STUDIES

ON

ESSENTIAL OILS

A. S. BAWDEKAR, M.Sc.

1966

ACKNOWLEDGMENT

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A. S. Bawdekar 12/3/66.

A. S. BAWDEKAR.

POONA
March 1966.

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INTRODUCTION

In the course of centuries, various, more or less independent branches, have separated from the common trunk of natural science and each one has made a history of its own. A fairly exact knowledge of those plant products, known as volatile oils has been gained only recently. It seems almost certain that not only the grace and vivid colouring of the flowers but also the fragrance of the vegetation must have aroused the curiosity of man. Thus, early in his history, man evinced a great interest in the preservation of the fragrant exhalations of the plants. The early chemists then occupied themselves with attempts to separate the essences of the perishable plants.

Man, sensitive to all manifestations of beauty, sought to draw out from the plants, their colour and their perfume so that even after the flower

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The author gratefully acknowledges the liberal use made of the following books in compiling the Introduction.

(1) The Volatile Oils, Vol. I by Gildemeister and Hoffman (English translation).

(2) The Essential Oils, Vol. I, by E. Guenther (1948; D. Van Nostrand Co. Inc., New York).

(3) Natural Perfume Materials, by Y.R. Naves and Mazuyer (English translation) (1947; Reinhold Publishing Corporation).

withered, the colour and the perfume might be preserved. He discovered that the woods and the foliage of the trees, of herbs and the flowers would give up their aroma to water, to oils and to fats. It was probably observed that heating of moist plant material caused the odoriferous principle to evaporate along with water which on cooling condensed and formed a mixture consisting of two layers, water and oil. Additional water or steam was later introduced in 'stills' to obtain better yields and quality.

In early work, therefore, the term 'essential oil' or 'ethereal oil' has been defined as the volatile oil obtained by steam distillation of plants. Such a definition is clearly intended to make a distinction between the fixed oils and the oils which are easily volatile. Their volatility and plant origin are the characteristic properties of these oils. For this reason, it is necessary to include in our definition also the volatile plant oils obtained by methods other than direct steam distillation.

The main advantage of this process is the simplicity of equipment and ease of working and it is reasonably satisfactory for many of the oils. But

in the case of those oils which contain constituents likely to be affected by heat and water the process is found to be unsatisfactory, since it is unable to yield all the constituents in their natural condition.

On the other hand, the treatment of freshly gathered plant material, particularly flowers, with fats or volatile solvents, which act only as solvents for the essential oil without any risk of chemical alteration, conserves in the extracted odorous material all the fullness and delicacy of the natural aroma. If the solvent is properly chosen all the chemical constituents responsible for the characteristic odour should be practically unchanged in the extracted product. It will therefore be understood that despite the technical progress of steam distillation, the ancient methods of maceration, digestion and diffusion have been conserved and their practice modernised, and even to this day they constitute the true 'art' of the extraction of natural perfumes.

In the early stages study of essential oils was restricted mainly to the isolation of the oils from the plant material and determination of their physico-chemical properties and their evaluation as perfumery materials.

With the improvements in the techniques of isolation of the constituents of the oils by vacuum distillation, fractionation and chromatography, it has now been possible to isolate the various constituents of the oil in the pure form and also ascertain the fragrant principles of the oils. In addition to the normal chemical methods of reductive and oxidative degradations, the extensive use of spectroscopy such as IR, UV and NMR, along with ORD and mass spectrometry, has greatly helped in establishing the structure and stereochemistry of these constituents.

Examination of the structures of the components obtained from various essential oils has now revealed that a great majority of them belong to a class of compounds known as "Terpenoids". Terpenoids are a class of compounds the basic carbon skeleton of which can be considered to be made up of one or more isoprene units, usually arranged in a head to tail fashion.

In the present discussion the examination of some of the constituents of costus root oil obtained from roots of Saussurea lappa Clarke, by a low temperature solvent extraction procedure using petroleum ether (40-60°) has been dealt with.

Saussurea lappa Clarke, belonging to the family 'compositae', is a tall, sturdy, herbaceous perennial, upto 8 ft. high with large radical leaves and a robust stem bearing a cluster of several bluish-black flower heads. Costus plant, locally called 'kuth' grows abundantly in the Kishengang Valley of Kashmir and in the higher elevations of the Chenab valley. It thrives in shady moist places beneath birch and dwarf willow, at altitudes ranging from 9000 to 11000 ft.[1] It also grows in the upper regions of Kulu valley in the Punjab.

In India, it is known under various names[2,3] such as kuth, kut, kashmirja, kushta, etc. The plant possesses medicinal properties and has been widely used in the indigenous sytem of medicine.[2-4] Its roots are known to contain a fragrant principle. The roots used to be exported to China and Japan for burning as incense in Buddhist temples,[1] and also for the preservation of silk fabrics. The essential oil contained in the roots has attracted the attention of perfumers in India and abroad and is being used in high grade perfumery.

In the past, various workers have used diverse methods for the extraction of the essential oil from the

roots. Hydrodistillation and steam distillation of the roots as well as vacuum distillation of the resinoid were commonly used by many workers.[5-7] The yields obtained were invariably low. The oil has been known to be very rich in lactonic constituents which are not so volatile in steam. Some of these constituents are thermolabile and are denatured on prolonged heating. For this reason, solvent extraction procedure using, petroleum ether,[8] benzene,[9] ether and alcohol[7] has also been employed.

It appears that many of the workers did not fully appreciate the labile nature of the lactonic materials and did not take adequate care during the extraction procedure and subsequent processing. Some of them subjected the oil to vacuum distillation at temperatures as high as 190-200°/11 mm. pressure, to collect different fractions, including the thermolabile lactones.[6,7,10,11] Considering the boiling points of these lactones, the bath temperatures during the distillation must have been as high as 250°, resulting in substantial polymerisation or denaturing of these constituents. For this reason, the information about the constituents reported by different workers is somewhat contradictory and the yields generally poor.[6,7,10-13]

Costus root is a typically valuable Indian raw material and we, in the National Chemical Laboratory, were naturally interested in it. Our objective was to isolate the essential oil in its natural form and characterise its various constituents. We realised at the initial stage, that since the oil was unusually thermolabile, the methods of extraction employed by previous workers were inadequate. Since lactonic constituents which formed a major portion of the oil were the most thermolabile products we felt it necessary to develop a solvent extraction procedure using mild conditions. For this purpose pet.ether (40-60°) was chosen and the extraction was carried out at room temperature. The whole process was so designed that at no stage the operational temperature was allowed to exceed 40 ± 2°. The process was carried out through the pilot plant stage using stainless steel operational units. Strict adherance to temperature control yielded spectacular results. The oil was obtained in a yield of 6.6 % from the best grade Kashmir roots and about 4 % from the Lahaul (Punjab) variety of roots. The oil thus obtained is practically free from polymerisation or denaturing and is in the form in which it exists in the roots. It is fundamentally different from costus root oil isolated by any of the previous workers.

With a view to characterising as many of the constituents of costus root oil as possible, a project was initiated in this Laboratory. The crystalline lactones, from the oil, were initially separated by cooling the petroleum ether solution of the oil successively at 0° and -18°. The residual oil was treated with caustic alkali to remove the free acids. The neutral oil thus obtained was then chromatographed on alumina (grade III) using pet.ether ($40-60^{\circ}$), pet. ether-benzene (1:1), benzene, ether and methanol as eluents. A critical examination of each of these fractions was undertaken.** Part I of this thesis deals with the critical chemical examination of the petroleum ether-benzene fraction (J) and also the water insoluble acids of the oil.

From the water insoluble free acids obtained from costus oil by extraction with alcoholic alkali a new sesquiterpenic acid called costic acid has been isolated. Part IIa of the thesis deals with the structure and absolute configuration of this acid.

** The task of examination of costus root oil was so enormous that a team of six workers, including the author, was working on it simultaneously. This thesis will mainly deal with the work carried out by the author. A mention of other work in this connection will be made in order to maintain continuity in the narrative.

Among the alcohols obtained from costus root oil a sesquiterpenic primary alcohol, costol, forms a major portion of the oil. Costol isolated by previous workers has been shown to be a non-homogeneous product (containing atleast three components) by GLC/TLC analysis. Its presence in the alcohol fraction has been shown by its oxidation to a mixture of acids from which costic acid could be isolated as a major product. This work is described in Part IIb of this thesis.

A biogenetic relationship of the various constituents of this oil has also been brought out in the Appendix.

Part III of the thesis consists of the preparation of mono- and diepoxides of costunolide and dihydrocostunolide. These compounds were prepared with a view to correlate them with the corresponding compounds from the known epoxy lactone, parthenolide, and thus arrive at the absolute configuration of parthenolide.

With a view to elucidate the stereochemistry of dehydrocostus lactone at the lactone ring juncture, hydrobromination of dihydrodehydrocostus lactone was carried out. The dibromolactone thus obtained was dehydrobrominated with a view to get the conjugated

lactone. It was intended to be degraded by ozonolysis to give a keto-lactone carboxylic acid, that could be compared with a compound of the same structure obtained from dihydrocostunolide, since the stereochemistry of the latter is known.

But contrary to our expectations, the desired conjugated lactone which was obtained only in low yield could not be isolated in pure condition. Instead, some other lactones along with a large quantity of acidic material were formed during the dehydrobromination.

Part IV of the thesis deals with an examination of these compounds.

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P A R T I

EXAMINATION OF THE CONSTITUENTS of COSTUS ROOT OIL

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SUMMARY

Costus oil, obtained from the roots of the plant, Saussurea lappa (Clarke), belonging to the Compositae family has been previously examined by several workers. The methods of extraction followed by them were invariably drastic and inadequate, which accounted for the poor yield and unsatisfactory quality of the oil.

A new, low temperature solvent extraction procedure has been developed in our laboratory, which employs petroleum ether (40-60°) as the solvent. The extraction is carried out at room temperature and at no stage the operational temperature is allowed to exceed 40 ± 2°. This led to spectacular results, giving a high yield and a superior quality of the oil. The oil obtained by this procedure was fundamentally different from the oil obtained by any of the previous workers. It also revealed the presence of hitherto undetected compounds some of which are described in this chapter.

The oil is extremely rich in lactonic constituents. Costunolide, a ten-membered ring lactone was isolated from this oil for the first time.

A systematic investigation of the constituents of this oil was undertaken in this Laboratory. This chapter describes the work done by the present author as a part of this project.

Solid, crystalline lactones, costunolide and dehydrocostus lactone, were initially obtained by stage-wise cooling of the oil at 0° and -18°. Free acids were removed by extraction with dilute alkali.

Large scale chromatography of the residual, partially delactonised, neutral oil afforded fractions, I, J, K, L, M and N (Chart II; p. 20). The present discussion deals only with the examination of the Fraction J, eluted with a mixture of petroleum ether-benzene (Charts III and IV, pp..24).

Amongst the hydrocarbons, β-elemene, β-selinene and aplotaxene have been identified by their physical properties and IR spectra.

The other constituents isolated are, a C_{13}-ketone, α- and β- ionones, dehydrocostus lactone, dihydrodehydro costus lactone, saussurea lactone and costol. All these compounds were identified by their physical properties, IR spectra and by preparation of suitable derivatives.

A small quantity of β-sitosterol was also isolated. A solid hydrocarbon, an oxide and two unidentified solid alcohols were obtained in very small amounts.

The water-insoluble free acids of costus root oil were examined and they form the subject matter of Part IIa.

EARLIER WORK

Semmler and Feldstein[1] first examined costus root oil obtained through Schimmel & Co. They subjected it to vacuum distillation and collected several fractions. They have described the presence of several hydrocarbons, ketones, alcohols, lactones and free acids. Despite limited technical facilities available at that time their pioneering work on this oil has proved of considerable importance to the later workers.

Ukita[2] in 1939 extracted costus root oil with petroleum ether, distilled the oil under vacuum, b.p. 179-190°/6 mm., and isolated a lactone, $C_{15}H_{18}O_2$, m.p. 60.5°, in low yield. Carabalona[3] probably isolated the same lactone from the oil after several years standing. This lactone is presumably the same as the dehydrocostus lactone isolated by Naves[4] by vacuum distillation of the oil at 2 mm.

More recently, Šorm and coworkers[5] have critically examined this oil, which they obtained from the Dutch firm of Pollak & Schwarz, Zaandam, presumably isolated by conventional method.

Šorm has reported the presence of humulene, β-elemene, caryophyllene, cedrene, aplotaxene, cedrol,

costol, dehydrocostus lactone and certain other minor components.

They also isolated the same dehydrocostus lactone of Naves and assigned structure (I)[6] to it.

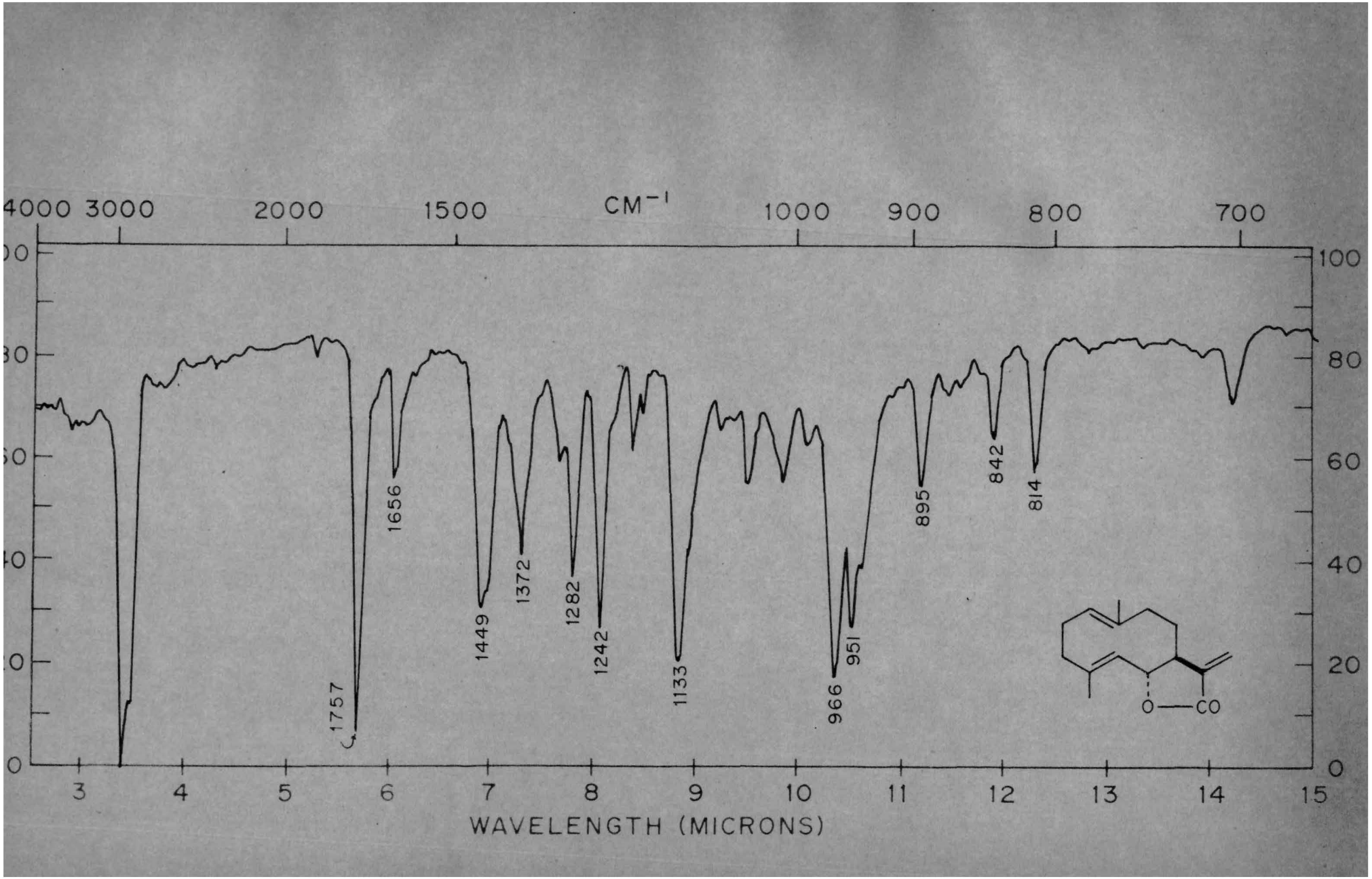
In India, Rao and Varma[7] extracted costus roots with alcohol on a pilot plant scale and then finally subjected the resinoid to vacuum distillation and obtained a fraction, b.p.180-200°/11 mm. which on crystallisation gave a lactone m.p.145-47°. Rao, Varma, Ghosh and Dutta[8] subsequently carried out structural investigations on this lactone which they named as saussurea lactone. Its structure (II, IR Fig.8) has recently been established in our Laboratory.[9]

So far, dehydrocostus lactone was probably the only crystalline lactone isolated from the oil by different workers, though always in poor yield.

PRESENT WORK

The oil used for the present investigation was obtained by following the mild solvent extraction procedure already referred to in the Introduction.

The oil which is extremely rich in lactonic constituents[10] (over 50% of the total weight of the oil), gave a mixture of crystalline lactones by stagewise cooling of the pet.ether solution of the oil at 0° and -18°. This mixture was found to consist of


4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
100
80
60
40
20
0
1757
1656
1449
1372
1282
1242
1133
966
951
895
842
814
O—CO
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

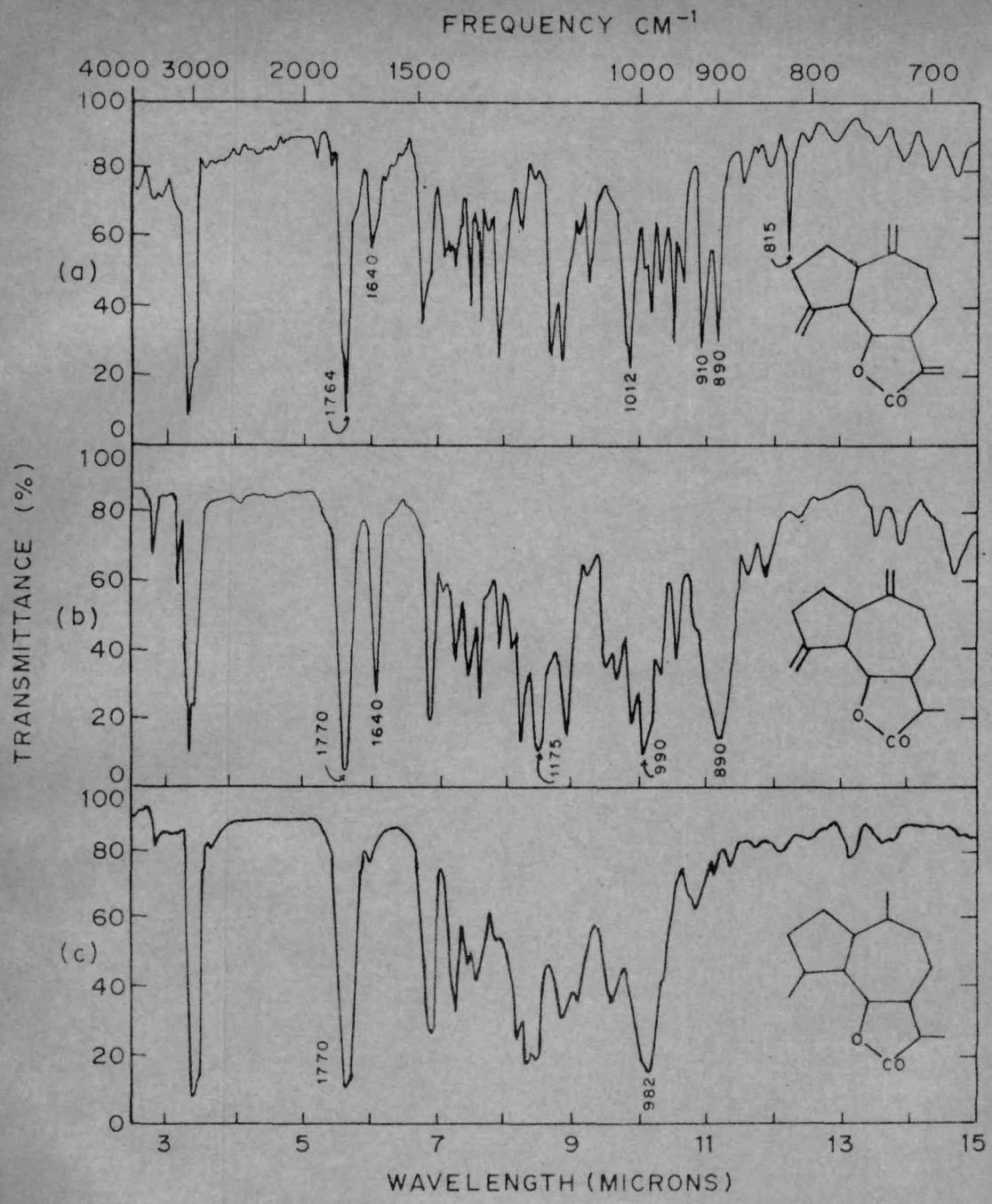


FIG 2.

a) IR SPECTRUM OF DEHYDROCOSTUS LACTONE (IN NUJOL).

b) IR SPECTRUM OF DIHYDRODEHYDROCOSTUS LACTONE (IN LIQUID FILM).

c) IR SPECTRUM OF HEXAHYDRODEHYDROCOSTUS LACTONE (IN LIQUID FILM).

costunolide (III; IR Fig.1) (15% of the oil) and dehydrocostus lactone (I; IR Fig.2a) (35% of the oil). It is of interest to note that costunolide was isolated from this oil for the first time by us. The previous workers missed it because the oil used by them was obtained by a method involving heat treatment. The structure and stereochemistry of costunolide were determined in our laboratory and also by Sorm and coworkers.[11,12] The oil left behind (mother liquor, D, see Chart I) after removal of solid lactones showed an acid value of 27.5. On the basis of the acid value the requisite amount of alcoholic potash was added to the ethereal solution of the above oil and the mixture stirred mechanically and the acids were isolated in the usual manner (details are given in Part IIa).

