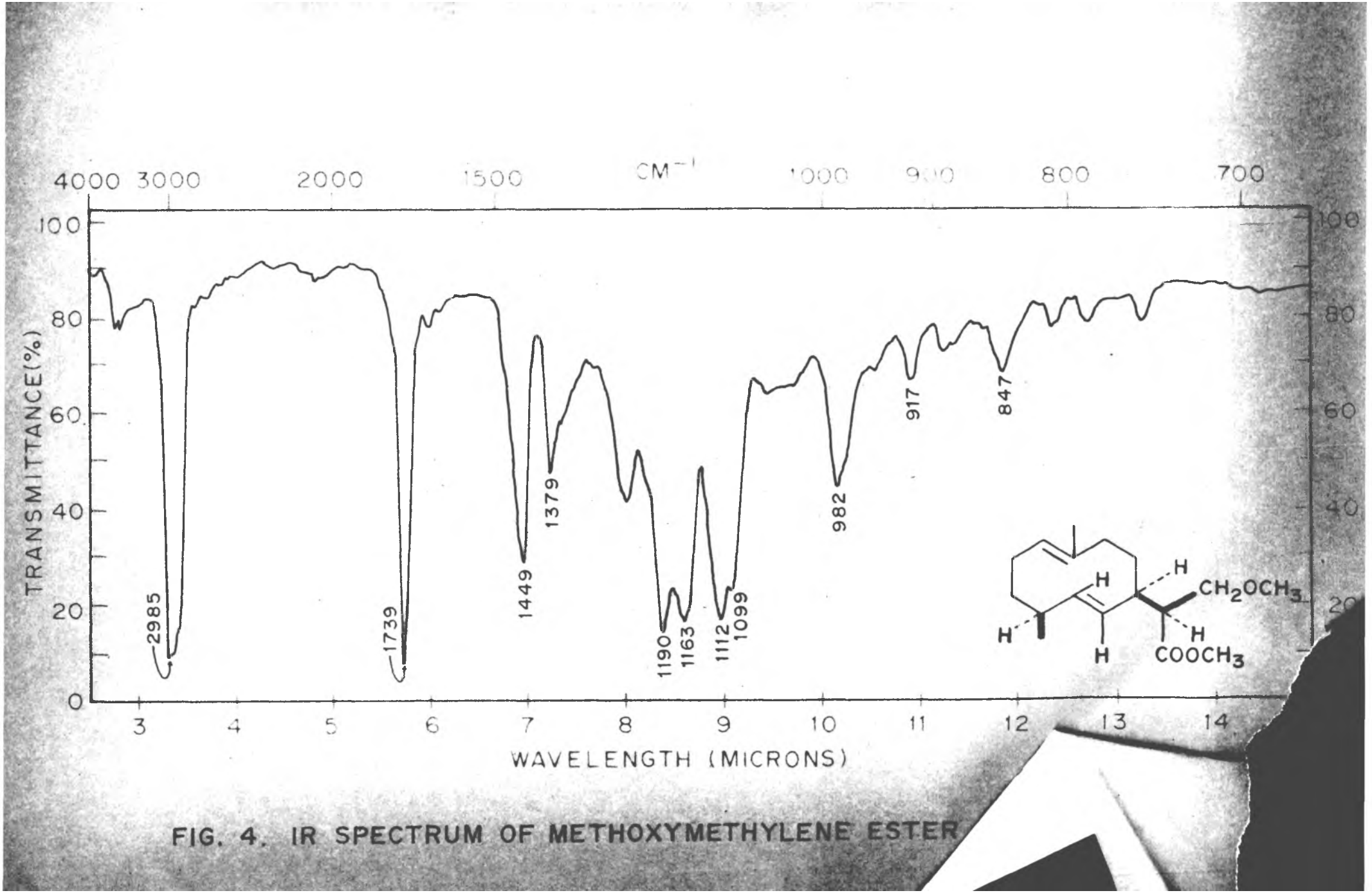


FIG. 4. IR SPECTRUM OF METHOXYMETHYLENE ESTER

bands at: 1660 cm^{-1} due to trisubstituted double bond and also at 980, 970 cm^{-1} due to a trans disubstituted double bond.