The neutral oil (F) was chromatographed on neutral alumina in one lot and the column was developed by elution with solvents in the following order: (1) Petroleum ether 40-60°(I); (2) pet.ether-benzene mixture (1:1) (J); (3) benzene (K and L); (4) ether (M), and (5) methanol (N). Each fraction was then processed separately by evaporation of the solvent under vacuum at 40 ± 2°.

The petroleum ether fraction[13] (I) had a saponification value of 26.43 and consisted chiefly of hydrocarbons. It contained β-elemene (IV; IR Fig. 3),

CHART No. I

Scheme of separation of the constituents of Costus root oil

COSTUS ROOT OIL
(8 Kg.)

Mixed with 4 l petroleum ether (40–60°) and kept at 0° for 12 days

CRYSTALS A
(1·9 Kg.)

Chromatographed

Consists mostly of
i) Costunolide
ii) Dehydrocostus lactone
iii) Traces of other products

MOTHER LIQUOR B

Kept in deep freeze at −18° for one week

CRYSTALS C
(2·5 Kg.)

Chromatographed

Consists mostly of
i) Dehydrocostus lactone
ii) Costunolide
iii) Other products in traces

MOTHER LIQUOR D
(Material present 3·6 kg. in solution)

Step-wise extraction with aqueous alcoholic KOH at 0°

AQUEOUS FRACTION E

Acidified

NEUTRAL OIL F
(3·3 Kg)

WATER INSOLUBLE ACIDS G
(100g)

WATER SOLUBLE ACIDS H

(See chart no. 2)

CHART No. II

NEUTRAL OIL F

(3·3 Kg.)

Chromatographed in one lot on 45 Kg. alumina

PETROLEUM ETHER FRACTION I

(672 g.)

(Sap. value 26·43)

Chromatographed thoroughly on grade I alumina

Constituted of

i) β-selinene

ii) aplotaxene

iii) other hydrocarbons

iv) smaller quantities of oxygenated bodies

BENZENE FRACTION K

(335 g.)

(Sap. value 160·4)

Chromatographed

Constituted of oxygenated compounds

BENZENE FRACTION L

(200 g)

(Sap. value 161·4)

Chromatographed

Constituted of oxygenated bodies

METHANOL FRACTION N

(160 g)

(Sap. value 174·9)

Chromatographed

Constituted of oxygenated bodies including a methoxy lactone $C_{16}H_{24}O_3$

m.p. 128°

$[\alpha]_D$ + 107·3°

PETROLEUM ETHER: BENZENE FRACTION J

(560 g.)

(Sap. value 174·8)

Chromatographed

Constituted of series of oxygenated bodies

ETHER FRACTION M

(370 g.)

(Sap. value 167·8)

Chromatographed

Constituted of oxygenated bodies including a diterpenic alcohol $C_{20}H_{34}O$

m.p. 135-136°; $[\alpha]_D$ -40-45°

(The chromatography was done on neutral grade III alumina unless otherwise stated)

β-selinene (V; IR Fig.4), aplotaxene (VI; IR Fig.5); α-ionone (VII; IR Fig.6b), a C_{13} ketone (IR Fig.6d), dihydrodehydrocostus lactone (VIII; IR Fig.2b) and a few unidentified hydrocarbons. Details of the composition of this fraction will not be described here, but a mention may be made of the interesting straight-chain hydrocarbon- aplotaxene-present in the oil. Aplotaxene, $C_{17}H_{28}$, having four double bonds is a major constituent of costus oil and the somewhat disagreeable odour of the oil can be attributed partly to this hydrocarbon. We undertook the structure elucidation of this compound and while we were carrying out initial experiments, a paper by Šorm and coworkers,[14] on the structure of aplotaxene, appeared. We, therefore, discontinued the work on this hydrocarbon. The NMR spectrum (Fig.5a) is in agreement with the proposed structure (VI). The physical constants of our compound were in good agreement with those reported by the Czech workers.

Examination of pet.ether-benzene fraction J.[15]
(Charts III and IV)

Fraction J (Chart II) eluted with pet.ether-benzene mixture (1:1) in the main chromatography, was rechromatographed on grade III alumina and a large number of fractions were collected employing solvents

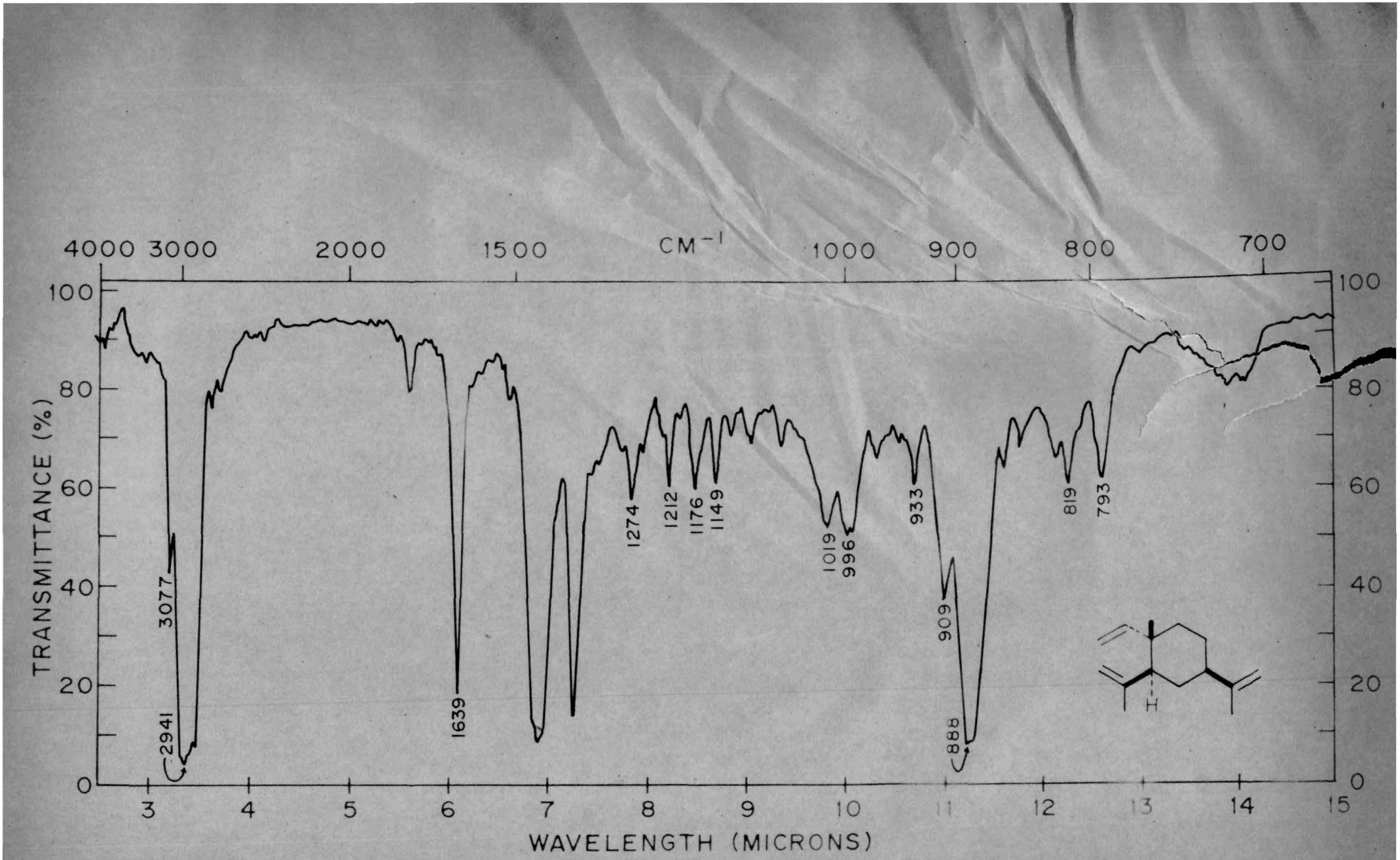


FIG. 3. IR SPECTRUM OF β-ELEMENE (LIQUID FILM).

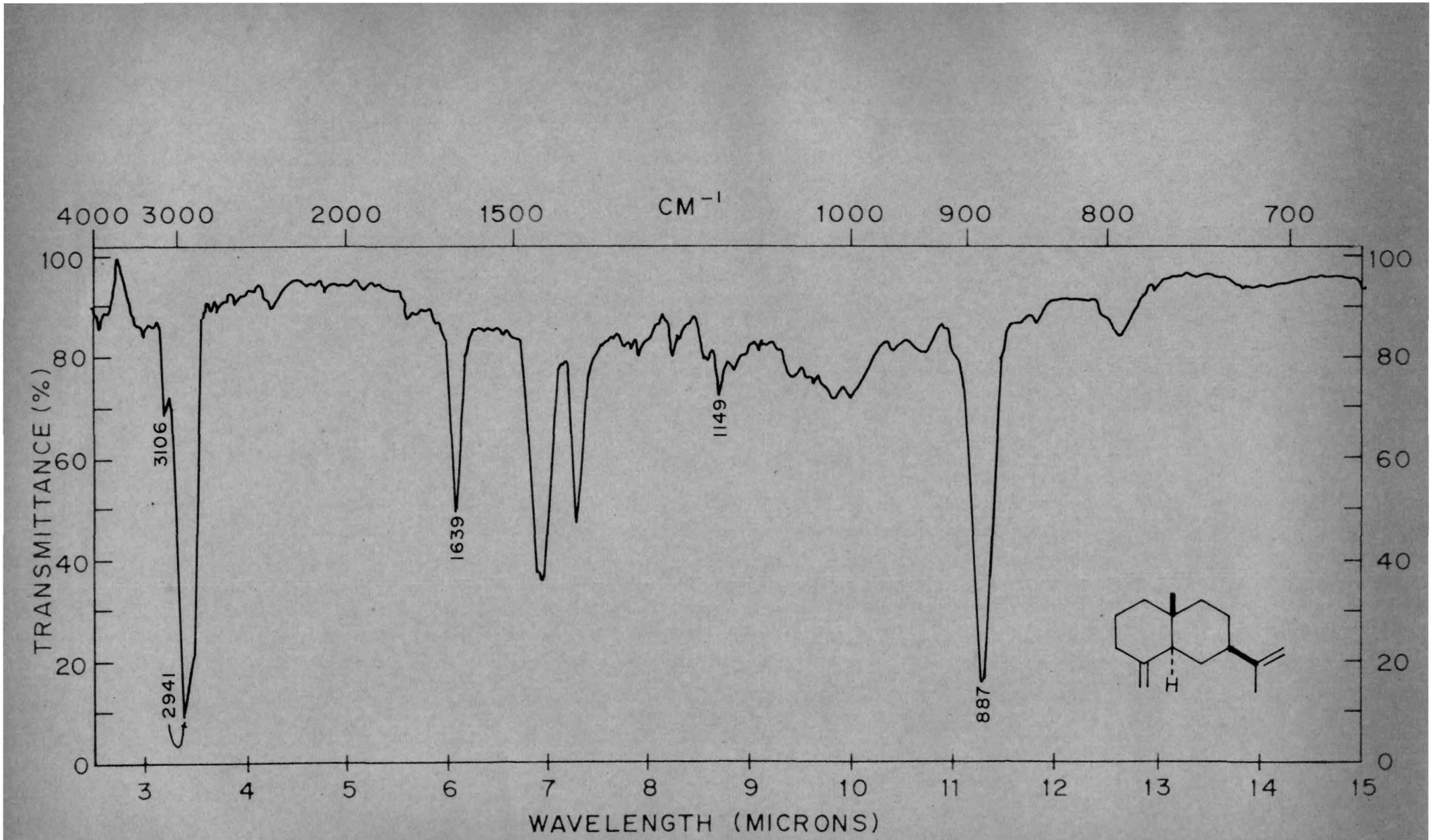


FIG. 4. IR SPECTRUM OF β-SELINENE (LIQUID FILM).

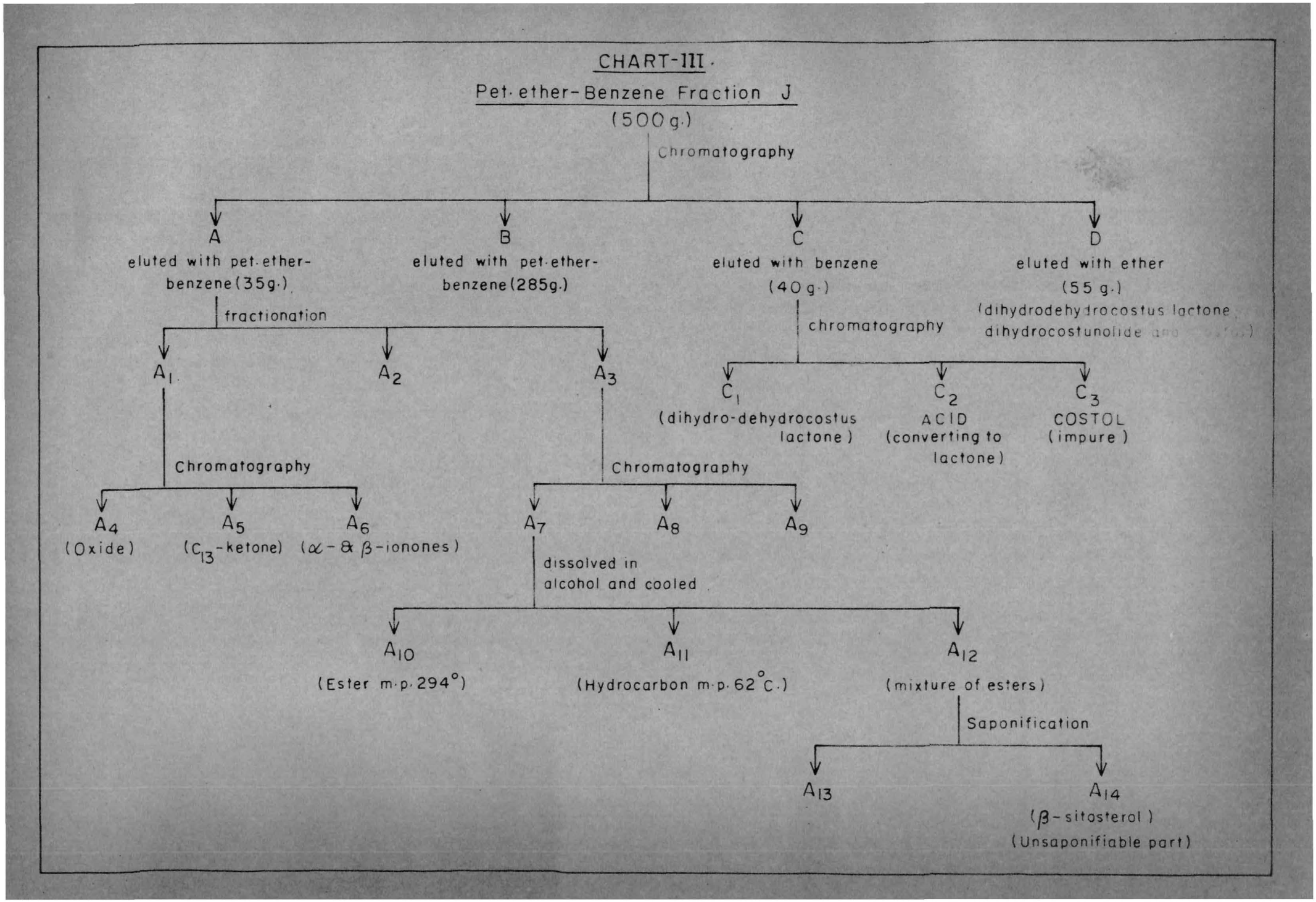


CHART-III
Pet. ether-Benzene Fraction J
(500 g.)
Chromatography
A
eluted with pet. ether-benzene (35g.)
B
eluted with pet. ether-benzene (285g.)
C
eluted with benzene (40 g.)
D
eluted with ether (55 g.)
(dihydrodehydrocostus lactone dihydrocostunolide and
fractionation
chromatography
A1
A2
A3
C1
(dihydro-dehydrocostus lactone)
C2
ACID
(converting to lactone)
C3
COSTOL
(impure)
Chromatography
Chromatography
A4
(Oxide)
A5
(C13-ketone)
A6
(α- & β-ionones)
A7
A8
A9
dissolved in alcohol and cooled
A10
(Ester m.p. 294°)
A11
(Hydrocarbon m.p. 62°C.)
A12
(mixture of esters)
Saponification
A13
A14
(β-sitosterol)
(Unsaponifiable part)

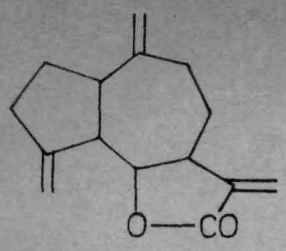
or solvent mixtures of increasing polarity for elution. The eluted fractions were combined suitably depending on their physical constants to get four major fractions, A, B, C, D. Fractions A, C and D will be described here, together with the unsaponifiable portion of Fraction B.

Examination of Fraction A:

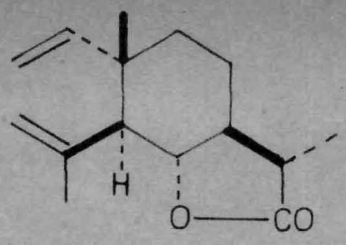
Isolation of a hydrocarbon, β-sitosterol (XII) and an oxide.

Fraction A (Chart III) was indicated to be a mixture of ketones, ester and lactone by IR spectrum. It was fractionally distilled and collected into two fractions A1 and A2. The undistilled residue A3 was chromatographed to get fractions A7, A8 and A9. Fraction A7 was dissolved in alcohol and cooled to get a small quantity of a solid ester (IR spectrum), m.p.294°. On further cooling a sticky solid was obtained which after purification by sublimation gave a saturated hydrocarbon, probably having 27 carbon atoms and m.p.62°. The mother liquors from the above after saponification gave β-sitosterol in the unsaponifiable portion. From the saponifiable portion, no water insoluble acids could be isolated.

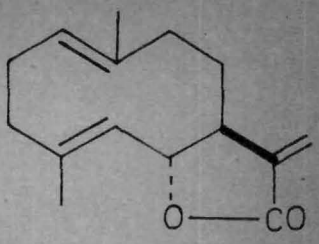
Fractions A8 and A9 being in small quantities and dark coloured were not studied.



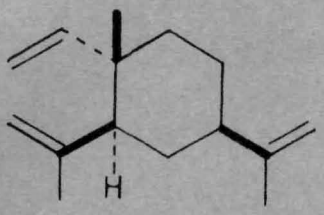
I



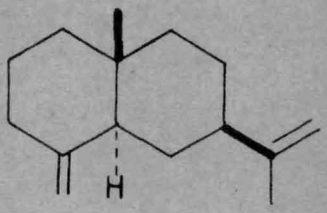
II



III



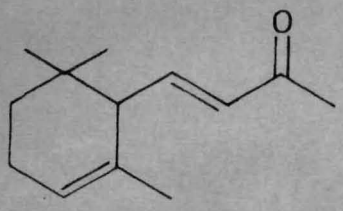
IV



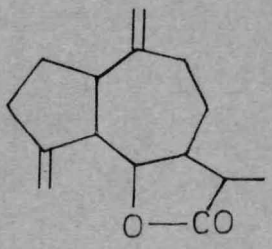
V

$$CH_2=CH\cdot(CH_2)_5\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH_3$$

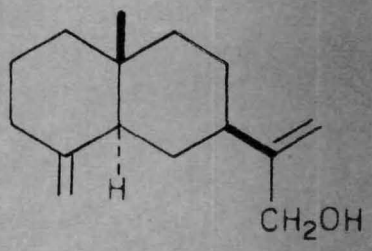
VI



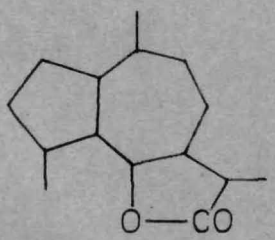
VII



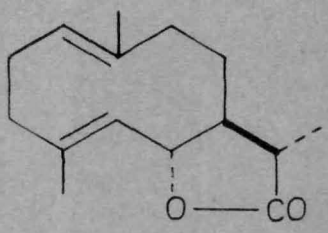
VIII



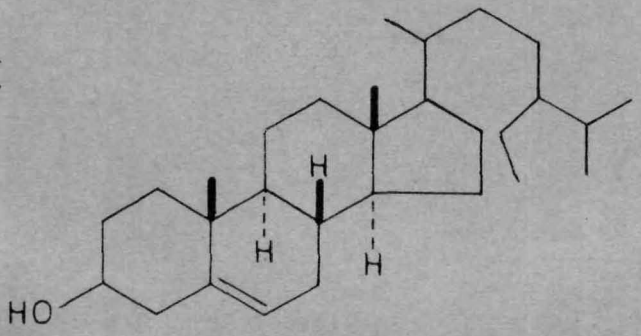
IX



X



XI



XII

Isolation of a C_{13}-ketone and α-ionone (VII)

Fraction A1 was chromatographed and collected into fractions A4, A5 and A6. Fraction A4 probably contained an oxide with two double bonds (IR). Fraction A5 was found to consist of an unidentified C13 ketone from its IR (Fig.6a) and elemental analysis. Fraction A6 consisted of a mixture of α- and β- ionones as indicated by its IR; (Fig.6b) and UV spectra and formation of derivatives.

Fraction A2, indicated by its IR spectrum to be a mixture of lactone and ester, was not investigated further.

Fraction C: (Chart III)

Isolation of dihydrodehydrocostus lactone (VIII)

Fraction C was saponified and the saponifiable portion was chiefly dihydrodehydrocostus lactone (VIII), identified by its physical constants and IR spectrum (Fig.2b). The unsaponifiable portion was somewhat impure costol (IX).

Fraction D (Chart III)

Isolation of costol (IX)

Fraction D consisted mostly of costol (IX) with a small proportion of lactones (IR, Fig.7).

Fraction B (Chart IV)

Isolation of dehydrocostus lactone(I), dihydrodehydrocostus lactone (VIII), and saussurea lactone (II)

Fraction B was saponified and separated into lactonic, acidic and unsaponifiable portions. The composition of the unsaponifiable portion only will be discussed in this thesis. The lactonic component consisted of dehydrocostus lactone (I), dihydrodehydrocostus lactone (VIII) and saussurea lactone (II), an artifact arising from dihydrocostunolide (XI; IR Fig.9).

Isolation of α- and β-ionones, β-sitosterol (XII) and two solid alcohols

The unsaponifiable portion (B3) was chromatographed to get fractions B15 to B18. Fractions B15 and B16 were shown to cosnsist of the C13-ketone, and α- and β- ionones which were also present in the petroleum ether fraction (I) of the main chromatography and described earlier. Fraction B17 was fractionally distilled and separated into fractions B19, B20, B21. Fraction B20 was found to be a mixture of dihydrodehydrocostus lactone and a small amount of alcohols giving a maximum at 233 mμ in the UV spectrum (ε 20,000). On cooling a solution of fraction B3 in alcohol at -18° a solid was obtained which after purification by crystallisation was identified as β-sitosterol by m.p.

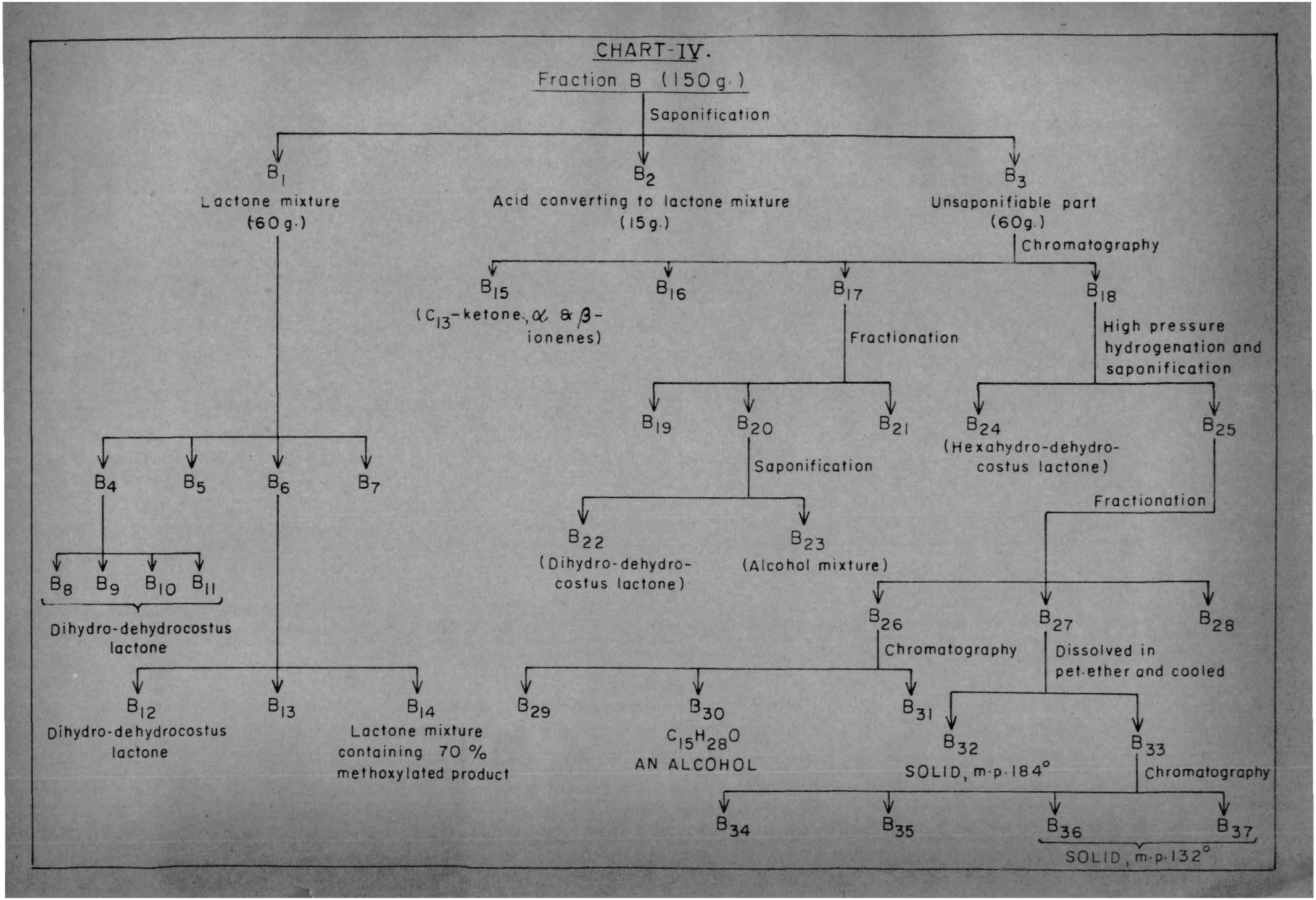


CHART-IV.
Fraction B (150 g.)
Saponification
B1
Lactone mixture
(60 g.)
B2
Acid converting to lactone mixture
(15 g.)
B3
Unsaponifiable part
(60 g.)
Chromatography
B15
(C13-ketone, α & β-ionenes)
B16
B17
Fractionation
B18
High pressure hydrogenation and saponification
B19
B20
B21
Saponification
B22
(Dihydro-dehydro-costus lactone)
B23
(Alcohol mixture)
B24
(Hexahydro-dehydro-costus lactone)
B25
Fractionation
B4
B5
B6
B7
B8
B9
B10
B11
Dihydro-dehydrocostus lactone
B12
Dihydro-dehydrocostus lactone
B13
B14
Lactone mixture containing 70 % methoxylated product
B26
Chromatography
B27
Dissolved in pet-ether and cooled
B28
B29
B30
C15H28O
AN ALCOHOL
B31
B32
SOLID, m·p·184°
B33
Chromatography
B34
B35
B36
B37
SOLID, m·p·132°

and IR spectrum. Fraction B18 was indicated to be a mixture of ketone, lactone and alcohol by its IR spectrum. It was saturated by pressure-hydrogenation and then saponified. The saponifiable part was identified as hexahydrodehydrocostus lactone (X; IR Fig.2e) From the unsaponifiable part two crystalline alcohols melting at 184° and 132° have been isolated in small quantities.

The other fractions of the main chromatography namely benzene fractions[13] K and L,[16] ether fraction M,[16] and methanol fraction N,[17] are not described in this thesis as they were investigated by other workers in our group.

Examination of water-insoluble acids (G)

This fraction was redissolved in aqueous caustic alkali (10%) and was extracted with ether to remove neutral matter, if any. The clear alkaline solution was carefully acidified with dilute hydrochloric acid. The regenerated acids were extracted with ether, the ether extract was washed free of mineral acid. The ether solution was then extracted successively with sodium bicarbonate and sodium carbonate solution (10% solution). Each extract was processed separately. This thesis deals with acids obtained by sodium carbonate extraction. Details of the carbonate soluble acids will be described in Part IIa of the thesis. Mention may, however, be made

here that the sodium bicarbonate soluble fraction contained hydroxy acids as revealed by the examination of its IR spectrum.

Water soluble acids (H) were not examined further.

EXPERIMENTAL*

Extraction of costus root oil

Finely-powdered costus roots (36 kg) were stirred mechanically with petroleum ether (40-60°; 65 L) at room temperature for two hours in a narrow mouthed stainless steel tank of adequate size and fitted with a perforated false bottom, a tap for drawing the extract and a powerful spark-proof vertical motor stirrer. The extract was allowed to settle for about half an hour and then drawn through the tap and initially filtered through cloth. The residual plant pulp was then extracted similarly twice with 45 L of petroleum ether each time. The combined extracts (135 L) were then filtered through filter paper and the filtrate concentrated in a double jacketed stainless steel distillation vessel under vacuum at a bath temperature not exceeding 40 ± 2°. The concentrate was then taken out and the last traces of solvent were removed in a glass vessel using higher-vacuum (4 mm) at 40 ± 2° when practical grade costus root oil was obtained. The yield depends upon the quality and storage period of the root. The yield from the finest grade Kashmir costus roots, October variety, is 2.4 kg, (6.66%). The oil was clear and pale brown in colour,

* When the work discussed in this part of the thesis was carried out, VPC facilities were not available in our laboratory. As a result, some of the minor fractions could not be characterised.

d_{30}^{30} 1.048; n_D^{25} 1.5288; $(\alpha)_D$ + 31.47° ($CHCl_3$). Acid value 15.23; saponification value 180.6.

Refined grade costus root oil: The practical grade oil (9 kg) was mixed with petroleum ether (18.5 L; 40-60°) and the mixture stirred thoroughly at room temperature, in a stainless steel tank as described above. The extract was drawn and resinous matter, if any (quantity depends upon the quality of roots and storage period) was filtered off and extracted again with adequate quantity of petroleum ether. The combined extracts (100 L) were then stirred in a stainless steel tank with activated charcoal (500 g) for one hour. The solution was filtered through filter paper and the charcoal washed thoroughly with petroleum ether. The combined petroleum ether filtrate was then concentrated according to the procedure described under practical grade oil. The yield of the refined grade oil was 8.5 kg, and it had the following properties: colour pale brown, d_{30}^{30} 1.039; n_D^{30} 1.5240; $(\alpha)_D$ + 34.44° ($CHCl_3$); acid value 12.18; saponification value 172.32; solubility: more than 99% of the oil dissolved in 25 vol. of alcohol(97%).

Separation of the constituents of costus root oil

Practical grade costus root oil (8 kg) was mixed with pet.ether (40-60°; 16 L) and the solution kept at 0° for 12 days. The crystals thus obtained were

filtered (crystals A: 1.9 kg) and washed with a little cold pet.ether. The combined filtrate (mother liquor B) was then kept at -18° in a deep freeze for one week, when a second crop of crystals (crystals C) separated. These were collected by filtration (2.5 kg) and the mother liquor (mother liquor D) set aside for further processing.

Isolation of costunolide (III) and dehydrocostus lactone (I)

Crystals A and C and mother liquor B were found to consist of a mixture of costunolide and dehydrocostus lactone in varying proportions. The individual constituents were separated by repeated crystallisations and chromatography. Costunolide (III), m.p.106-107° (collapses to a glassy mass); $(\alpha)_D$ + 128° ($CHCl_3$, C, 0.45).

Analysis

Found: C, 77.89; H, 8.78.

mol. wt. by sap. 236.1

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

mol. wt. 232.30

IR bands at: 1764 cm^{-1} (α,β-unsaturated γ-lactone); $\lambda_{max.}$ 213 mμ, log ε 4.21.

Dehydrocostus lactone (I), m.p.60°, $(\alpha)_D$ - 12°.

Analysis

Found: C, 72.11; H, 7.62.

$C_{15}H_{18}O_2$ requires: C, 72.23; H, 7.88%.

IR bands at: 1754, 1627, 891, 814 cm^{-1}.

Examination of mother liquor D

It had the following properties: d_{25}^{25} 0.9938; n_D^{25} 1.5110; $(\alpha)_D$ + 20.92°. Saponification value 110.17.

Separation of free acids

Mother liquor D was concentrated in vacuum at 40° to give an oil (3.6 kg) having an acid value 27.5. On the basis of the acid value the requisite amount (1 L; 10%) of alcoholic potash was added to it in two instalments and the mixture was stirred mechanically with a stirrer. The mixture was then diluted with ether (20 L) and the ether solution then extracted with 5% sodium chloride solution (3 X 3 L). The ethereal layer after washing with water was dried over anhydrous sodium sulphate. After removal of ether in vacuo, the 'neutral oil F' was obtained which was processed by chromatography.

The aqueous alkaline extract obtained above was extracted twice with ether to remove any adhering neutral material and then acidified with cold, dilute sulphuric acid. The regenerated organic acids were then extracted with ether, as usual. Evaporation of ether gave water-insoluble acids (G, 100 g), which are described later. The water-soluble acids (H), which remained in aqueous solution were not examined.

Chromatography of the Neutral oil F

The neutral oil (3.3 kg) was chromatographed in one lot on neutral alumina (grade III, 40 kg) and the column developed by elution with solvents in the following order:

1. Pet.ether (40-60°) 90 Lit; 38 fractions.
2. Pet.ether-benzene mixture (1:1; 90 Lit; 32 fractions).
3. Benzene (90 Lit; 32 fractions).
4. Ether (45 Lit; one combined fraction)
5. Methanol (45 Lit; one combined fraction).

The course of chromatography was followed by quantitatively measuring the residue obtained by evaporation of a definite volume of the eluate, from almost every alternate fraction, on the basis of which the solvent was changed at appropriate stages. In all about 110 fractions of about three litres each, one combined ethereal extract of 45 lit. and a methanolic extract of 45 lit. were collected. The respective fractions were then combined together and the solvents from the combined fractions were removed under reduced pressure at a bath temp. of 40 ± 2° in a stainless steel distillation unit, and the residue collected. These were then processed separately for characterisation of the constituents. Thus, following six major fractions were obtained: 1. Pet.ether fraction (I) (672 g);

(2) pet.ether-benzene fraction J (560 g); (3) benzene fraction K (335 g); (4) benzene fraction L (300 g); (5) ether fraction M (370 g); and (6) methanol fraction N (160 g).

Petroleum ether fraction I

Examination of aplotaxene (VI). Pet.ether fraction I which was investigated by other workers in our group gave on repeated chromatography and fractional distillation, a hydrocarbon, aplotaxene, b.p. 125-130°/2.5 mm., n_4^{27} 0.8181; n_D^{27} 1.4730; $(\alpha)_D \pm 0°$. IR spectrum had bands at: 910, 991, 1645 cm^{-1} due to a vinyl group and a band at 720 cm^{-1} due to an isolated cis-double bond. The IR spectrum was identical with that of aplotaxene reported in literature.

Analysis

Found: C, 87.4; H, 11.7.

$C_{17}H_{28}$ requires: C, 87.9; H, 12.2%.

Isolation of the ketone $C_{13}H_{22}O$ from the pet.ether fraction I.

Pet.ether fraction I (672 g) was initially chromatographed on neutral alumina (grade III; 1.4 kg) and eluted with pet.ether (40-60°) and ether. The pet. ether eluted fraction A1 and the ether eluted fraction B1 were processed separately.

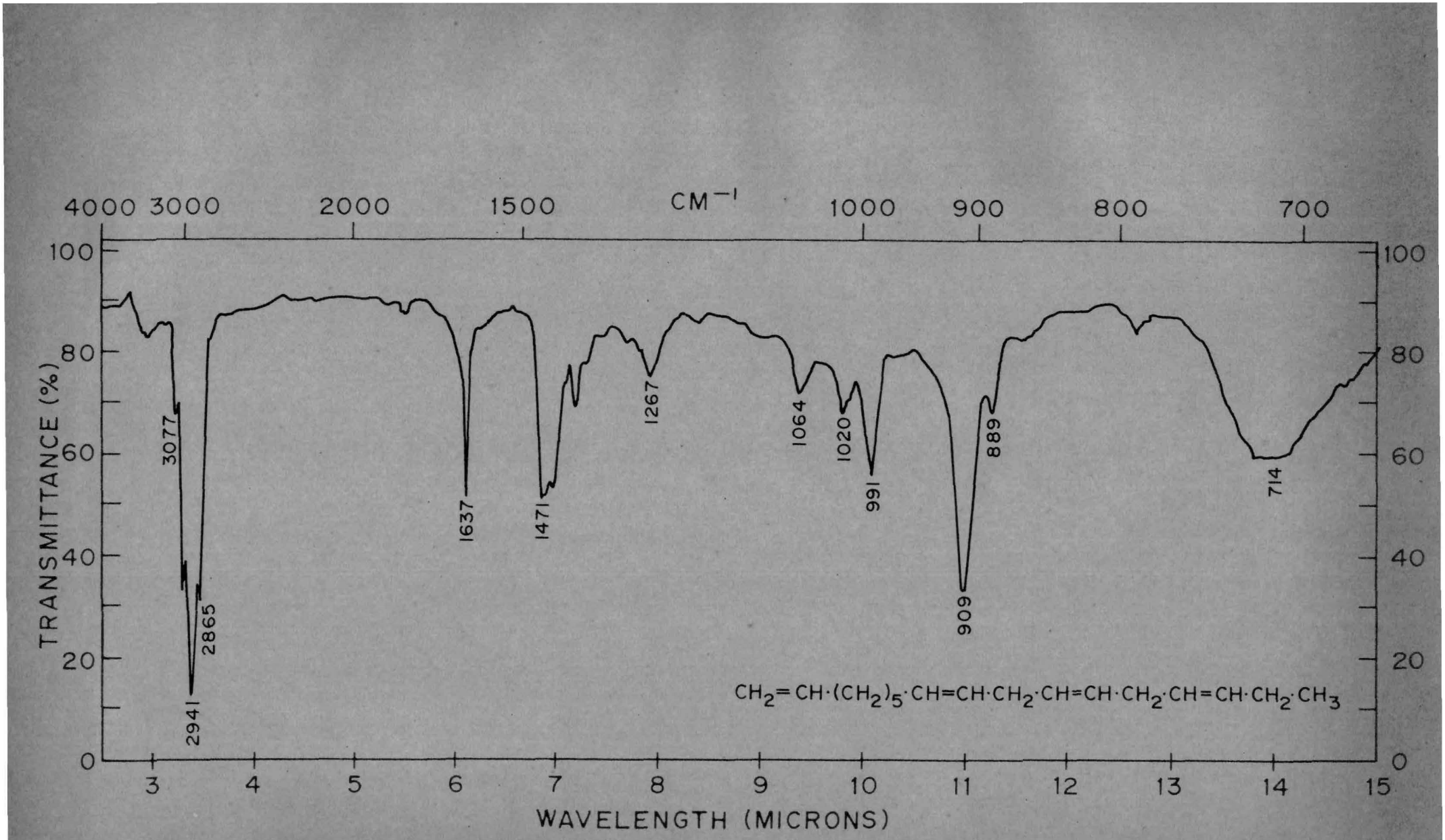


FIG. 5. IR SPECTRUM OF APLOTAXENE (LIQUID FILM).

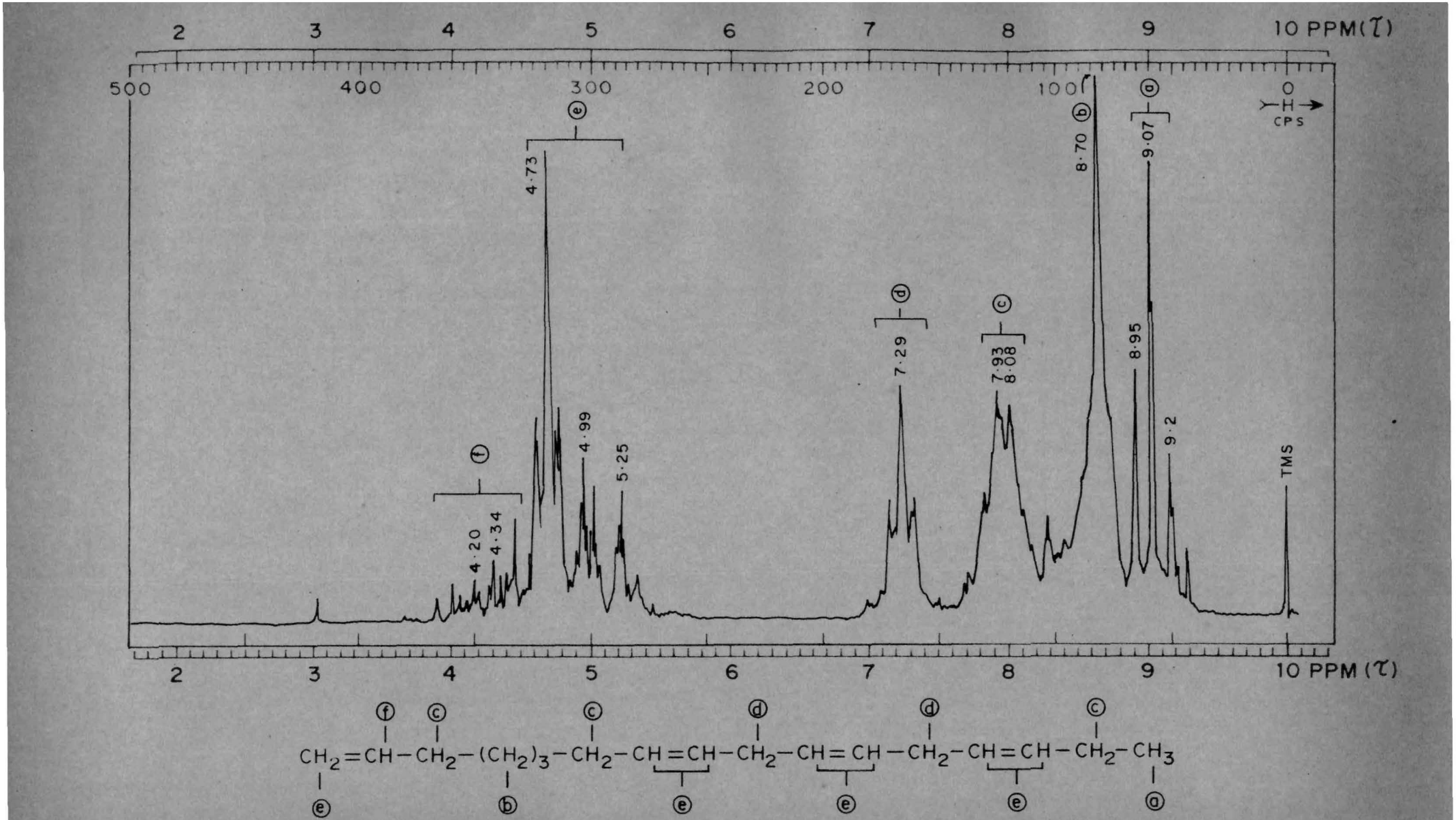


FIG. 5a. NMR SPECTRUM OF APLOTAXENE.