The ester 20 was converted into the corresponding alcohol 21 (IR spectrum shown in Fig. 5) by LAH reduction, which was further transformed into methyl ether 22, by tosylation of 21, followed by LAH reduction. This methyl ether 22, showed $(\alpha)_D$ - 133° (IR and NMR spectra shown in Figs. 6 & 7 respectively). The series of reactions from 12-methodydihydrocostunolide (1a) leading to methyl ether 22, have been shown in Scheme II.

The methyl ether 18a (from Scheme I) and 22 (from Scheme II) obtained respectively from dihydrocostunolide(4) and 12-methoxydihydrocostunolide (1a) were found to be identical in all respects. The mixture of two ethers showed single spot on TLC analysis and on GLC analysis give one peak only. Their IR and NMR spectra were superimposable and physical properties were practically identical.

A close examination of the reactions leading to the same methyl ether 22, from both dihydrocostunolide(4) and 12-methoxydihydrocostunolide (1a) carried out under mild conditions, clearly indicates that the methoxymethylene group in 12-methoxydihydrocostunolide (1a) and methyl group in dihydrocostunolide (4) are oriented in

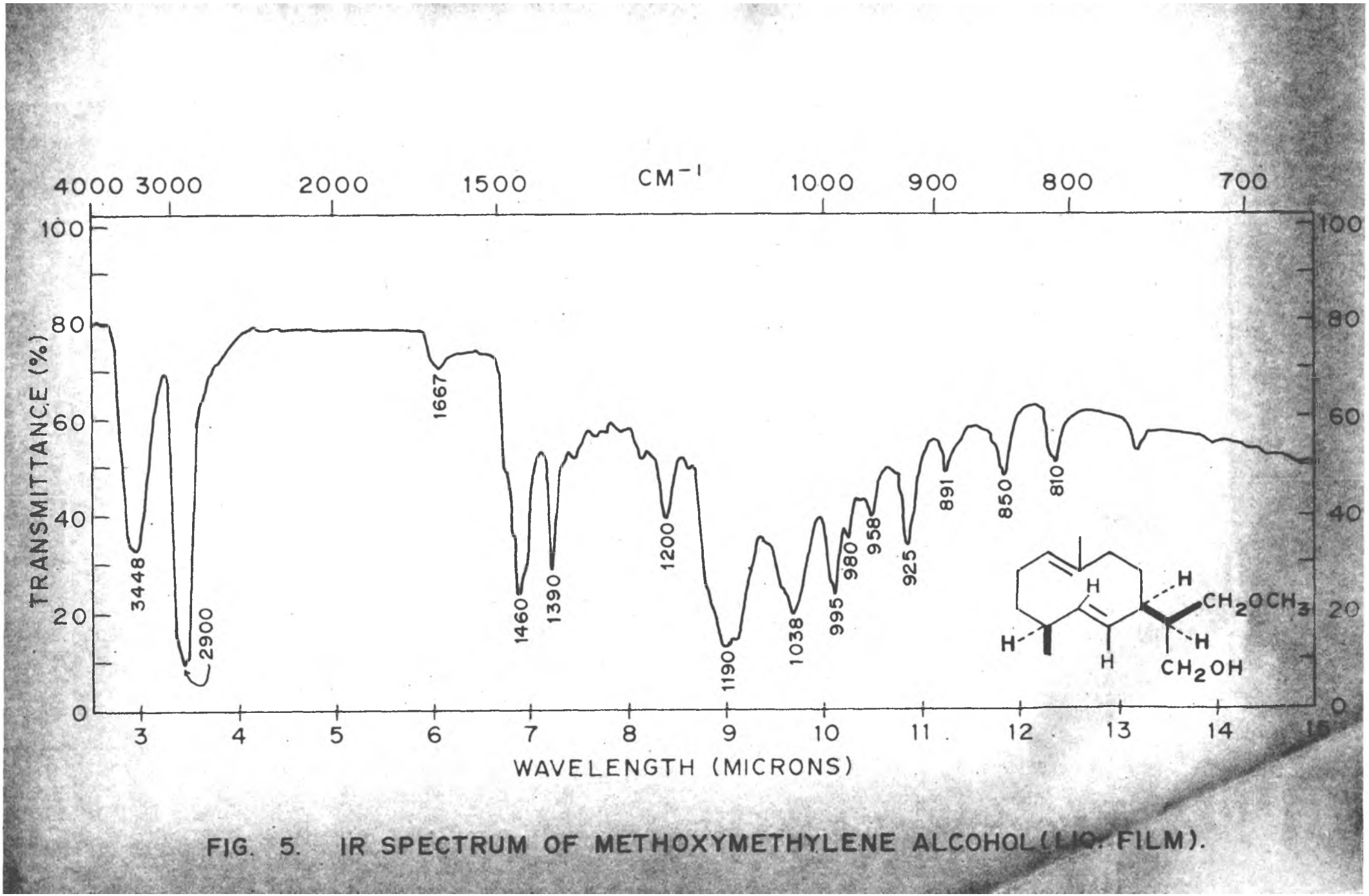
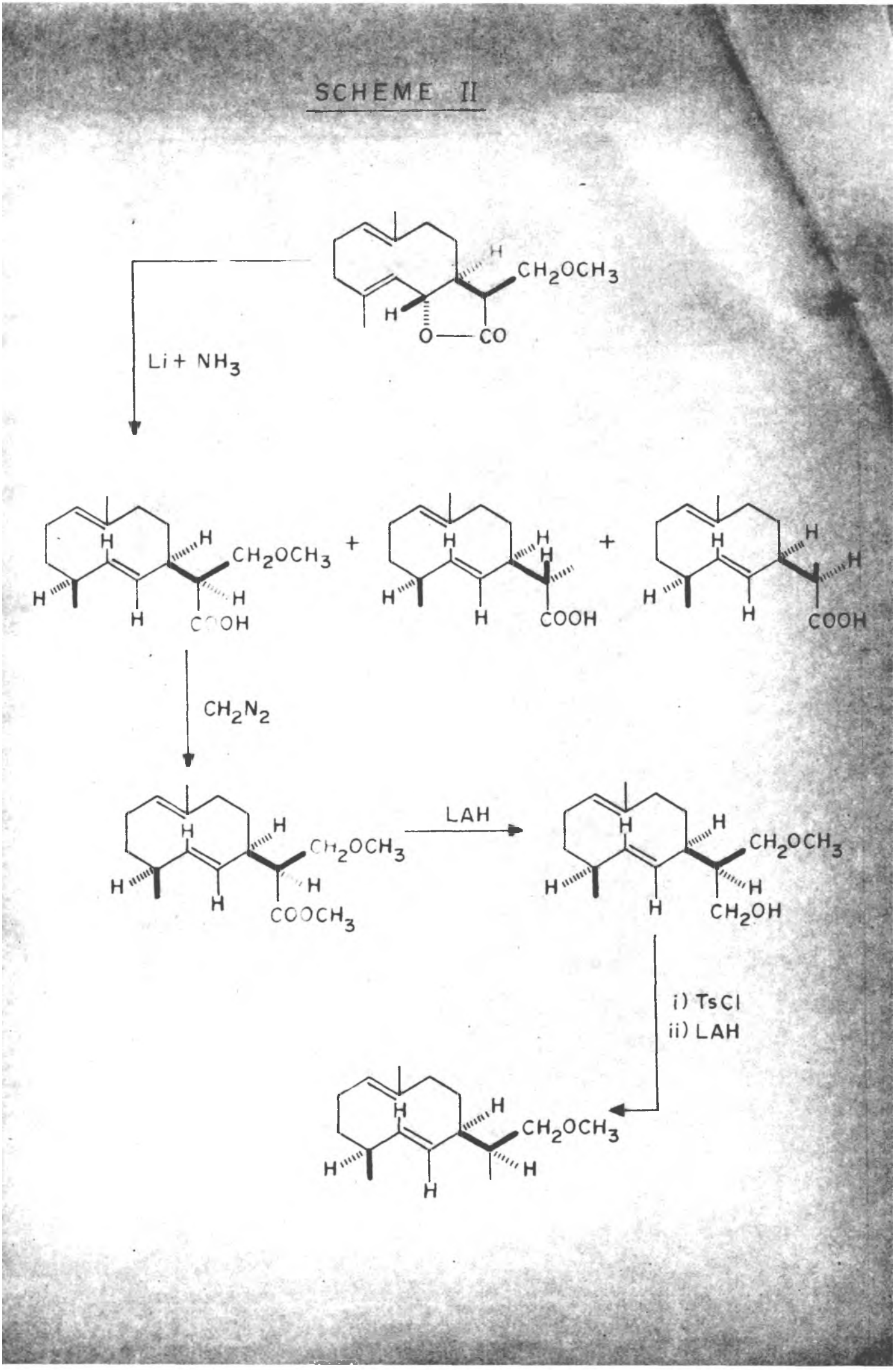


FIG. 5. IR SPECTRUM OF METHOXYMETHYLENE ALCOHOL (LIQ. FILM).

SCHEME II



different manner, i.e. the $-CH_2OCH_3$ group at C_{11} in 12-methoxydihydrocostunolide should be β-oriented.

Further, it was felt necessary to give some additional proof in support of the structure and stereochemistry assigned to the compounds (20), (21), and (22), because firstly it is quite likely that during the metal amine reduction the migration of double bond from position C_4-C_5 to C_5-C_6 may not take place and compounds (20), (21) and (22) may have the double bonds at the same positions as in 12-methoxydihydrocostunolide and dihydrocostunolide. Secondly, the orientation of methyl groups at C_4 in 22 obtained from 12-methoxydihydrocostunolide and of 18a obtained from dihydrocostunolide may be different. This doubt was clarified by subjecting the ether 22 obtained from 12-methoxydihydrocostunolide (1a) to ozonolysis, which gives rise to S(+)-α-methyl glutaric acid(identified and characterised through its methyl ester 23b), and a ketocarboxylic acid 24a, methyl ester 24b, $C_{10}H_{18}O_4$. The formation of S(+)-α-methylglutaric acid establishes the stereochemistry at C_4 in 20, 21 and 22 and shows that the methyl group at C_4 in all the three compounds is β-oriented, as in the case of the corresponding compounds obtained from dihydrocostunolide. This also clearly establishes that the stereochemistry at C_4 and C_7 in methyl ethers prepared from 12-methoxydihydrocostunolide and dihydrocostunolide