A part of the fraction B1 was treated with alcohol (70%), and the soluble portion was shaken with pet.ether. The pet.ether layer, on removal of solvent furnished a thick brown oil (37 g., fraction B4). The 70% alcohol-layer on removal of solvent furnished fraction B5 (20 g).

Fraction B4 (37 g) was chromatographed on alumina (grade IV, 800 g) and the results are shown in Table 1.

TABLE 1

Fr.No.	Weight (g)	$n_D^{22.5}$	$(\alpha)_D$	solvent	Volume ml.
1	8.4	1.4935	+41°	Pet.ether	400
2	4.5	1.4958	+50°	"	300
3	2.0	1.5029	+51°	"	300
4	11.3	1.5189	+20°	Pet.ether-benzene (1:1)	1000
5	3.3	1.5163	+14°	Benzene	1000
6	2.9	-	+15°	Ethanol	1000

Fractions 1,2 and 3 from the above chromatography were mixed together and rechromatographed on alumina grade III. The results are given in Table 2.

TABLE 2

Fr.	Weight g.	n_D^{23}	$(\alpha)_D$	Solvent	Volume ml.
1	0.6	1.4828	+24°	Pet.ether	2500
2	4.1	1.4820	+18°	"	500
3	2.0	1.4872	+19°	Pet.ether-benzene (1:1)	250
4	1.5	1.4920	+32°	"	250
5	0.9	1.4973	+56°	"	500
6	0.5	1.5075	+103°	Benzene	500
7	4.6	1.5189	+33°	Ethanol	1000

Fractions 1,2 and 3 of the above chromatography were combined and rechromatographed on alumina (grade II, 200 g). The results are shown in Table 3.

TABLE 3

Fr.	Weight g.	n_D^{23}	$(\alpha)_D$	Solvent	Volume ml.
1	1.5	1.4795	+26°	Pet.ether	400
2	0.7	1.4800	+29°	Pet.ether-benzene (1:1)	100
3	1.5	1.4812	+15°	"	100
4	0.8	1.4830	+13°	"	100
5	0.8	1.4890	+24°	Benzene	700
6	Traces	-	+19°	Methanol	250

Isolation of the ketone $C_{13}H_{22}O$

Fractions 1 and 2 above, were combined and distilled to give a liquid b.p.140-165°/5 mm.(bath) n_D^{23} 1.4720; d_{23}^{23} 0.9031; $(\alpha)_D$ + 27°.

Analysis*

Found: C, 80.27; H, 10.60.

$C_{13}H_{22}O$ requires: C, 80.35; H, 11.4%.

UV spectrum of this compound did not show strong absorption in the region 220-300 mμ. IR spectrum showed bands at: 1711, 1640, 1445, 1412, 1377, 1358, 1252, 1231, 1161, 909, 889, 863 and 824 cm^{-1}.

Hydrogenation of ketone $C_{13}H_{22}O$

The compound (0.7 g) was hydrogenated in acetic acid in the presence of platinum oxide (30 mg) (vol. of absorbed hydrogen at NTP 80 ml). The hydrogenated product was isolated as usual. It was distilled, b.p.160-165°(bath)/ 10 mm., n_D^{23} 1.4505; $(\alpha)_D$ + 6°,

Analysis

Found: C, 79.2; H, 12.25.

$C_{13}H_{24}O$ requires: C, 79.6; H, 12.25%.

IR bands at: 1718, 1461, 1415, 1364 and 1165 cm^{-1}.

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* The ketone $C_{13}H_{22}O$ has been isolated from several fractions and a satisfactory analysis is reported on page 47.

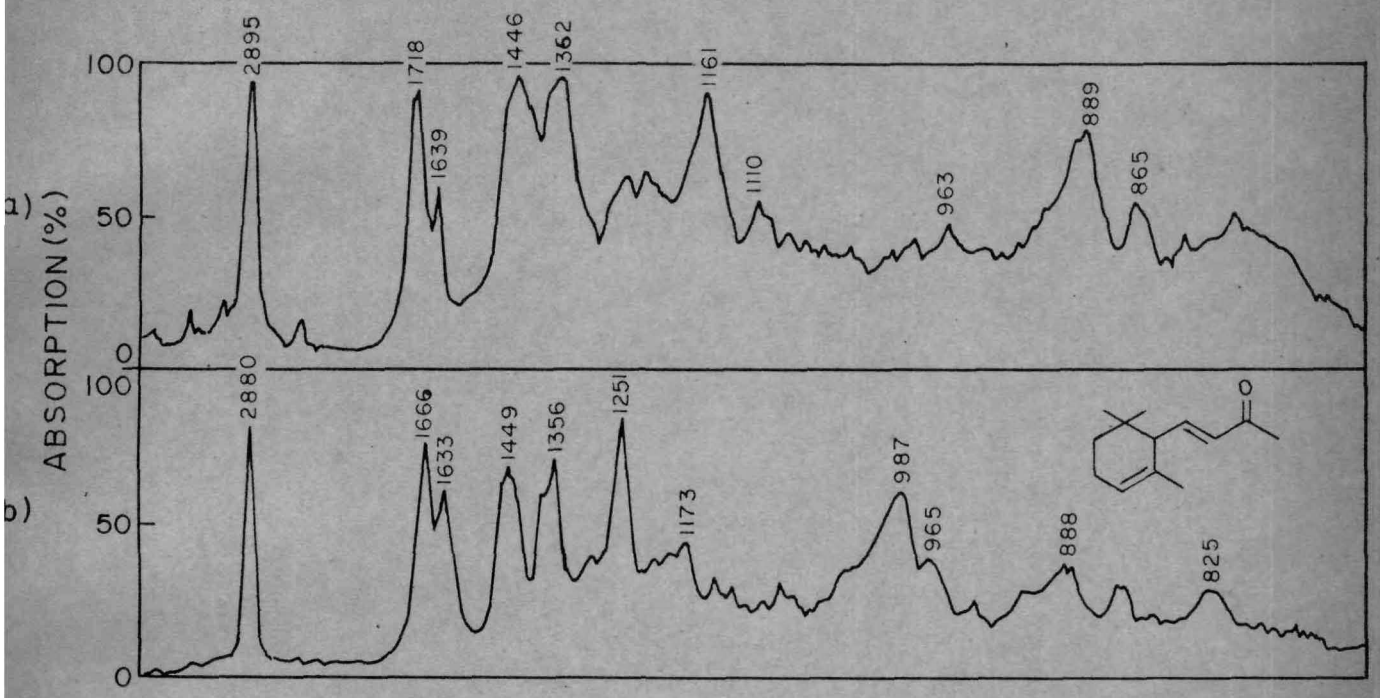

FIG. 6.

a) IR SPECTRUM OF C_{13} KETONE (LIQUID FILM)

b) IR SPECTRUM OF α – IONONE (LIQUID FILM)

Isolation of α-ionone (VII)

Fraction 35 (20 g) was chromatographed on alumina (grade IV; 500 g) and the results are shown in Table 4.

TABLE 4

Fr.	Weight g.	n_D^{23}	$(\alpha)_D$	Solvent	Volume ml.
1	3.23	1.4998	+58°	Pet.ether	250
2	1.20	1.5104	+40°	"	250
3	1.40	1.5130	+47°	"	500
4	3.70	1.5239	+19°	Pet.ether-benzene(1:1)	250
5	2.80	1.5225	+10°	"	250
6	1.30	1.5171	+18°	Benzene	500
7	2.20	-	-	Methanol	1000

Fractions 1,2 and 3 from the above chromatography were combined and chromatographed on alumina (grade II, 200 g). The results are described in Table 5.

TABLE 5

Fr.	Weight g.	n_D^{23}	$(\alpha)_D$	Solvent	Volume ml.
1	0.6	1.4790	+48°	Pet.ether	500
2	0.6	1.4858	+70°	Pet.ether-benzene (1:1)	100
3	0.6	1.5001	+116°	"	200
4	0.4	1.5105	+38°	"	300
5	1.0	1.5194	+32°	Benzene	300
6	0.9	1.5160	+19°	Methanol	1000

Fraction 6 of Table 2 and fraction 3 of Table 5 with specific rotation + 102° and + 116° respectively were mixed and distilled, b.p. 135-140°/1 mm., n_D^{25} 1.4969; $(\alpha)_D$ + 143°, (c, 3.7)

Analysis

Found: C, 80.9; H, 10.4.

$C_{13}H_{20}O$ requires: C, 81.25; H, 10.42%.

The IR spectrum was very similar to that of α-ionone, λ_{max}. 282 mμ, ε 2240 and 227 mμ, ε 7134. Some β-ionone was possibly present as an impurity.

A 2,4-dinitrophenyl hydrazone (ddep red) m.p. 114-116°, $(\alpha)_D$ + 266°, (c, 0.9)

Analysis

Found: C, 61.0; H, 6.3; N, 14.9.

$C_{19}H_{24}O_4N_4$ requires: C, 61.3; H, 6.5; N, 15.1%.

Its IR spectrum was in good agreement with that of 2,4-dinitrophenyl hydrazone of (+) α-ionone.

Dihydrodehydrocostus lactone (VIII)

Fractions 4, 5, 6 and 7 of Table 4 and fractions 5 and 6 of Table 5 were combined together and chromatographed on alumina (grade III, 300 g). The results are tabulated below:

TABLE 6

Fr.	Weight g.	n_D^{24}	$(\alpha)_D$	Solvent	Volume ml.
1	1.4	1.5158	+30°	Pet.ether	600
2	1.7	1.5229	+21°	Pet.ether-benzene 1:1	200
3	1.3	1.5220	+22°	"	200
4	0.6	1.5201	+28°	"	200
5	0.6	1.5184	+23°	Benzene	200
6	1.0	1.5163	+20°	"	300
7	2.2	1.5212	+16°	Methanol	1000

Fractions 2 and 3 of Table 6 were combined and distilled b.p.175-190° (bath)/0.9 mm., d_{25}^{25} 1.040; n_D^{25} 1.5225; $(\alpha)_D$ + 22°.

Analysis

Found: C, 77.7; H, 8.4.

$C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.7%.

IR spectrum was identical with that of a lactone isolated from a different fraction of costus root oil and shown to be dihydrodehydrocostus lactone (VIII).

Hydrogenation of dihydrodehydrocostus lactone

Hexahydrodehydrocostus lactone (X):

Dihydrodehydrocostus lactone (0.5 g) was hydrogenated initially in alcohol in the presence of 10% palladium charcoal (0.05g) and finally in acetic acid in the presence of platinum oxide at room temp. and atmospheric pressure. Total hydrogen uptake was 92 ml at NTP (2 moles). The product which was hexahydrodehydrocostus lactone (X) was worked up in the usual way. It was distilled, b.p.186-200° (bath)/0.6 mm., n_D^{32} 1.4980; $(\alpha)_D$ + 61°, (c, 1.8)

Analysis

Found: C, 75.3; H, 9.9.

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

The remaining portions of fraction B1 were shown to be a mixture of the C13-non-conjugated ketone

(described earlier), ∝-ionone, and dihydrodehydrocostus lactone, by chromatography of appropriate fractions and study of physical constants and IR spectra.

Examination of pet.ether-benzene fraction J

Pet.ether-benzene fraction J (500 g) was chromatographed on alumina (grade III, 18 kg) (in 3 batches) and the results are shown in the following table 7.

T A B L E 7

Fr.	Weight g.	n_D^{24}	$(\alpha)_D$	Solvent
A	35.0	1.5100 to 1.5190	+0° to +20°	Pet.ether-benzene
B	285.0	1.5220 to 1.5280	+18° to +15°	"
C	40.0	1.5220 to 1.5180	+20° to +40°	Benzene
D	55.0	-	-	Ether.

Fraction A (Chart III)

Fraction A (23 g) was fractionally distilled and separated into 3 fractions A1, A2 and A3 shown in Table 8.

TABLE 8

Fr.	Weight (g)	b. p.
A1	4.5	180-85° (bath)/1.5 mm.
A2	1.5	200-240° (bath)/1.5 mm.
A3	15.0	above 250° (bath)/1.5 mm.

Fraction A1

Isolation of an oxide:

Fraction A1 (4.5 g), indicated by its IR spectrum to be a mixture of ketones, was chromatographed on alumina (grade III, 300 g) using pet.ether, pet.ether-benzene, benzene, and ether as eluents. Fractions 1-3 eluted with pet.ether gave a liquid (A4; 0.3 g) whose IR spectrum indicated the presence of atleast two double bonds, one methylenic double bond (1647, 891 cm^{-1}) and the other vinyl group ($-CH=CH_2$; 910, 980 cm^{-1}), n_D^{27} 1.4720.

Analysis

Found: C, 80.6; H, 11.7.%

The IR spectrum did not show any absorption in the hydroxyl or carbonyl region. It is probably an oxide. No further work was done on this fraction due to paucity of material.

Isolation of the ketone $C_{13}H_{22}O$:

Fractions 4-10 eluted with pet.ether gave a liquid (A5; 1.5 g) which was purified by distillation, b.p. 130-145° (bath)/0.6 mm., n_D^{27} 1.4860.

Analysis

Found: C, 79.2; H, 11.2.

$C_{13}H_{22}O$ requires: C, 80.36; H, 11.41%.

The IR spectrum of A5 showed peaks at 1710 cm^{-1} due to a keto group, 1647 and 891 cm^{-1} due to a methylenic double bond.

Lithium aluminium hydride reduction of A5

Fraction A5 (0.9 g) was reduced with lithium aluminium hydride (0.5 g) in ether solution and the product was worked up in the usual manner. It was distilled, (0.7 g), b.p.135-140° (bath)/1 mm.

Analysis

Found: C, 78.4; H, 11.7.

$C_{13}H_{24}O$ requires: C, 79.53; H, 12.32%.

IR spectrum indicated the presence of methylenic double bond and hydroxyl group.

Selenium dehydrogenation of alcohol from A5

The above alcohol (0.4 g) was heated in the atmosphere of nitrogen with selenium (0.6 g) at 240-250° for 24 hours. The product was extracted with pet.ether

and chromatographed. The fraction eluted with pet.ether was distilled (0.1 g), $\lambda_{max.}$ 228 mμ; ε 8000, indicating // the presence of small quantities of naphthalenic compounds. Attempts to prepare the s-trinitrobenzene adduct were unsuccessful.

Fraction A2

α- and β- Ionones.

Fraction A 2 was found to be a mixture of α- and β- ionones by UV spectrum ($\lambda_{max.}$ 228 and 296 mμ)

Fraction A3

Fraction A3 (15 g) mostly containing high boiling constituents was chromatographed on alumina (grade III, 450 g) and only the fraction (7 g) eluted with pet.ether-benzene (fraction A6) was investigated. Fractions A7 and A8 eluted with benzene and ether respectively and each about 1.5 g. were highly dark coloured and were not investigated.

Isolation of a hydrocarbon:

Fraction A6 was dissolved in alcohol (250 ml) and cooled when a small amount of solid separated. It was crystallised from pet.ether, m.p.294° (A9). Its IR spectrum indicated it to be an ester (1720, 1247, 1023 cm^{-1}). The mother liquor was then cooled to -18°, when a solid was obtained, which was sublimed, m.p. 62°. Its IR spectrum indicated it to be a hydrocarbon.

Analysis

Found: C, 86.0; H, 14.0%. It was probably a C_{27} to C_{29} hydrocarbon.

Isolation of β-sitosterol (XII)

After the removal of the solids the mother liquors were concentrated and saponified with alcoholic potash (10%) for 4 hours, and separated into saponifiable and unsaponifiable parts. No water-insoluble acids could be isolated from the saponifiable part. The non-saponifiable part gave a semisolid which, after two crystallisations, from methanol gave a solid, m.p.135-136°.

Analysis

Found: C, 83.1; H, 12.1.

$C_{29}H_{50}O$ requires: C, 83.87; H, 11.99%.

From the melting point and IR spectrum, it was identified as β-sitosterol.

Fraction B (Chart IV)

Separation of fraction B into lactonic acidic and unsaponifiable portions.

A portion of fraction B (150 g) was refluxed for one hour with alcoholic potash (5%, 500 ml). Most of the alcohol was removed under reduced pressure and the residue was diluted with water and extracted with

ether. The ether extract was washed with water and dried. Removal of ether furnished fraction B3 (60 g). The aqueous layer was acidified at 0° with dilute sulphuric acid and extracted with ether. The ether extract was washed with sodium bicarbonate solution to remove acidic components. The remaining ether solution on evaporation of ether gave fraction B1(60 g). The bicarbonate solution was acidified with cold, dilute sulphuric acid and the regenerated acid was taken up in ether. The ether solution was washed free of mineral acid and dried. On evaporation of ether it gave fraction B2 (15 g).

Fraction B3

Chromatography of the unsaponifiable portion B3.

Fraction B3 (32 g) was chromatographed on alumina (grade III, 1 kg) and the results are shown in the following Table 9.

T A B L E 9

Fr.	Weight (g)	Eluent
B15	1.5	Pet.ether
B16	1.6	Pet.ether + benzene
B17	9.5	Benzene
B18	8.0	Ether.

The ketone $C_{13}H_{22}O$

Fractions B15 and B16 showed bands at: 1718 (a keto group), 1637, 891 cm^{-1} (exocyclic double bond) in the IR spectrum. The UV spectrum showed peaks at 290, 300 and 318 mμ. A portion of fraction B16 was distilled, b.p.120-130° (bath)/0.6 mm. This ketone was the same as the C13-ketone mentioned earlier.

Analysis

Found: C, 81.84; H, 11.2.

$C_{13}H_{22}O$ requires: C, 80.35; H, 11.41%.

Fraction B17 indicated it to be a mixture of ketone, lactone and alcohol from its IR spectrum (bands at: 1718, 1760 and 3360 cm^{-1}). It was fractionally distilled and separated into fractions B19, B20 and B21. Fraction B19 was redistilled, b.p.160-200° (bath)/0.7 mm. IR bands at: 3460, 1711, 1669, 1627 and 885 cm^{-1}. It failed to give any solid 2:4-dinitrophenyl hydrazone.

Fractions B16 and B19 were comparable in their IR spectra. They were mixed together and chromatographed on alumina (grade III, 90 g). The results are shown below.

T A B L E 10

Fr.	Weight (g)	Eluent
1	0.2	Pet.ether
2+3	0.5	Pet.ether
4	0.2	Pet.ether
5	0.7	Pet.ether + benzene

Fraction 1, above was distilled, b.p.120-130° (bath)/0.3 mm., $n_D^{23.5}$ 1.4975; $\lambda_{max.}$ 210 mμ (end absorption), ε 7021; IR bands at: 1708, 1647 and 891 cm^{-1}. This was identical with the earlier ketone.

Analysis

Found: C, 79.9; H, 11.44.

$C_{13}H_{22}O$ requires: C, 80.35; H, 11.41%.

Fractions (2+3) and 4 were identical in their IR spectra and hence were combined together and distilled, b.p.140-150° (bath)/0.5 mm., $n_D^{23.5}$ 1.4870; end absorption at 210 mμ, ε 8404.

Analysis

Found: C, 80.9; H, 11.4.

$C_{13}H_{22}O$ requires: C, 80.35; H, 11.4%.

It failed to give a DNP derivative.

Fraction 5 gave a peak at 296 mμ, ε 4430. The IR and UV spectra indicated it to contain β-ionone.

Isolation of dihydrodehydrocostus lactone (VIII)

Fraction B20 was found to be a mixture of alcohol and lactone by its IR spectrum. Fraction B20 (2 g) was saponified with alcoholic potash (10%; 50 ml) for 8 hours, and separated into saponifiable fraction B_{22} and the unsaponifiable fraction B_{23}. Fraction B_{22}

(1.5 g) was purified by chromatography and was distilled, b.p.140-145°/0.5 mm., n_D^{28} 1.5290; $(\alpha)_D$ + 27°. From the IR spectrum and physical constants, it was identified as dihydrodehydrocostus lactone (VIII). Fraction B23(0.4 g) was distilled, b.p.140-160°(bath)/0.7 mm., n_D^{25} 1.5040; $\lambda_{max.}$ 233, 270 and 282 mμ, ε 2000, 3000 and 2200 respectively. IR bands at: 3360, 1655, 1637, 980 and 910 cm^{-1}.

Analysis

Found: C, 80.95; H, 11.45.

$C_{13}H_{22}O$ requires: C, 80.35; H, 11.41%.

It appears to be a mixture of alcohols.

β-Sitosterol (XII)

An alcoholic solution of fraction B18 when cooled at -18° gave a solid, m.p.135-136°; $(\alpha)_D$ - 39°. It was identified as β-sitosterol from its physical properties and IR spectrum.

Hexahydrodehydrocostus lactone (X)

Fraction B18 (5.6 g) was hydrogenated in alcohol medium using palladium charcoal (5%) (0.3g) as catalyst under 1400-1600 p.s.i. pressure at 70-80°. The hydrogenated product was saponified with alcoholic potash (10%, 100 ml) and separated into saponifiable (fraction B24) and unsaponifiable (fraction B25) portions. Fraction B24 was distilled, b.p.170-190°(bath)/0.5 mm., n_D^{22} 1.5035;

$(\alpha)_D$ + 23° (c, 2·1), It was identified as hexahydrodehydrocostus lactone (X) from its IR spectrum.

An unidentified alcohol

Fraction B25 was distilled and collected into fractions B26 and B27. Fraction B26, b.p.120-138°/0.7 mm. was found to be a mixture of ketone, lactone and alcohol from its IR spectrum. Fraction B26 (1.5 g) was chromatographed on alumina (grade III, 50 g). The fraction eluted with a mixture of pet.ether and benzene was found to be a mixture of ketone and lactone from IR spectrum. The fraction eluted with benzene was distilled, b.p.140°-150°(bath)/1 mm., n_D^{26} 1.4900; IR spectrum indicated it to be an alcohol.

Analysis

Found: C, 80.79; H, 12.68.

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

The fraction eluted with ether was not investigated.

Isolation of two crystalline alcohols

Fraction B27, b.p.240-260°(bath)/0.7 mm. (0.5 g) was highly viscous. It was dissolved in pet.ether and cooled when a crystalline solid (30 mg) was obtained. It was crystallised from pet.ether, m.p.184°. The IR spectrum indicated it to be an alcohol. It was not examined further due to paucity of material. The mother liquors from the above solid alcohol were concentrated

and the residue chromatographed on alumina (grade III, 10 g). The fraction eluted with ether gave a solid, m.p.132-133°. It was an alcohol as revealed by IR spectrum.

Fraction C (Chart III)

Saponification of Fraction C

Isolation of dihydrodehydrocostus lactone (VIII)

Fraction C (18 g) was saponified with alcoholic potash (5%, 200 ml) for 8 hours, and separated into lactonic (C1) acidic (C2) and neutral (C3) portions, by the usual procedure. The acid-free saponifiable product C1 (7 g) was distilled, b.p.185-195°(bath)/0.5 mm., n_D^{27} 1.5305; $(\alpha)_D$ + 37°. IR spectrum was closely similar to that of dihydrodehydrocostus lactone.

Analysis

Found: C, 77.77; H, 8.68.

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

Selenium dehydrogenation of Fraction C1

Fraction C1 (2.5 g) was heated in an atmosphere of nitrogen with selenium (2 g) for 24 hours at 280-300°. The reaction product was extracted with pet.ether and chromatographed on a column of alumina (grade I, 20 g). The blue product thus obtained was taken up in pet.ether and extracted with phosphoric acid to remove azulenes.

The non-azulenic product was distilled. The distillate exhibited the characteristic UV absorption for a naphthalenic compound. It gave an adduct with s-trinitrobenzene, m.p.106-107° which was identified as the TNB adduct of 1-methyl 7-ethyl naphthalene by m.p. and mixed m.p. with an authentic sample. The naphthalenic compound amounts to about 3%. The small amount of azulenes obtained was not examined. The above lactone probably contained a small amount of dihydrocostunolide.

Acidic fraction C2

The dark coloured fraction C2 was distilled. The distillate (1.5 g) was dissolved in pet.ether and cooled at 0° for 24 hours when a sticky solid was obtained. It was purified by sublimation, m.p. 62°. The IR spectrum gave a peak at 1764 cm^{-1}, characteristic of a γ-lactone and no absorption for -CO of carboxyl group was observed. Bands at 1645, 890 cm^{-1} indicated the presence of exo-methylene group.

Analysis

Found: C, 76.8; H, 9.46.

$C_{15}H_{22}O_2$ requires: C, 76.93; H, 9.45%.

It appears that the acid obtained above cyclises slowly to a lactone on keeping.

Unsaponifiable part C3

Isolation of costol (IX)

Fraction C3 was distilled, b.p.150-160° (bath)/1 mm. n_D^{28} 1.5060; $(\alpha)_D$ + 16.53°.

Analysis

Found: C, 81.3; H, 11.4.

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

The UV spectrum showed low ε value peaks at 272, 282, 302 and 317 mμ. IR spectrum showed bands at: 3350, 1640, 890 and 1023 cm^{-1} and was closely similar with that of costol. In addition, it showed weak bands in carbonyl region. In order to isolate costol in a state of purity, the above liquid (5 g) was chromatographed on alumina (grade III, 250 g). The earlier fractions (Fr.1-3) eluted with pet.ether-benzene were indicated to be mixtures of lactone and ketone from IR spectrum. The middle fractions (Fr. 4-7) eluted with benzene contained lactone and alcohol as revealed by IR spectrum. The IR spectrum of fraction 8 eluted with ether, and also its physical constants were in good agreement with that of costol (IX), n_D^{28} 1.5156; $(\alpha)_D$ + 15°.

Fraction D

Dihydrodehydrocostus lactone (VIII) and dihydrocostunolide (XI)

Fraction D (55 g) was chromatographed on alumina (grade III; 1.5 kg) and a large number of fractions were

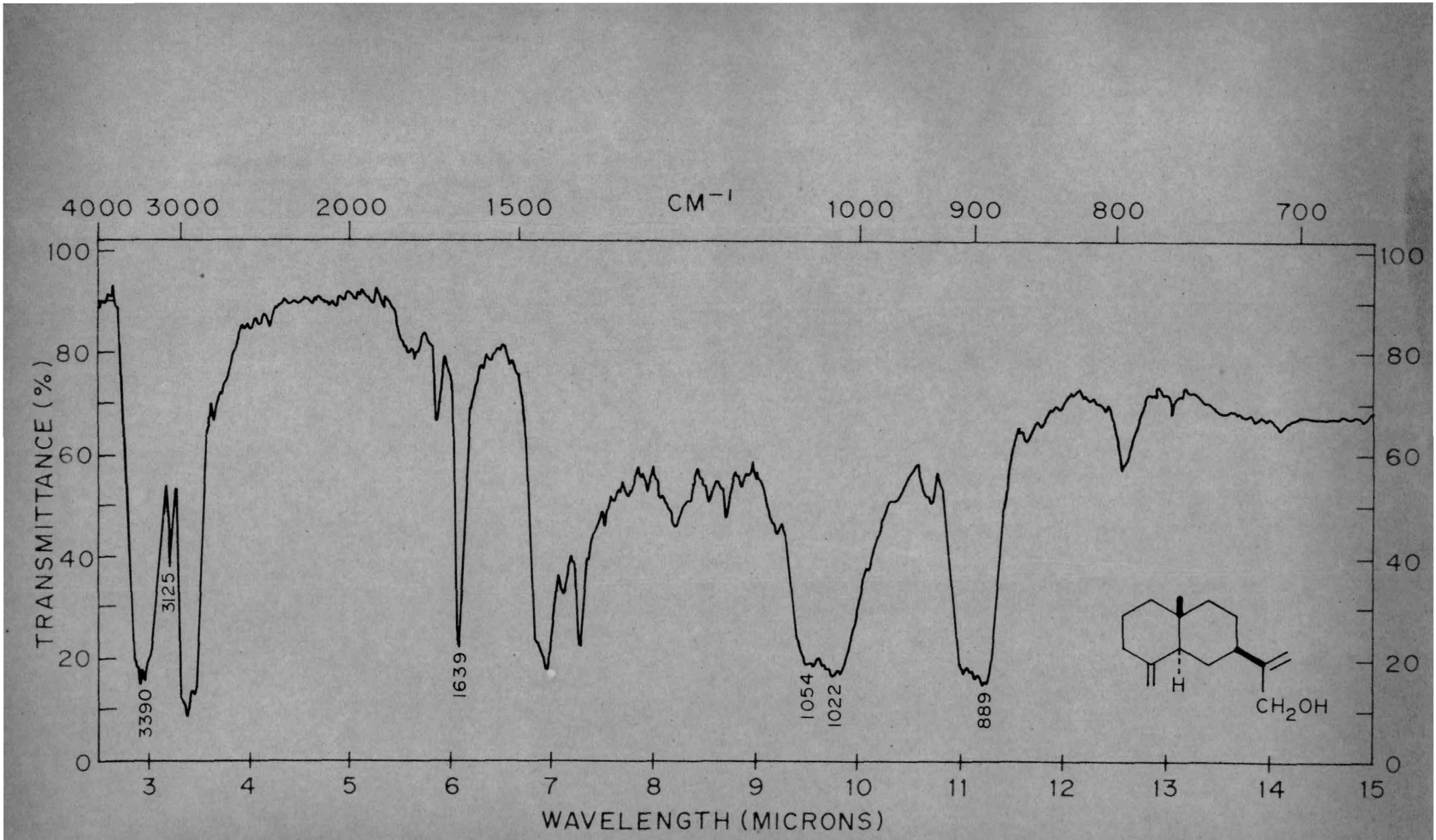

FIG. 7. IR SPECTRUM OF COSTOL (LIQUID FILM).

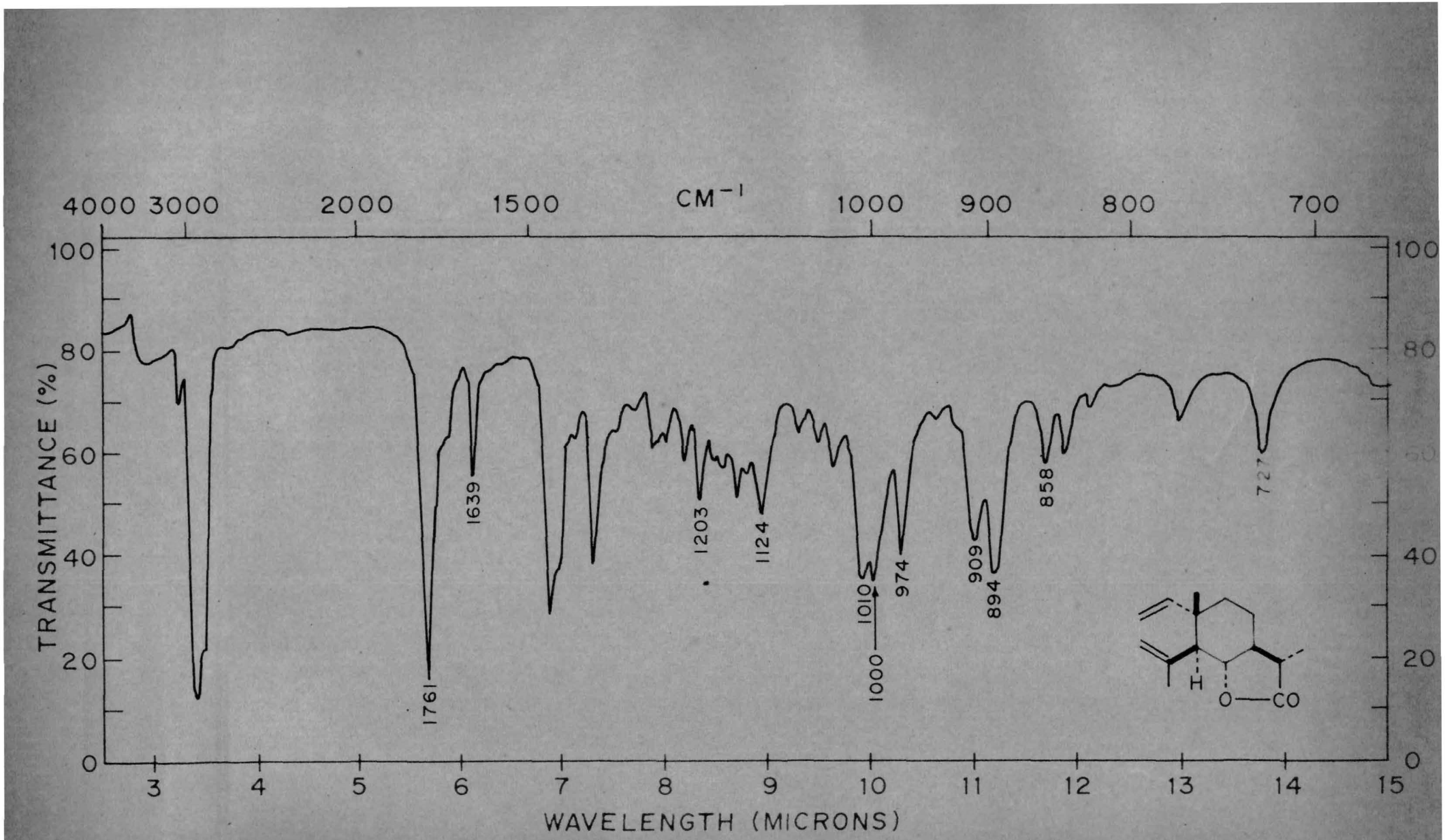

FIG. 8. IR SPECTRUM OF SAUSSUREA LACTONE (IN NUJOL).

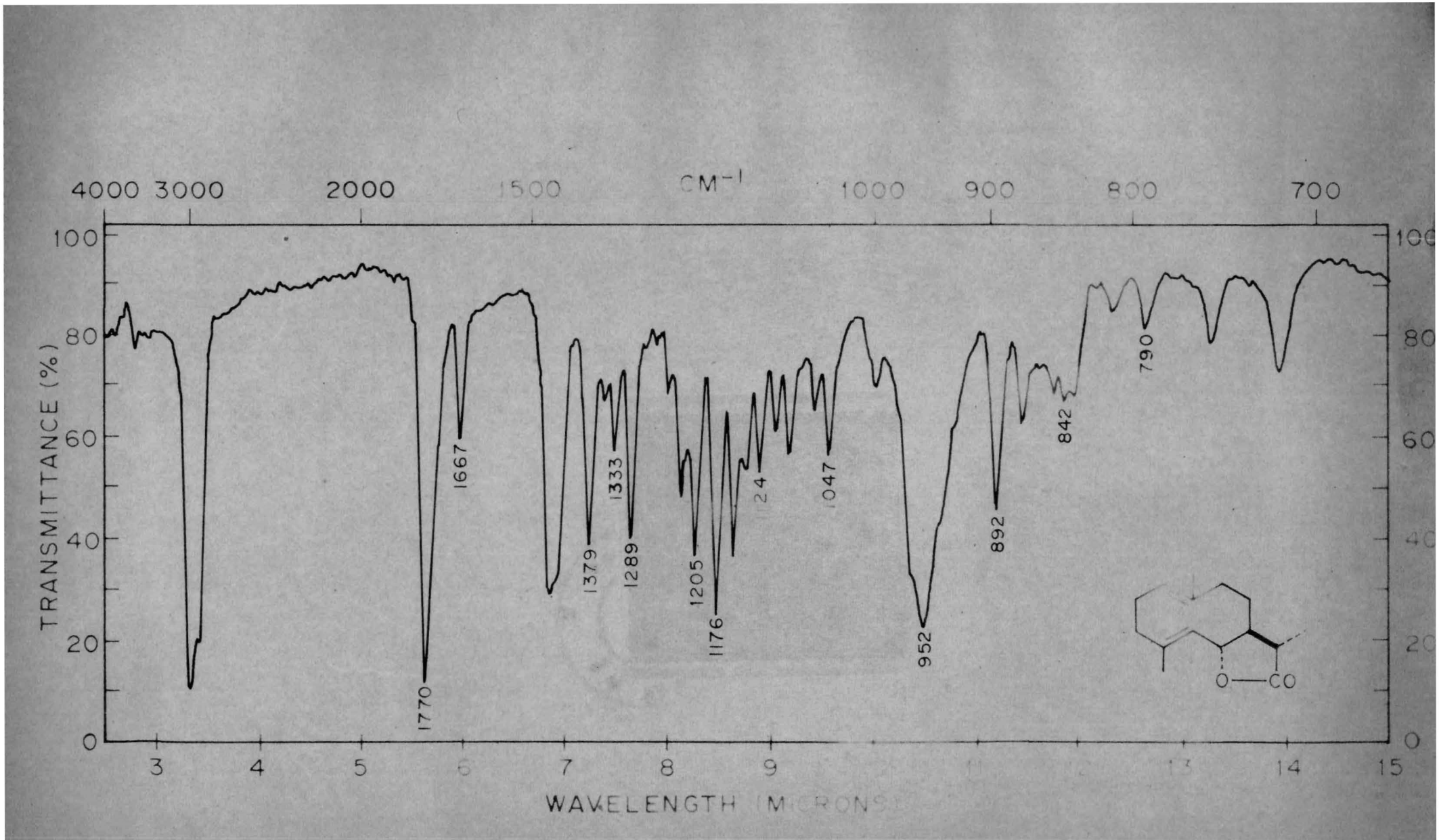

FIG. 9. IR SPECTRUM OF DIHYDROCOSTUNOLIDE (IN NUJOL).

collected. The fractions were suitably mixed and divided into 3 main fractions, D1, D2 and D3, depending on their physical constants, given in Table 11.

TABLE 11

Fr.	Weight g.	n_D^{27}	$(\alpha)_D$	Eluent
D1	25.0	1.5020 to 1.5120	+15° to +22°	Pet.ether
D2	18.0	1.5100 to 1.5170	+23° to +30°	Pet.ether + benzene
D3	9.0	1.5170	+35° to +42°	Ether.

Fraction D1 was saponified with alcoholic potash (5%, 500 ml) and separated into saponifiable and unsaponifiable parts. The saponifiable part (1.8 g) was indicated to be a mixture of lactones probably dihydrodehydrocostus lactone and dihydrocostunolide from the IR spectrum and physical constants, b.p.170-210° (bath)/0.7 mm., n_D^{28} 1.5121; $(\alpha)_D$ + 48.7°, (c, 1·8)

Analysis

Found: C, 77.2; H, 9.3.

$C_{15}H_{20}O_2$ requires: C, 76.93; H, 9.46%.

Costol

The unsaponifiable part was fractionated and the main component was found to be costol (IX) from

its IR spectrum and physical constants, b.p.115-117°/0.9 mm., n_D^{28} 1.5119; $(\alpha)_D$ + 8.9°, (c, 3.9)

Analysis

Found: C, 80.53; H, 10.95.

$C_{15}H_{24}O$ requires: C, 81.76; H, 10.9%.

Fractions D2 and D3 were similarly treated and found to consist mostly of costol and small amounts of lactone mixture.

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P A R T IIa

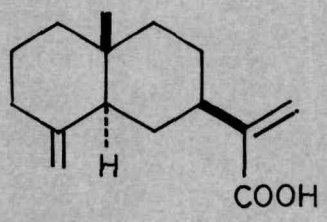
STRUCTURE AND ABSOLUTE CONFIGURATION OF COSTIC ACID.

***

SUMMARY

A new, crystalline, sesquiterpenic acid, costic acid, has been isolated from costus root oil. Its structure has been determined on the basis of physical properties, spectroscopic data and chemical degradations.

Costic acid has been smoothly converted into dihydroeudesmol by a series of stereospecific reactions, which establish its absolute configuration as shown below.



COSTIC ACID

Isolation of free acids from costus root oil

Costus root oil obtained by the low temperature solvent extraction procedure,[1] described earlier, contained free acids as revealed by its acid value of 15.2. The partially delactonised oil, obtained after the removal of solid, crystalline lactones from the above oil by stage-wise cooling at 0° and -18°, was further enriched in its free-acid content as indicated by its acid value which rose to 25.0. On the basis of the acid value, the partially delactonised oil was mixed with the requisite amount of alcoholic sodium hydroxide solution (10%) at 15°. The homogeneous mixture was shaken occasionally and kept for a short period. It was then diluted with water and neutral product extracted by shaking with ether. The aqueous alkaline solution contained the organic acids along with some hydrolysed lactones.

The aqueous alkaline solution was acidified with cold, dilute sulphuric acid. The regenerated acidic material was extracted with ether. The ether solution was then exhaustively extracted, first with 10% sodium bicarbonate solution, followed by a 10% solution of sodium carbonate. The two extracts were processed separately. The remaining ether solution which contained the lactones was not further examined.