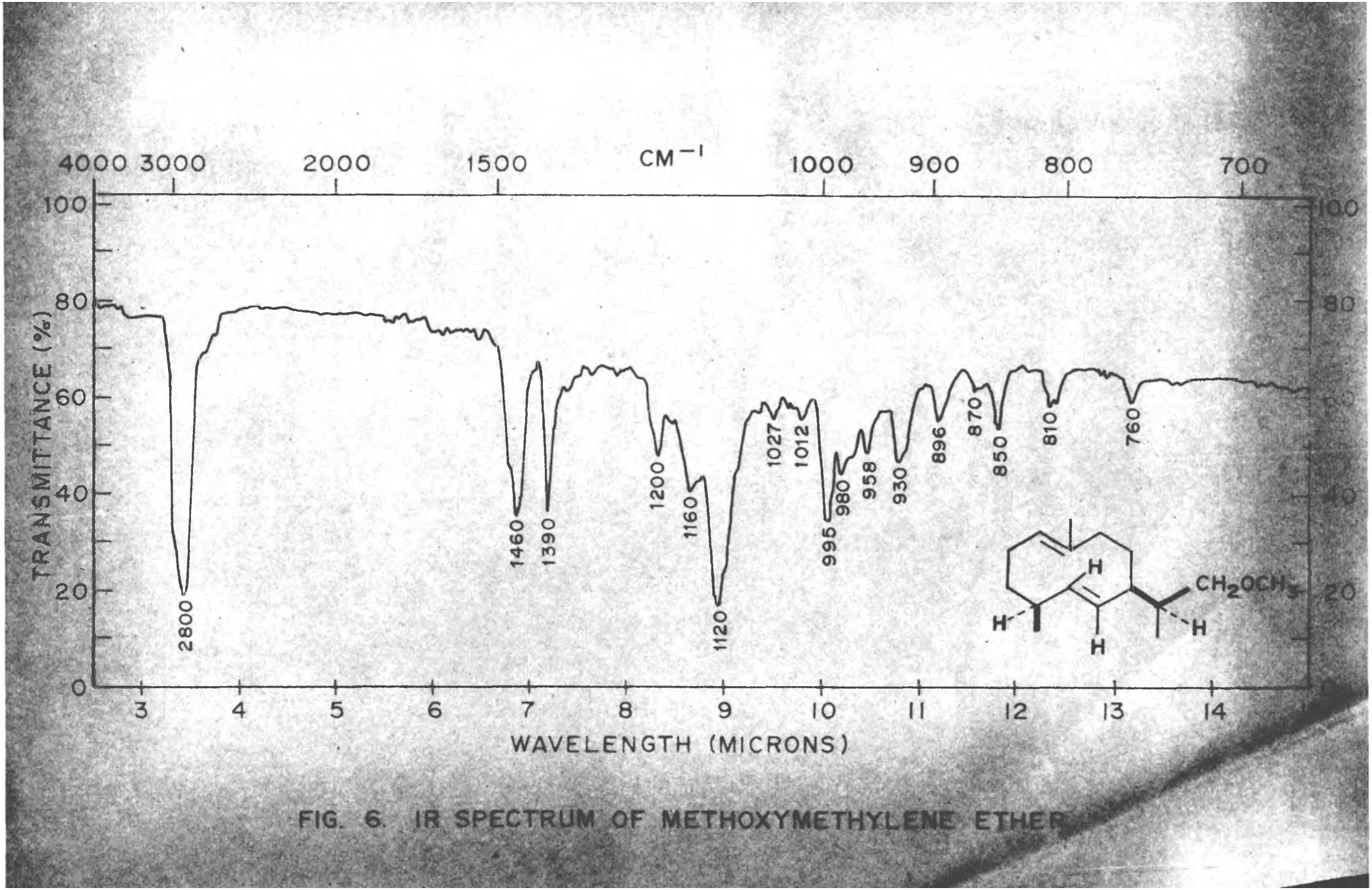


FIG. 6. IR SPECTRUM OF METHOXYMETHYLENE ETHER

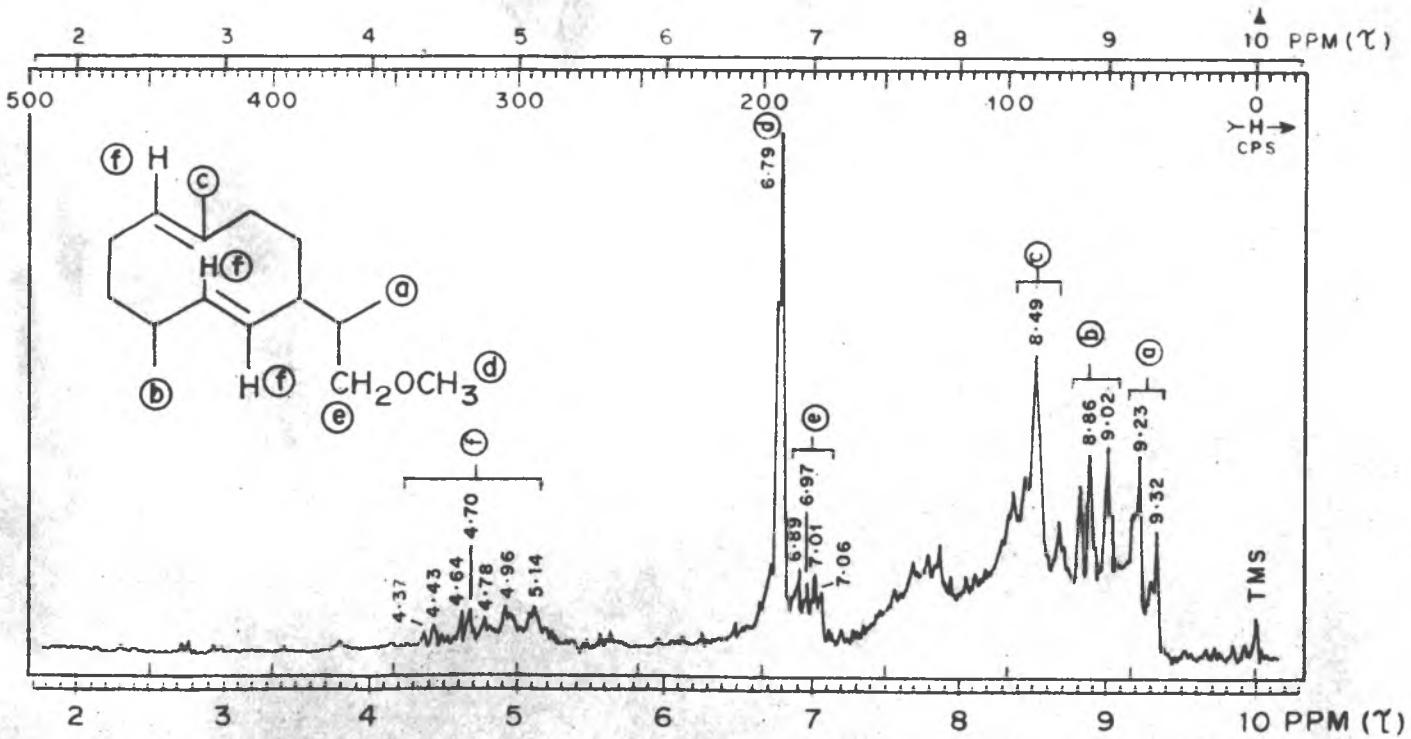


FIG. 7. NMR SPECTRUM OF METHOXY METHYLENE ETHER.

is the same, and the identity of the two ethers in physical constants, spectroscopic data, GLC and TLC behaviour can be explained, if only they are having identical stereochemistry at C_{11} as well.

The methyl ester of ketocarboxylic acid 24a formed a semicarbazone, m.p. 80-81° and which analysed for $C_{11}H_{21}O_4N_3$.

This new finding regarding the stereochemistry of the methoxymethylene group in 12-methoxy dihydrocostunolide would warrant critical re-examination of the stereochemistry of the various products derived from it via pyrolytic rearrangement.

EXPERIMENTAL

Metalamine reduction of 12-methoxydihydrocostunolide

In a 2 l. three necked flask equipped with a mercury sealed stirrer, liquid ammonia (800 ml) was drawn. Lithium (2.3 g) was carefully added during a period of 15-20 min. The ethereal solution of the lactone 1a (10.0 g. in 350 ml) was added dropwise and mixture stirred for 3 hr. Excess of ammonia was allowed to evaporate and the residue decomposed by dropwise addition of alcohol followed by water, which was then added in excess for dilution. It was acidified with dil. HCl (1:1) and extracted with ether. After washing the ethereal layer free of mineral acid, it was washed with sodium carbonate solution (10%) in order to remove organic acid. The sodium carbonate extract was then acidified with dil. HCl (1:1) and again extracted with ether. The ether extract was washed free of mineral acid, dried over anhydrous Na_2SO_4 and evaporated to give a mixture of acids (6.33 g). The methyl esters of acids were prepared by diazomethane in the usual manner. The TLC analysis indicated the presence of three esters, which were then separated by chromatography over alumina (gr.II; 240 g).