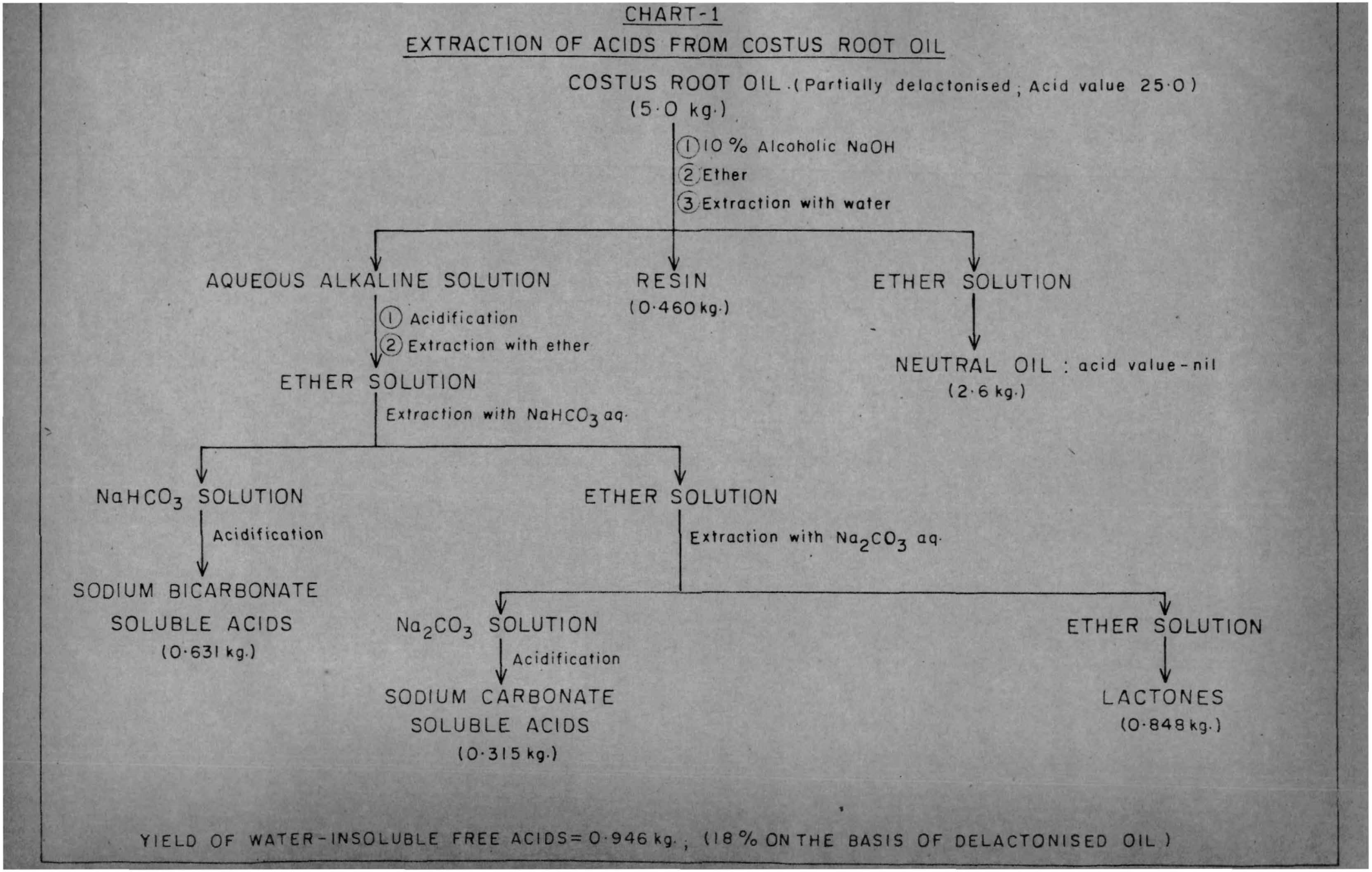


CHART-1
EXTRACTION OF ACIDS FROM COSTUS ROOT OIL
COSTUS ROOT OIL (Partially delactonised; Acid value 25·0)
(5·0 kg.)
① 10 % Alcoholic NaOH
② Ether
③ Extraction with water
AQUEOUS ALKALINE SOLUTION
RESIN
(0·460 kg.)
ETHER SOLUTION
① Acidification
② Extraction with ether
ETHER SOLUTION
NEUTRAL OIL : acid value - nil
(2·6 kg.)
Extraction with $NaHCO_3$ aq.
$NaHCO_3$ SOLUTION
ETHER SOLUTION
Acidification
Extraction with Na_2CO_3 aq.
SODIUM BICARBONATE
SOLUBLE ACIDS
(0·631 kg.)
Na_2CO_3 SOLUTION
ETHER SOLUTION
Acidification
SODIUM CARBONATE
SOLUBLE ACIDS
(0·315 kg.)
LACTONES
(0·848 kg.)
YIELD OF WATER-INSOLUBLE FREE ACIDS = 0·946 kg.; (18 % ON THE BASIS OF DELACTONISED OIL)

The sodium bicarbonate solution was extracted with ether to remove any adhering neutral material and then carefully acidified in the cold, with dilute sulphuric acid. The liberated acids were taken up in ether. A portion of it was converted into methyl ester and the GLC analysis of the distilled methyl ester revealed that it was a mixture of at least six components with very close retention times. A portion of the methyl ester was chromatographed on grade II neutral alumina using a high ratio, but a separation of the components could not be effected. The acid mixture did not form solid salts with cyclohexylamine, dicyclohexylamine and various other amines. It was distilled under high vacuum (10^{-5} mm) but a pure component could not be isolated. From the IR spectra of the acid mixture as well as its methyl ester, it was evident that hydroxy acids were present. The product contained non-conjugated acids as revealed by the UV data which did not show any characteristic absorption for α,β-unsaturated acid. This fraction was examined separately but the results are not incorporated in this thesis.

The sodium carbonate solution was similarly treated and the acids were extracted with ether. The ether extract was dried over anhydrous sodium sulphate,

removal of ether yielded the acids as a thick brown oil.

The present discussion will deal only with the acids obtained from the sodium carbonate extract.

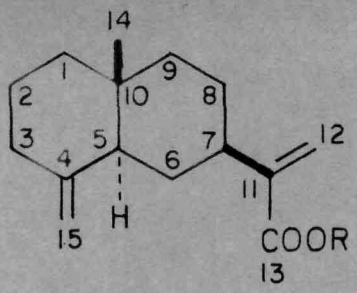
The acids thus isolated from the sodium carbonate solution were dissolved in acetone and the solution cooled at -18° for 24 hours, when a solid separated. It was collected by filtration. The mother liquor was concentrated and again chilled, when a second crop of the solid was obtained. When no further solid separated, the mother liquor was concentrated to yield a thick oil. The solid obtained from this operation was found to be a fatty acid from its IR spectrum. Several crystallisations from acetone-methanol mixture afforded pure palmitic acid (XIV) and behenic acid (XV).

A small portion of the acid mixture obtained after removal of fatty acids, was esterified with diazomethane. The distilled methyl ester was found to be a mixture of six components, four of which were in almost equal proportion, as revealed by its GLC analysis. In the UV spectrum it showed end absorption $\lambda_{max.}$ 210 mμ, ε 7,230, indicating the presence of an α,β- unsaturated ester. Its IR spectrum showed bands at: 1730, 1258 (ester grouping), 1653, 819 (conjugated methylene group), 1639, 891 cm^{-1} (end methylene group).

Methyl ester of this acid was then chromatographed on neutral alumina (grade II) using petroleum ether, petroleum ether-benzene, benzene and ether as eluents. Small fractions were collected and the course of separation was followed by physical properties, IR spectrum and GLC/TLC analyses. Physical properties of all the fractions varied within the limits of experimental error and IR spectra of every alternate fraction tested remained unaltered. The GLC analyses of every alternate fraction revealed no change in the pattern, from the original starting methyl ester. All these results showed that no separation could be effected by column chromatography.

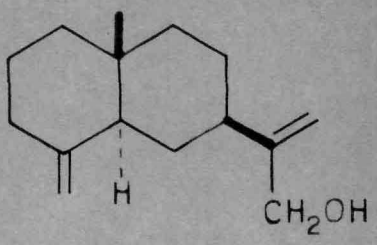
Isolation of costic acid.[2]

At this stage a recourse was taken to the amine salt formation of the acids.[3] A small sample of the acid was dissolved in methyl ethyl ketone and treated with requisite quantity of cyclohexylamine. The mixture became warm and a solid separated. This gave us a hint that amine salt formation may prove a key to the separation of individual components of the acid mixture. Accordingly, all the sodium carbonate soluble acid was dissolved in an excess of methyl ethyl ketone and a calculated amount of cyclohexylamine was added to it. The solid thus obtained was filtered, and washed with a little methyl ethyl ketone, crude m.p. 165-180°.

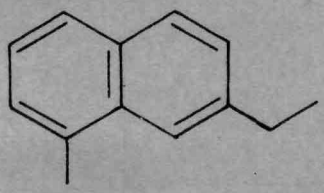


I , R = H

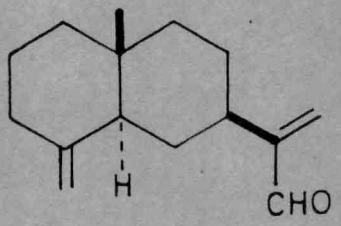
I a, R = CH_3



II



III



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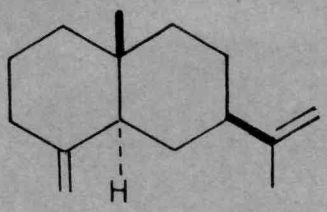

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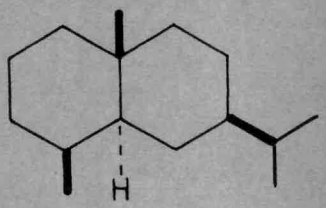

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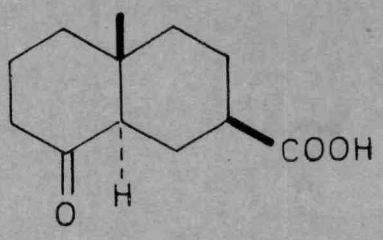


VII

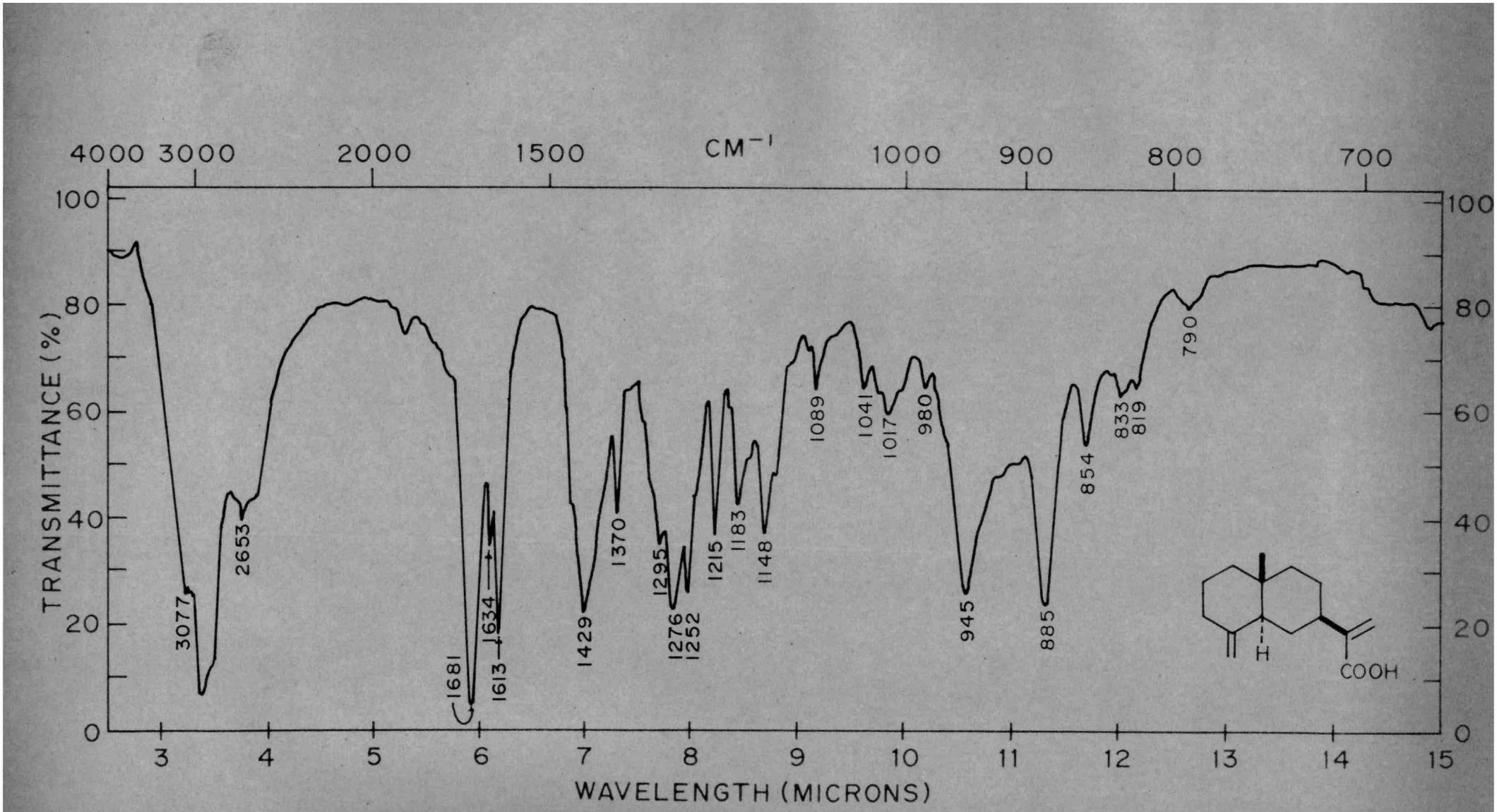


FIG. 1. IR SPECTRUM OF COSTIC ACID (IN NUJOL).

It was crystallised from the same solvent thrice to a constant melting solid, m.p.184-185°. The mother liquors of the cyclohexylamine salt were preserved.

The cyclohexylamine salt was suspended in ether and treated with aqueous alkali (5%) until all the solid dissolved in the alkaline solution. The ether layer, which contained the cyclohexylamine was removed. The aqueous alkaline solution was carefully acidified and the regenerated acid mixture was taken up in ether. Ether solution was dried and the ether evaporated. The residue was obtained as a colourless viscous oil.

A small quantity of this acid mixture was esterified with diazomethane and the distilled methyl ester on GLC analysis showed it to be composed of four components, three of which were in almost equal proportion. This result clearly indicated that a partial separation of components had taken place. Further purification was achieved by preparing a constant melting dicyclohexylamine salt, m.p.90-91°. The salt was decomposed with dilute alkali, as described earlier. The acid obtained from dicyclohexylamine salt, solidified. It was crystallised twice from dilute alcohol, m.p. 87-88°. It was found to be homogeneous by GLC and TLC examination of the acid and its methyl ester.

Structure of costic acid.[2]

The acid (I) analysed for the molecular formula $C_{15}H_{22}O_2$ and its equivalent weight was found to be in agreement with the calculated value for a mono-carboxylic acid. It was hydrogenated, in the presence of platinum oxide catalyst in ethanol solution when it absorbed two moles of hydrogen, indicating the presence of two double bonds. The acid was thus bicyclic. Its IR spectrum (Fig.1) exhibited bands at 1681 cm^{-1} due to a carboxyl group conjugated with a double bond; at 1634 and 885 cm^{-1} due to an exocyclic methylene group and 1613 cm^{-1} due to the methylene group in conjugation with the carboxyl group. The UV spectrum showed absorption for an α,β- unsaturated carboxyl group ($\lambda_{max.}$ 210 mμ; ε 8030).[4] This acid has been named as 'costic acid'.

Methyl ester of costic acid (Ia) also supported the α,β-unsaturated nature of the carboxyl group from the UV and IR spectra. It showed high end absorption, $\lambda_{max.}$ 210 mμ; ε 5624. Its IR spectrum (Fig.2) exhibited bands at: 1706 (conjugated ester group), 1629, 882 (exocyclic methylene group), 1613 and 814 cm^{-1} (methylene group in conjugation with the carbonyl of the ester). The NMR spectrum (Fig.3a) of the methyl ester showed signals at 9.25 τ (3H) due to the quaternary methyl group at C_{10}; 6.30 τ (3H) due to the methyl of the ester grouping;

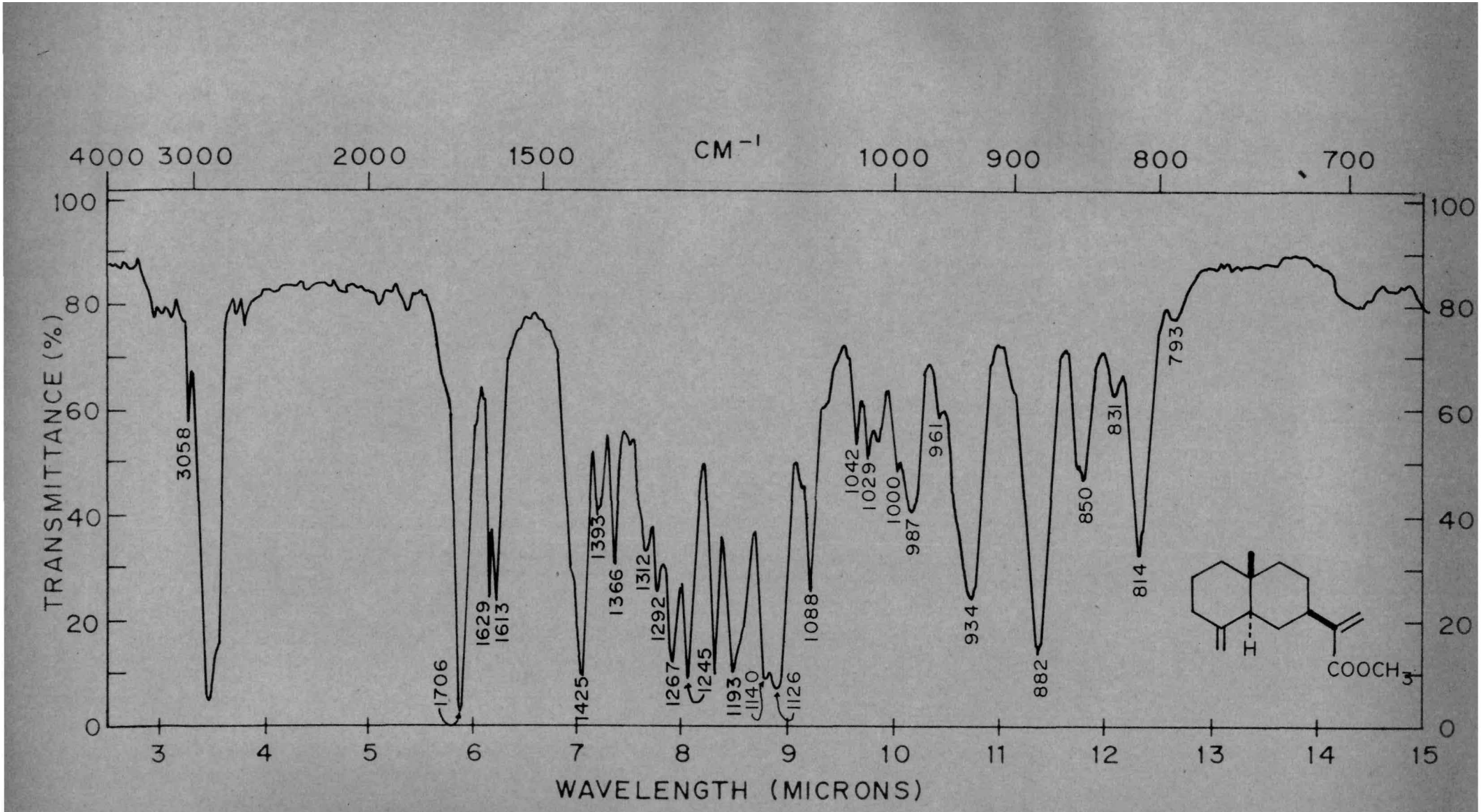


FIG. 2. IR SPECTRUM OF METHYL COSTATE (LIQUID FILM).

at 5.63, 5.37τ(2H) due to the two olefinic protons of the exocyclic methylene group at C_4; and at 4.52, 3.97τ (2H) due to the two protons of the methylene group at C_{11}, in conjugation with the ester carbonyl group. The spectral data were thus in complete agreement with structure Ia for the methyl ester of costic acid.

Costic acid on dehydrogenation in presence of selenium gave a 30% yield of 1-methyl-7-ethyl naphthalene[5] (III), characterised as its sym-TNB-adduct. The formation of 1-methyl-7-ethyl naphthalene accounted for 13 of the 15 carbon atoms present in the acid. Of the remaining two, one can be accounted as an angular methyl group which is eliminated during dehydrogenation and the other lost due to decarboxylation. From this, it appears that the carboxyl group was present in the isopropyl side chain.

The methyl ester of costic acid (Ia) on reduction with lithium aluminium hydride gave a mixture of two alcohols as revealed by the GLC analysis. The presence of two compounds was possible only by the formation of a small amount of dihydroalcohol, which would arise from the reduction of the conjugated methylenic double bond.