No.	Solvent	Volume (ml)	Weight (g)
1	Pet.ether + benzene (3:1)	1800	2.11
2	Pet.ether+benzene (1:1)	1500	2.29
3	Ether	500	0.162

The second fraction contained methoxy ester (20) which was further purified by chromatography and distillation. The purified sample showed the following properties: b.p. 140-45° (bath)/0.1 mm., n_D^{23} 1.4920; $(\alpha)_D^{23}$ - 100.9° (c, 3.3).

IR bands at: 2985, 1739, 1449, 1379, 1190, 1163, 1112, 1099, 982, 917 and 847 cm^{-1}.

Analysis

Found: C, 72.36; H, 9.89.

$C_{17}H_{28}O_3$ requires: C, 72.83; H, 10.06%

LAH reduction of methoxyester (20)

To a slurry of LAH (0.471 g in 30 ml dry ether) was added dropwise, an ethereal solution of the ester (152 g. in 50 ml) at 0°. The stirring was continued at room temperature for 5 hr. Excess of LAH was decomposed by addition of alcohol followed by water. The reaction mixture was worked up in the usual way to give the corresponding alcohol (21) (1.02 g) which was purified

by chromatography and distillation, b.p. 150-155° (bath)/ 0.1 mm., n_D^{25} 1.5040; $(\alpha)_D^{25}$ - 107.2° (c, 3.3).

IR bands at: 3448, 2900, 1667, 1460, 1390, 1200, 995, 925, 891, 850 and 810 cm^{-1}.

Analysis

Found: C, 75.28; H, 11.32.

$C_{16}H_{28}O_2$ requires: C, 76.14; H, 11.18%.

Tosylation of alcohol (21) and its LAH reduction

The alcohol 21 (0.82 g) was dissolved in dry pyridine (15 ml) and freshly crystallised p-toluenesulphonyl chloride (3.2 g) was added. The reaction mixture was kept for 48 hr. at room temperature and then worked up in usual way to give the tosylate (0.913 g).

Ethereal solution of the tosylated (0.91 g in 30 ml) was added dropwise to a solution of LAH in ether (0.5 g. in 25 ml) at 0°. The stirring was continued for 5 hr. at room temperature. Excess of LAH was decomposed with alcohol followed by water and worked up in the usual manner to get the crude product (0.51 g). The crude product was purified by chromatography and distillation to give pure methyl ether 22, 10.20 g, b.p. 120-125° (bath)/0.4 mm., n_D^{24} 1.4911; $(\alpha)_D^{24}$ - 133.4° (c, 2.4).

IR bands at: 2900, 1460, 1390, 1200, 1160, 1120, 995, 930, 890, 850, 810 and 760 cm^{-1}.

Analysis

Found: C, 81.19; H, 12.00.

$C_{16}H_{28}O$ requires: C, 81.29; H, 11.94%.

Ozonolysis of methoxy ether (22)

The methoxy ether (22) (2.5 g) was ozonised in chloroform solution. The ozonide was decomposed by refluxing with water (10 ml) and hydrogen peroxide (5 ml) for 10 hr. After cooling the solution to room temperature, potassium hydroxide pellets (about 1.5 g) were added. The solution was extracted with ether to remove unreacted material. The aqueous layer was then acidified with dilute HCl and saturated with common salt and again extracted with ether repeatedly. The ether layer was washed with brine, dried over anhydrous sodium sulphate and evaporated to give mixture of acids (1.2 g). The methyl ester (1.1 g) was prepared in the usual manner with diazomethane, the mixture of esters thus obtained was fractionated according to their boiling points and following fractions were collected.

Fraction 1. (0.272 g), b.p. 95-100° (bath)/6.0 mm., n_D^{23} 1.4321; $(\alpha)_D$ + 18.73° (c, 1.1).

Analysis

Found: C, 54.91; H, 8.50.

$C_8H_{14}O_4$ requires: C, 55.16; H, 8.10%.

It was identified as α-methyldimethylglutarate (23b).

Fraction 2 (0.3388 g), b.p.110-115° (bath)/0.7 mm., n_D^{23} 1.4409; $(\alpha)_D$ + 6.8° (c, 3.6).

Analysis

Found: C, 58.61; H, 9.06.

$C_{10}H_{18}O_4$ requires: C, 59.38; H, 8.97%.

It was identified as keto ester (24b) which formed a semicarbazone, m.p. 80-81°.

Analysis

Found: C, 51.44; H, 8.18; N, 15.63.

$C_{11}H_{21}O_4N_3$ requires: C, 50.95; H, 8.16; N, 16.26%.

Liquid ammonia reduction of dihydrocostunolide (4)

Liquid ammonia (1500 ml) was drawn in a R.B. flask which is fitted with a mercury sealed stirrer, a dropping funnel and a condenser. Lithium pieces (2.5 g) were added during a period of 15 min. and the stirring continued for another 15 min. The dihydrocostunolide (10.2 g. in 350 ml dry ether) was added slowly using dropping funnel. After the addition of the compound is over the stirring was continued for another 5 hr. Excess of ammonia was allowed to evaporate and lithium was decomposed by adding alcohol. It was then diluted with water, acidified and extracted with ether several times. The ethereal layer washed free of mineral acid and extracted with sodium carbonate solution thoroughly. The carbonate solution acidified and again

extracted with ether. The ether layer was then washed with water to remove free acids and then dried over anhydrous Na_2SO_4. The ether on evaporation gave the acid, which was esterified with diazomethane in usual manner to give the ester 16 (8.35 g). It was purified (GLC/TLC) by chromatography and showed following properties: b.p. 115-120°(bath)/0.1 mm., n_D^{28} 1.4892; $(\alpha)_D$ - 91.4°(c,3.7).

Analysis

Found: C, 75.95; H, 10.52.

$C_{15}H_{26}O_2$ requires: C, 76.75; H, 10.47%.

LAH reduction of the ester 16 to the alcohol 17

The ethereal solution of the ester 16 (5.1 g. in 100 ml. dry ether) was added to a slurry of LAH (0.81 g in 70 ml dry ether) with stirring at 0° during a period of 20 min. When the addition is over, the stirring was continued for another 5 hr. at room temp. Excess of LAH was decomposed by alcohol followed by water. The product was worked up in the usual manner to give the alcohol 17 (4.82 g) and purified further to get pure alcohol (GLC/TLC) and showed the following properties: b.p.125-130°(bath)/0.1 mm., n_D^{28} 1.5034; $(\alpha)_D$ - 98° (c, 3.6).

Analysis

Found: C, 79.79; H, 11.96.

$C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%.

Methylation of the alcohol 17 using CH_2N_2 and BF_3

The alcohol (3.1 g) was dissolved in ether (50 ml containing a drop of BF_3 ethereate) and cooled to 0°. Ethereal solution of diazomethane was added till the yellow colour develops with shaking and the mixture allowed to stand at the same temperature for 45 min. Excess of diazomethane was removed and the ether concentrated to give a product (3.0 g) which on TLC analysis indicated the presence of two compounds, the more polar compound corresponded to the unreacted alcohol 17 on comparison.

This mixture was separated by chromatography over alumina gr.I (1.25) and eluted as follows:

No.	Solvent	Volume (ml)	Weight (g)
1	Pet. ether	600	2.1
2	Benzene	500	0.75

The fraction (1) was found to be pure (GLC/TLC) and comparative TLC indicated it to be identical with ether 22 obtained in Scheme II. The ether obtained by two schemes were identical in all respects. IR spectra were superimposable.

The second fraction was the unreacted alcohol.

The ether thus obtained showed the following properties: b.p.115-120°(bath)/0.5 mm., n_D^{24} 1.4914, $(\alpha)_D^{24}$ - 133.52° (c, 1.2).

IR bands at: 2900, 1460, 1390, 1200, 1160, 995, 930, 890, 850, 810 and 760 cm^{-1}.

Analysis

Found: C, 81.39; H, 12.05.

$C_{16}H_{28}O$ requires: C, 81.29; H, 11.94%.

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