The methyl ester of costic acid was then reduced with lithium aluminium hydride-aluminium chloride complex[6] in which case the actual reduction is by aluminium hydride. The alcohol, costol (II), obtained by this procedure was a

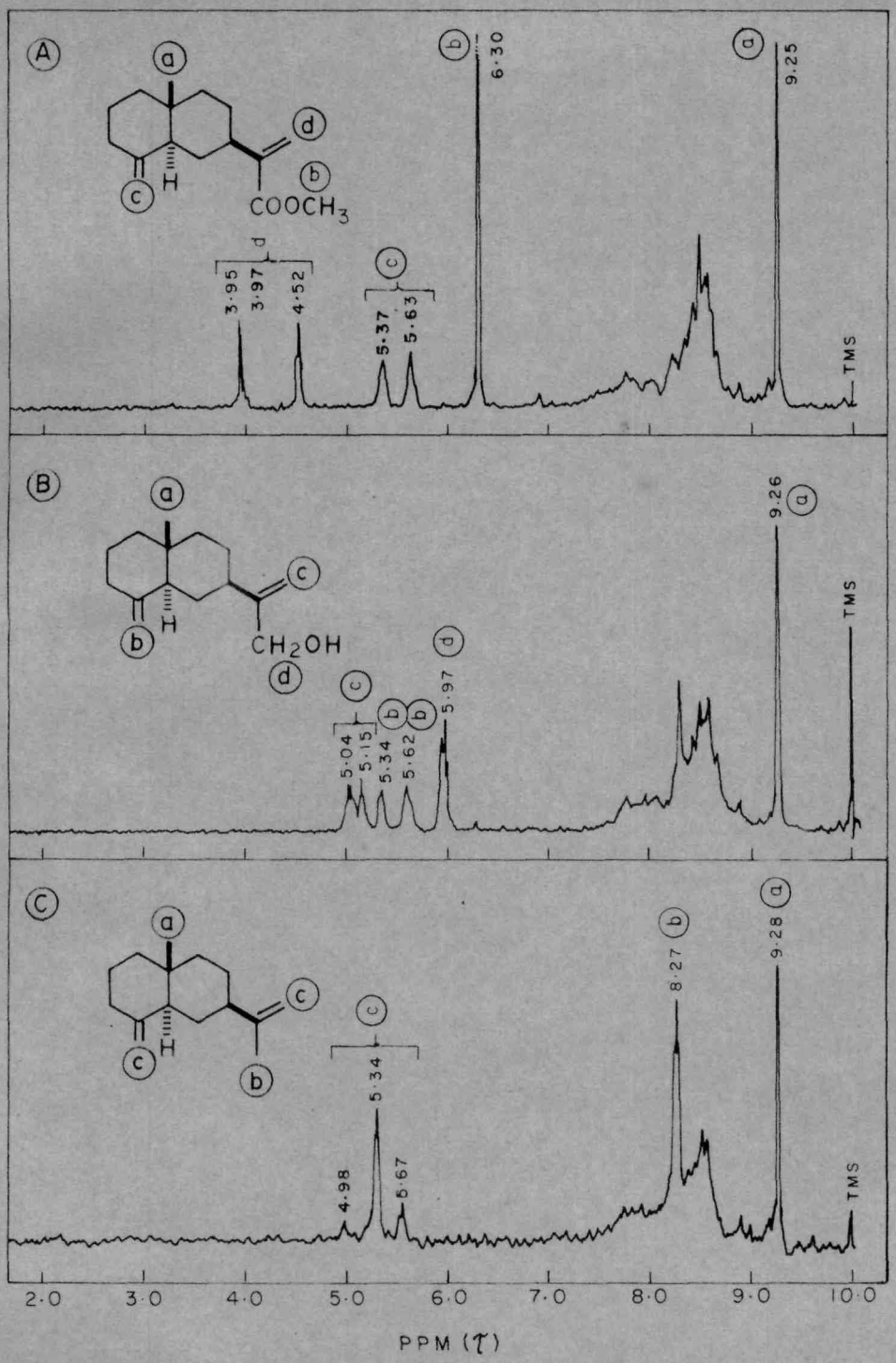


FIG.3

NMR SPECTRA OF : (A) METHYL COSTATE (B) COSTOL (C) β-SELINENE

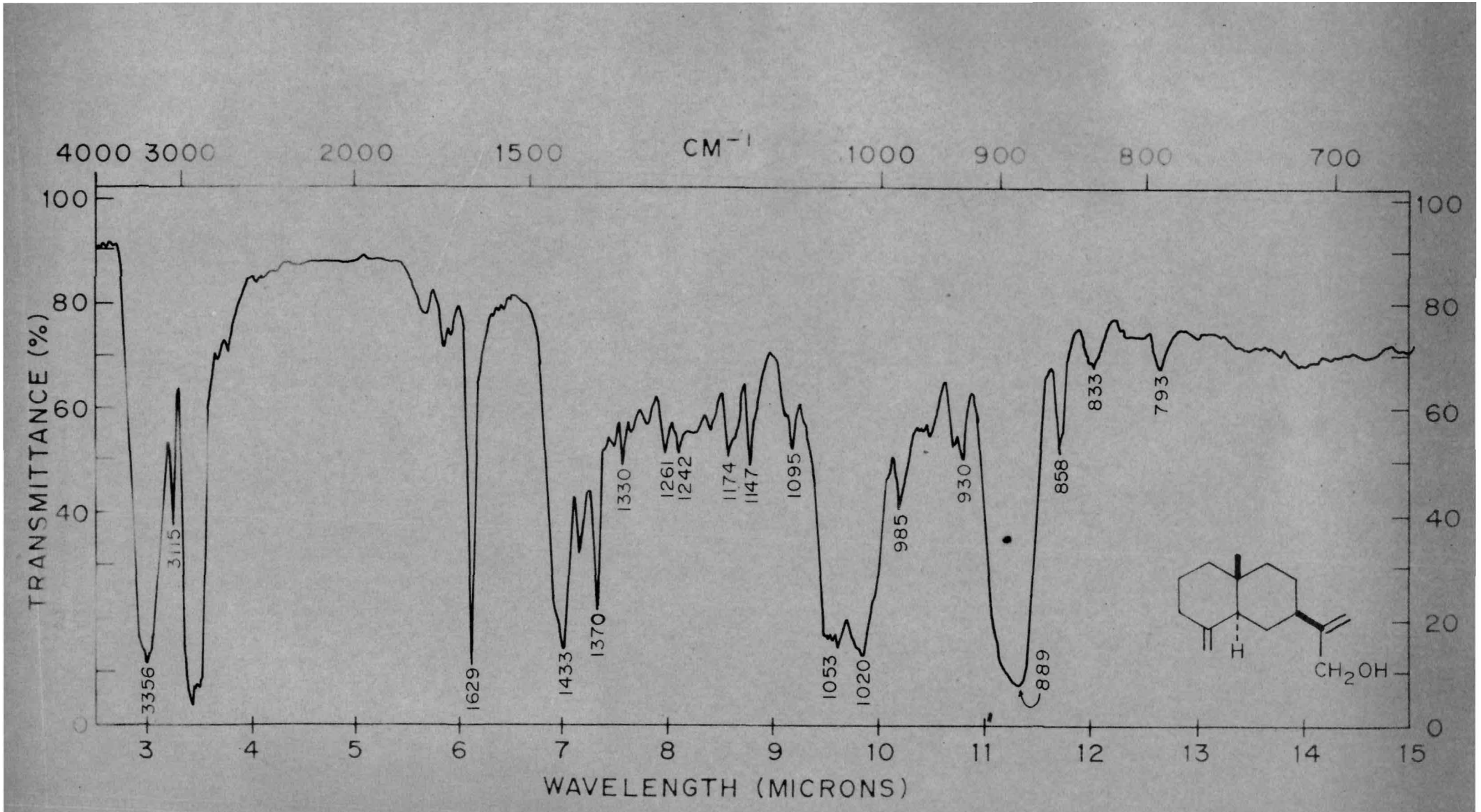


FIG. 4. IR SPECTRUM OF COSTOL (LIQUID FILM).

single, homogeneous product as revealed by GLC analysis. Its IR spectrum (Fig.4) showed bands at: 3356, 1020 (primary hydroxyl group) and 1639, 889 cm^{-1} (exocyclic methylene group). Its NMR spectrum (Fig.3b) exhibited signals at 9.26 τ(3H) due to angular methyl group at C_{10}; at 5.34, 5.62 τ (2H) due to the two olefinic protons of the exocyclic methylene group at C_4; at 5.04 and 5.15 τ(2H due to the two protons of the dissymmetrically disubstituted double bond at C_{11} and a multiplet at 5.97 τ(2H) due to the two protons of the allylic hydroxymethylene group. This was in complete agreement with the desired structure II of this alcohol.

On oxidation with Jones' chromic acid reagent[7] costol (II) gave an aldehyde (IV) (IR, Fig.5) in 75% yield, $\lambda_{max.}$ 216 mμ; ε 17,240. Its semicarbazone m.p. 223°, showed UV absorption at 262 mμ; ε 26,530. The UV absorption characteristics of the aldehyde as well as of its semicarbazone indicated that the aldehyde was an α,β-unsaturated aldehyde. This further confirmed the allylic nature of the parent alcohol and the α,β-unsaturated nature of costic acid.

The allylic alcohol (II) was reduced with lithium in liquid ammonia,[8] to give a hydrocarbon in 80% yield. The hydrocarbon was purified by chromatography and subsequent distillation on metallic sodium. It was a single, homogeneous product and was identified as

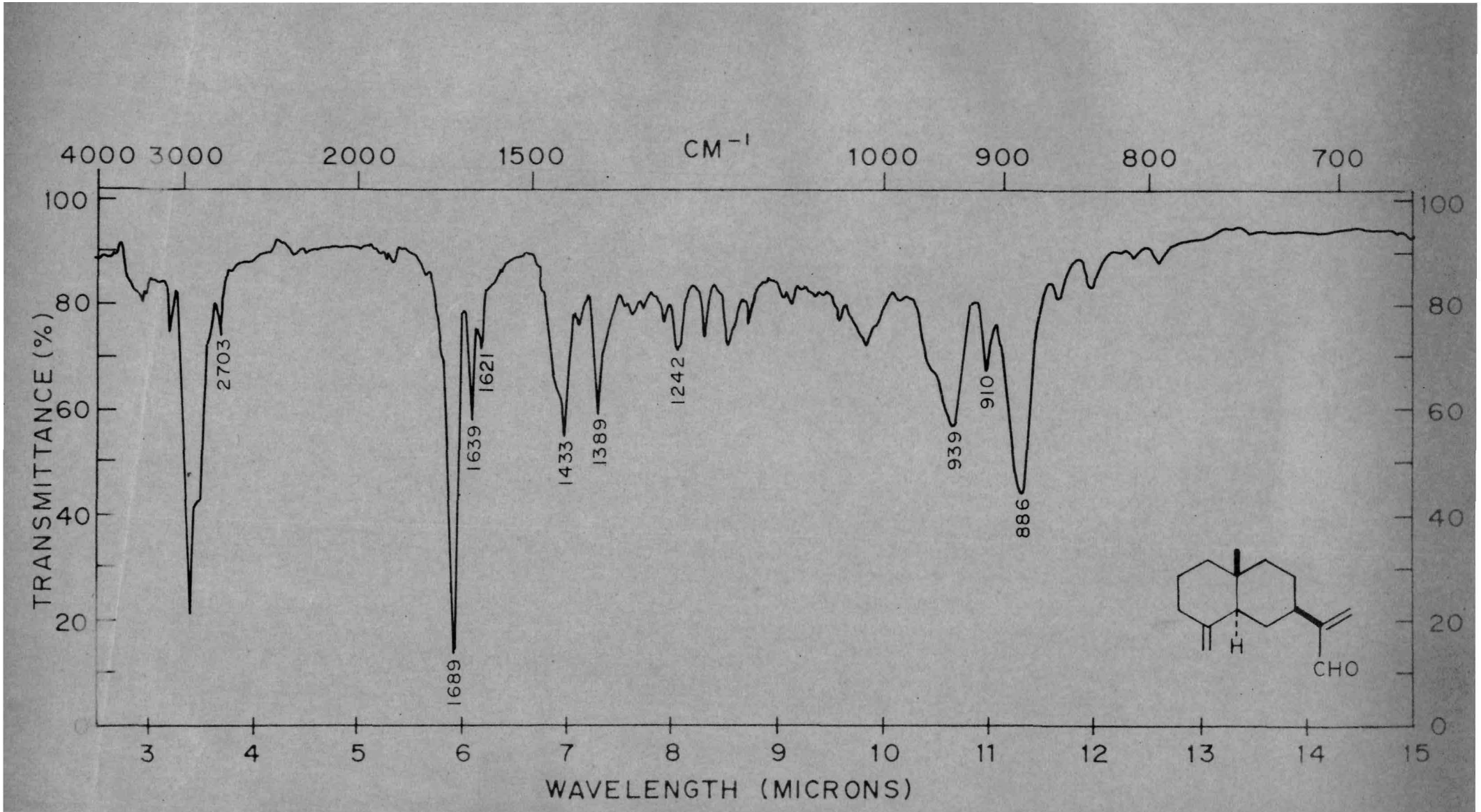


FIG. 5. IR SPECTRUM OF COSTAL (LIQUID FILM).

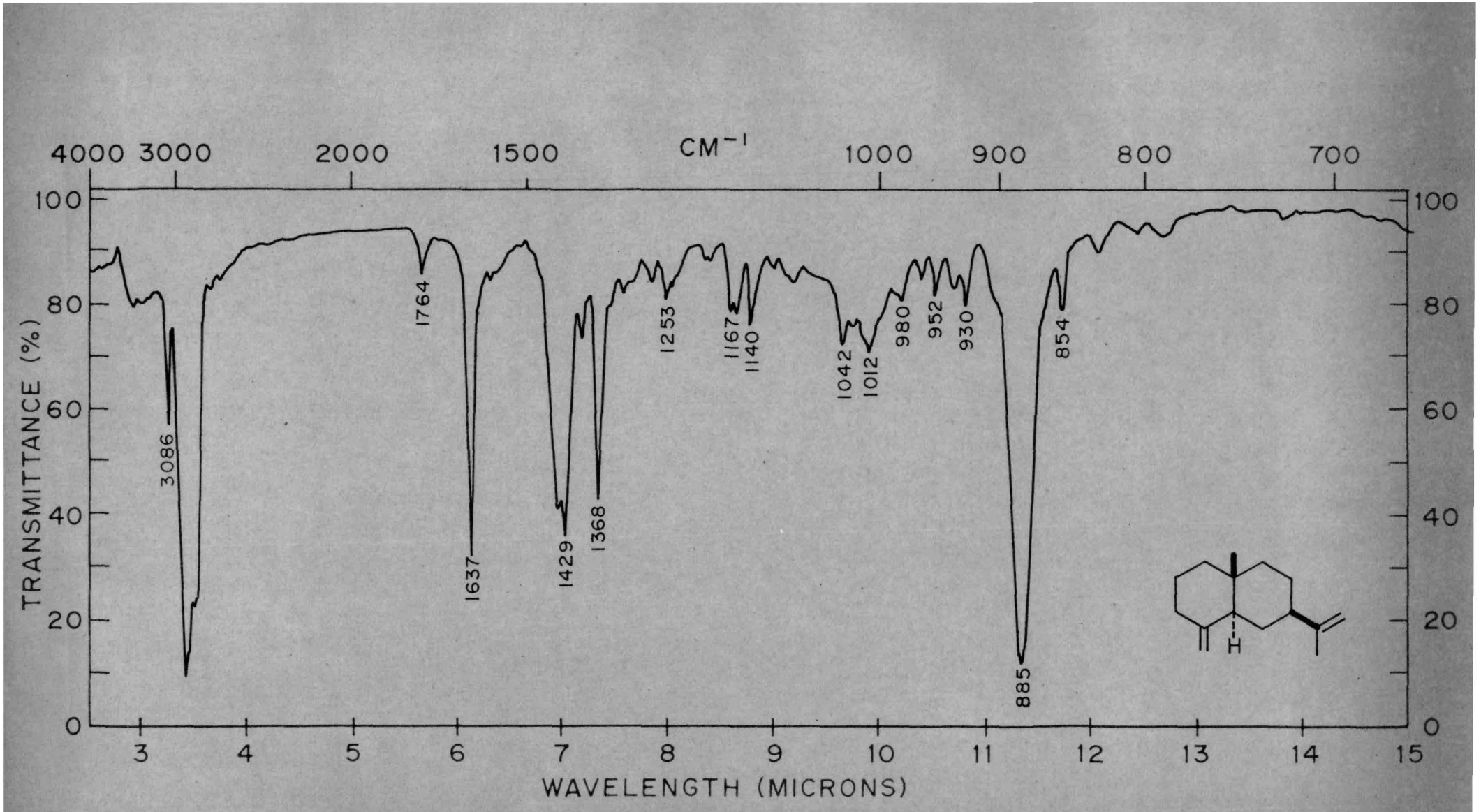


FIG. 6 IR SPECTRUM OF β-SELINENE (LIQUID FILM).

(+) β-selinene (V) from its physical properties, IR (Fig.6) and NMR spectra. It may specially be mentioned here that the β-selinene obtained from costic acid, via the alcohol II had a high specific rotation {+ 59.51°} which must be due to its high isomeric purity. The highest value recorded in the literature is + 40.0°.[9] The NMR spectrum (Fig. 3c) of the hydrocarbon showed signals at 9.28 τ(3H) due to the quaternary methyl group at C_{10}, at 8.27 τ(3H) due to the methyl group on a double bond at C_{11}; and at 5.67, 5.34 and 4.98 τ (4H) due to the four olefinic protons at C_4 and C_{11}.

The allylic nature of the alcohol II was further confirmed by the formation of selinane (VI) as a result of hydrogenolysis, when it was subjected to catalytic hydrogenation in ethanol in presence of palladised carbon. Selinane was identified by its physical properties, IR spectrum (Fig.7) and mixed GLC analysis with an authentic sample. Tetrahydrocostol was also obtained from this experiment.

Methyl costate (Ia) was ozonised in acetic acid solution[10] when a keto carboxylic acid (VII) was obtained. The methyl ester of VII gave a semicarbazone, m.p. 236°. This acid and the semicarbazone were previously obtained by Sorm by ozonisation of costol[11], a sesquiterpenic allylic alcohol, present in costus root oil.

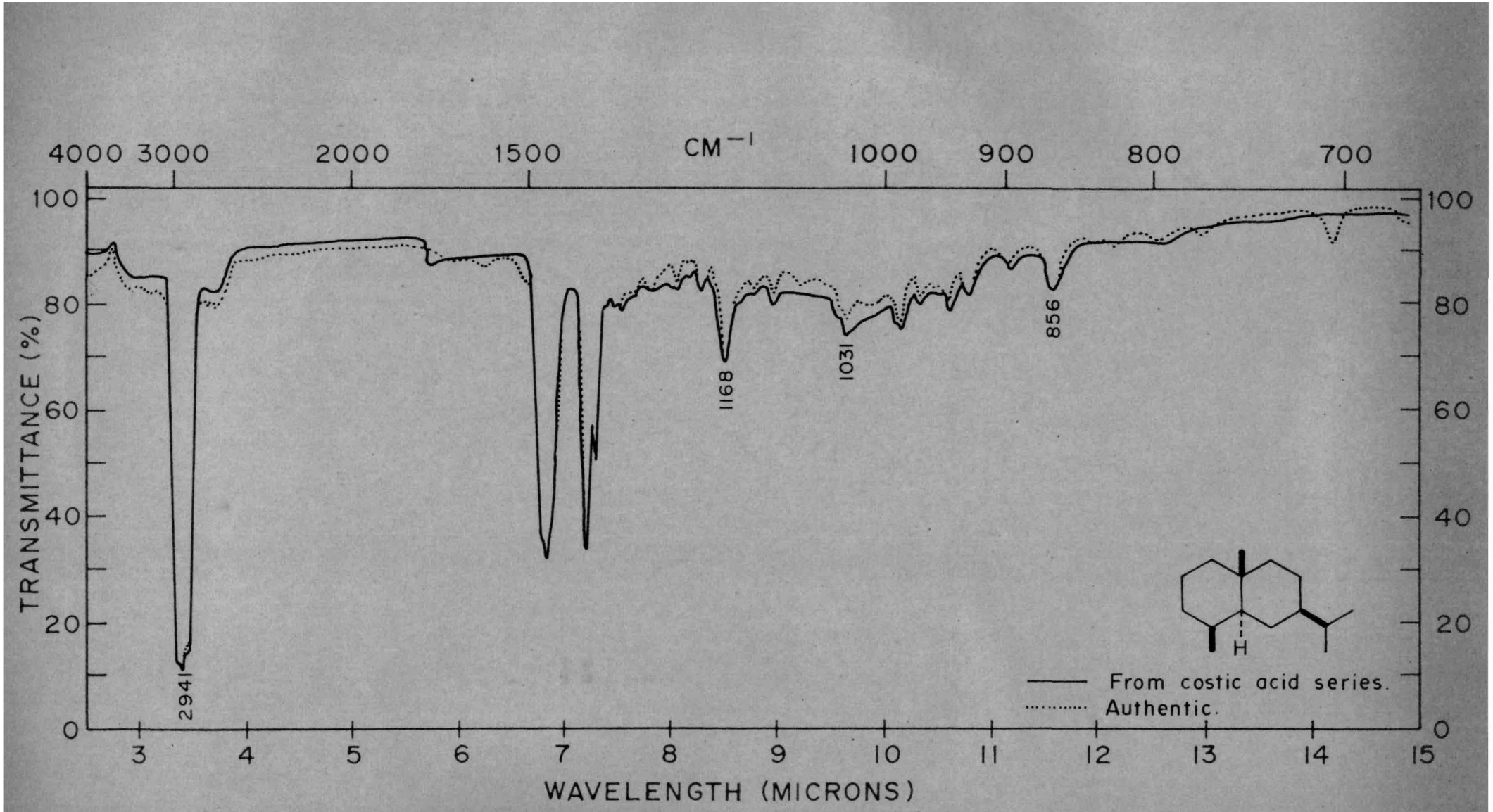


FIG. 7. IR SPECTRUM OF SELINANE (LIQUID FILM).

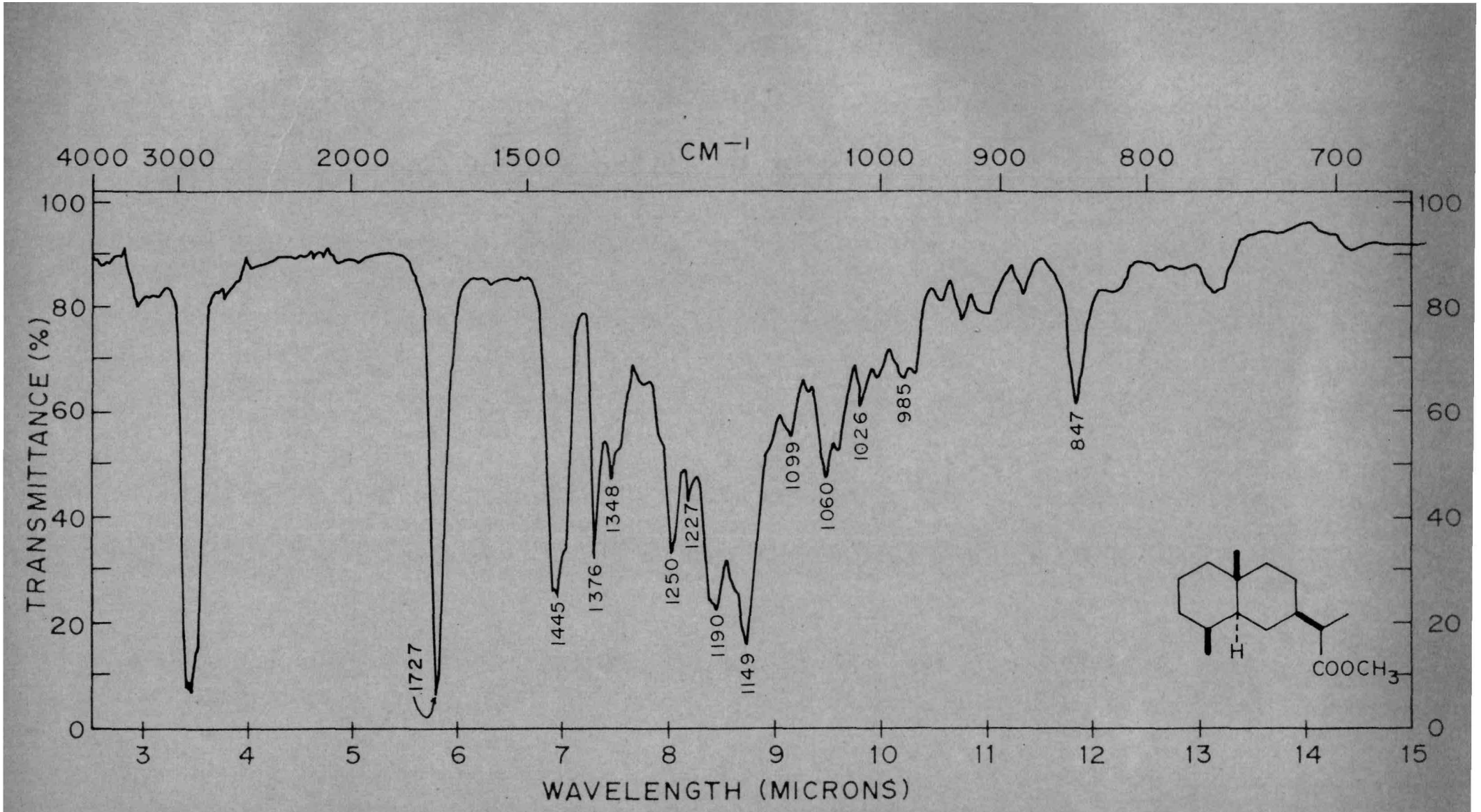


FIG. 8. IR SPECTRUM OF TETRAHYDRO METHYL COSTATE (LIQUID FILM).

From all these evidences it is clear that costic acid is represented by the structure I.

Absolute configuration of costic acid

Costic acid on hydrogenation in ethanol in presence of Adam's platinum oxide catalyst gave tetrahydrocostic acid (VIII) which did not solidify. Tetrahydrocostic acid has been systematically converted into dihydroeudesmol.

Tetrahydrocostic acid, on treatment with methyl lithium in dry ether, was converted into the methyl ketone (IX), which on Baeyer-Villiger oxidation with perbenzoic acid at room temperature gave the acetate (X). Saponification of the acetate yielded the alcohol (XI), which on oxidation with Jones' chromic acid reagent afforded the C_{14}-ketone (XII). The IR spectrum of XII (Fig.9) showed bands at: 1709, 1449, 1418 and 1351 cm^{-1}. This ketone was characterised by preparing its semicarbazone, m.p. 206-207°, agreeing with that reported in the literature.[12]

On treatment with methyl magnesium iodide in dry ether, the C_{14}-ketone (XII) was converted, in good yield, into dihydroeudesmol[12] (XIII), m.p.83-85°, $(\alpha)_D^{27}$ + 15.6°; mixed melting point with an authentic sample was undepressed and the IR spectra (Fig.10) were superimposable.

This proves conclusively the stereochemistry of tetrahydrocostic acid (VIII) at all the centres and hence that of the parent compound costic acid (I).

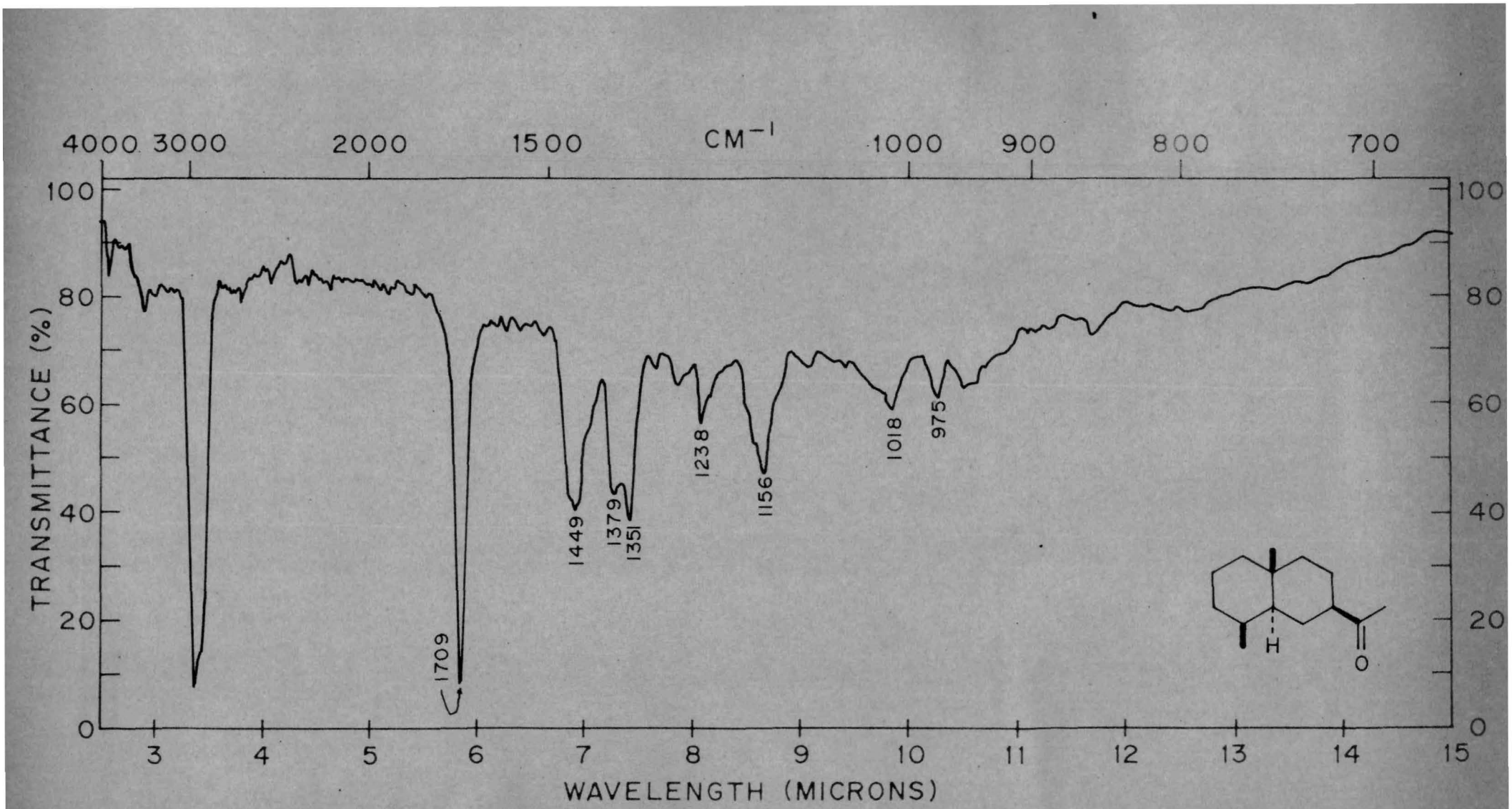
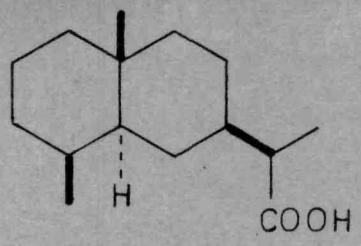
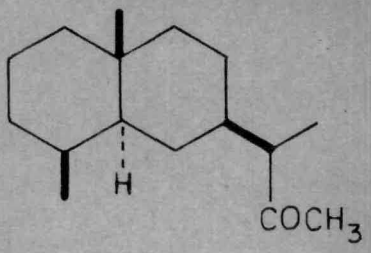


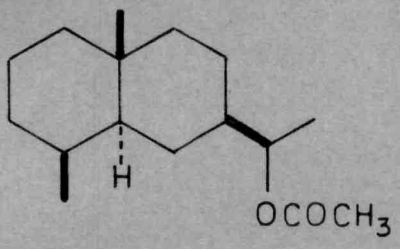
FIG. 9. IR SPECTRUM OF C_{14}-KETONE (LIQUID FILM).



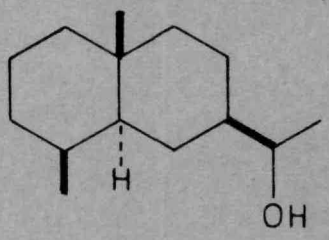
VIII



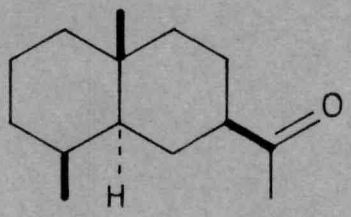
IX



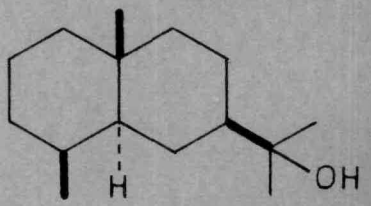
X



XI



XII



XIII

$CH_3 \cdot (CH_2)_{13} \cdot CH_2 \cdot COOH$

XIV

$CH_3 \cdot CH_2 \cdot (CH_2)_{18} \cdot CH_2 \cdot COOH$

XV

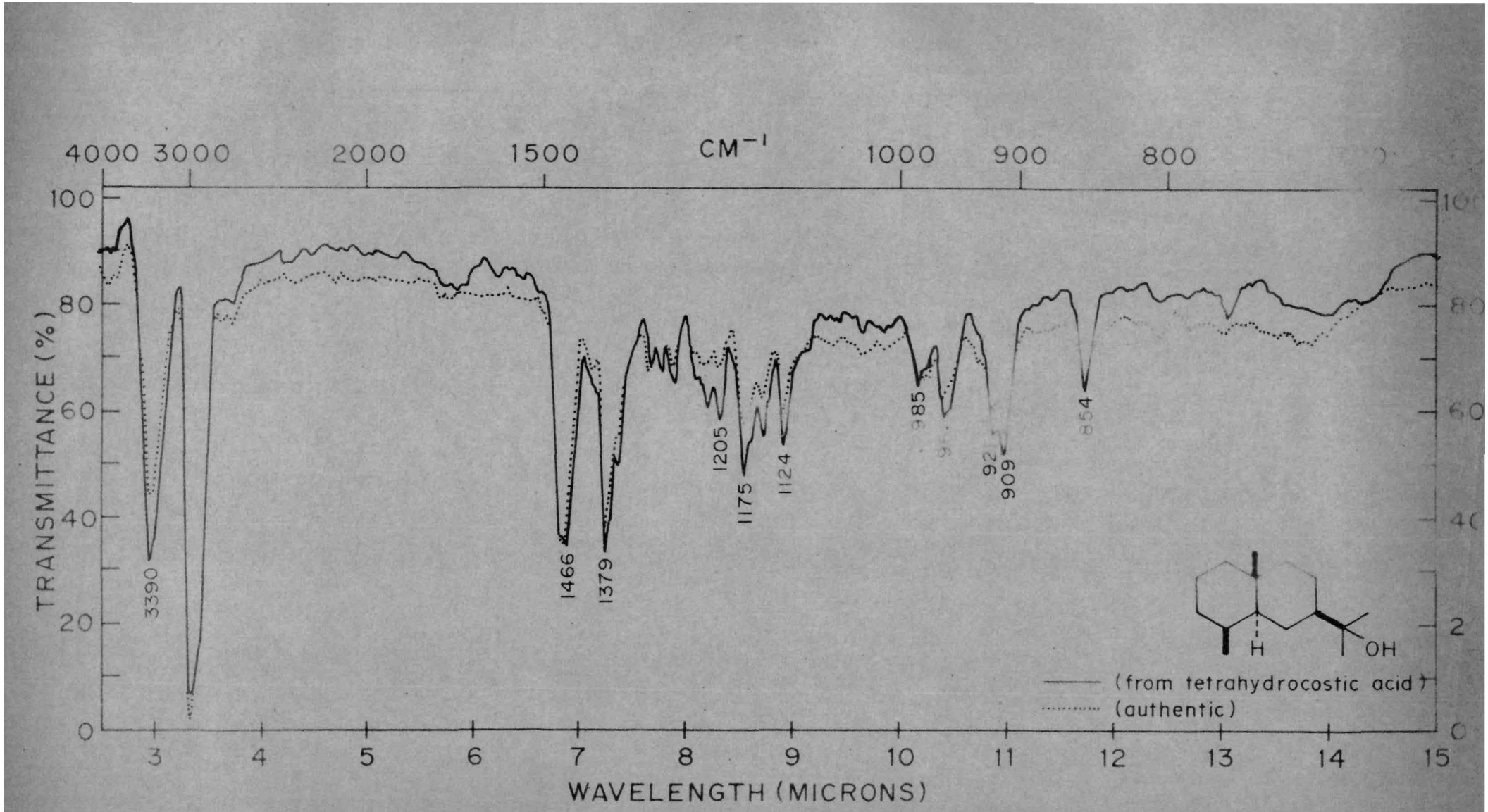


FIG. 10. IR SPECTRUM OF DIHYDRO EUDESMOL (IN NUJOL).

EXPERIMENTAL

Isolation of free acids from costus root oil

Partially delactonised costus root oil which was prepared by stagewise cooling of the petroleum ether solution of the oil at 0° and -18°, when most of the solid lactones crystallised out, was used for isolation of the free acids.

Partially delactonised costus root oil (500 g, acid value 25) was mixed with alcoholic sodium hydroxide solution (10%; 200 ml) at room temperature with thorough shaking. The mixture was allowed to stand for two hours with occasional shaking. It was then diluted with sufficient amount of water and stirred vigorously. Adequate quantity of ether was added to the mixture and the ether layer and the aqueous alkaline solutions were separated. The ether solution was again extracted with aqueous sodium hydroxide (10%; 50 ml). The combined aqueous alkaline solution was extracted twice with ether to remove all the adhering neutral oil, and then carefully acidified with cold, dilute sulphuric acid. The liberated acid was taken up in ether. The ether solution was washed free of mineral acid and dried over sodium sulphate. On removal of ether a residue (75 g) remained behind which was again dissolved in ether and extracted

thoroughly with 10% sodium bicarbonate solution. It was further extracted thoroughly with 10% sodium carbonate solution. The sodium bicarbonate extract and sodium carbonate extract were kept separately. The remaining ether solution which contained some lactones was preserved.

The sodium bicarbonate extract, on preliminary examination, was found to contain a mixture of hydroxy acids. They were investigated separately, but the results are not incorporated in this thesis.

The sodium carbonate solution was extracted with ether to remove adhering neutral matter and then acidified with cold, dilute sulphuric acid. The liberated acid was extracted with ether and the ether solution washed free of mineral acid and dried over anhydrous sodium sulphate. Evaporation of ether gave a dark, viscous liquid (29 g).

Isolation of palmitic (XIV) and behenic acids (XV).

The above liquid (29 g) was dissolved in acetone (75 ml) and kept at -18° overnight when a solid separated. It was collected by filtration and the mother liquor was concentrated to half the volume and left in the deep freeze for another 24 hr, when a further crop of solid was obtained. The mother liquor was preserved. The crude solid (3.0 g) was fractionally crystallised from a mixture of methanol-acetone. The first crop yielded almost pure palmitic acid (XIV). It was recrystallised from acetone m.p.61-62°.

Analysis

Found: C, 75.1; H, 12.2; Eq.wt. 255.10.

$C_{16}H_{32}O_2$ requires: C, 74.94; H,12.58%; Eq.wt. 256.42.

The mother liquor after removal of palmitic acid was concentrated to half the volume and cooled at 0°. The solid that separated was crystallised thrice from methanol-acetone (1:1) when behenic acid, (XV), m.p.75°, was obtained.

Analysis

Found: C, 78.16; H,13.10; Eq.wt.337.30.

$C_{22}H_{44}O_2$ requires: C, 77.58; H,13.02%; Eq.wt.34057.

Isolation of costic acid (I)

The mother liquor after removal of fatty acids was evaporated under vacuum and at a low temperature, the residue (25 g) was dissolved in methyl ethyl ketone (200 ml) and cyclohexylamine (12 g) was added to it. The mixture was shaken vigorously and left at room temperature for one hour. The solid amine salt thus obtained was filtered (23 g), crude m.p. 165-180°. It was crystallised three times from methyl ethyl ketone when a salt m.p. 184-185° was obtained.

The cyclohexylamine salt (20 g) was suspended in ether (100 ml) and 5% aqueous sodium hydroxide solution (350 ml) was added. The solid gradually disappeared and

aqueous and ethereal layers were distinctly formed. The ether layer containing cyclohexyl amine was removed and the aqueous alkaline layer was further extracted with ether to remove the residual amine. The alkaline solution was acidified with dilute hydrochloric acid, the regenerated acid was taken up in ether. Ether extract was washed with water and dried. Evaporation of ether gave a mixture of acids (15 g), as a clear, almost colourless liquid.

A small quantity of this acid was converted into its methyl ester (diazomethane). The distilled methyl ester revealed three peaks in the GLC analysis.

The above acid mixture (15 g) was dissolved in acetone (75 ml) and dicyclohexylamine (12 g) was added. The mixture was allowed to stand for 30 minutes at room temperature with occasional shaking when a solid separated. It was filtered and crystallised twice from acetone m.p. 91-92°.

The acid regenerated from its dicyclohexylamine salt by treatment with 5% alkali as before was obtained as a solid (10 g) and when crystallised from dilute alcohol yielded pure costic acid (8 g), m.p. 87-88°; $(\alpha)_D^{26}$ + 23.42° ($CHCl_3$; c, 1.3).

Analysis

Found: C, 76.79; H, 9.31; Eq.wt. 231.0.

$C_{15}H_{22}O_2$ requires: C, 76.88; H, 9.46%; Eq.wt. 234.33.

It showed end absorption in UV spectrum, $\lambda_{max.}$ 210 mμ; ε 5,030; and exhibited IR bands (Fig.1) at 1681, 1629, 1613 and 885 cm^{-1}.

Methyl costate (Ia)

Costic acid (1 g) was dissolved in ether (15 ml) and an ethereal solution of diazomethane (prepared from 1 g. nitrosomethyl urea) was added. Excess reagent was destroyed by adding two drops of acetic acid. The ethereal solution was washed free of acetic acid and dried over anhydrous sodium sulphate. The oil obtained after evaporation of ether was distilled (0.9 g), b.p. 135° (bath)/0.3 mm., n_D^{30} 1.5062; $(\alpha)_D^{27}$ + 30.71° ($CHCl_3$, c, 3.0), $\lambda_{max.}$ 210 mμ; ε 5,624. GLC showed a single peak. IR (Fig.2) bands at: 1706, 1629, 1613, 1245, 882, 814 cm^{-1}.

Analysis

Found: C, 77.41; H, 9.79.

$C_{16}H_{24}O_2$ requires: C, 77.37; H, 9.74%.

Selenium dehydrogenation of costic acid.

The acid (1 g) was heated with selenium (1.5 g) at 280-290° in an atmosphere of nitrogen for fifteen hours. The product was diluted with petroleum ether (40-60°) and passed through a column of alumina (grade II; 10 g). The column was eluted with pet.ether (40-60°; 100 ml). On

evaporation of the solvent a hydrocarbon (0.360 g) was obtained. It was distilled over metallic sodium to yield a liquid (0.215 g), b.p.140°(bath)/10 mm., $\lambda_{max.}$ 280 mμ, ε 2143; and $\lambda_{max.}$ 228 mμ, ε 48,700.

s-Trinitrobenzene (0.25 g) was added to the alcoholic solution of the above hydrocarbon (0.21 g). Yellow crystals separated, which after three crystallisations from methanol melted at 107-108°. Mixed melting point with an authentic sample of T.N.B. adduct of 1-methyl, 7-ethyl naphthalene was undepressed.

Analysis

Found: N, 11.14.

$C_{19}H_{17}O_6N_3$ requires: N, 10.96%.

Lithium aluminium hydride reduction of methyl costate.

Methyl costate (Ia, 0.6 g) dissolved in dry ether (20 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0.35 g) in dry ether (50 ml) at 0°. The mixture was stirred at 0° for 30 minutes and then refluxed for 6 hours. Excess reagent was destroyed by careful addition of moist ether. Ether solution was decanted and washed with a little water. It was dried over anhydrous sodium sulphate. Evaporation of ether gave a viscous liquid (0.45 g), b.p. 150°(bath)/0.5 mm., n_D^{24} 1.5098; $(\alpha)_D^{27}$ + 52.36° ($CHCl_3$; c, 2.3).

Analysis

Found: C, 81.94; H, 11.39.

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

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

GLC and TLC analyses showed the presence of two components in this product.

Aluminium hydride reduction of methyl costate.

Methyl costate (Ia; 0.73 g), dissolved in dry ether (25 ml) was added to a stirred slurry of aluminium hydride (prepared by adding 0.33 g anhydrous aluminium chloride to an ethereal suspension of 0.35 g. lithium aluminium hydride) in dry ether (50 ml) at room temperature. The stirring was continued for 45 minutes at room temperature. The excess reagent was destroyed by addition of moist ether and the product was isolated as described above. It was distilled to give pure costol (II; 0.53 g), b.p. 150°(bath)/0.5 mm., n_D^{26} 1.5180; $(\alpha)_D^{27}$ + 34.33° ($CHCl_3$; c, 4.6).

Analysis

Found: C, 81.79; H, 11.19.

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

The product was a single, homogeneous compound as revealed by GLC and TLC analyses. It showed IR (Fig.4) bands at: 3366, 1639, 1020, ~~886~~ 885 cm^{-1}.

Costal (IV) from costol (II)

To costol (II, 1 g) dissolved in acetone (20 ml) was added Jone's chromic acid reagent at 0° till brown colour persisted. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed free of sulphuric acid with water, followed by sodium bicarbonate solution to remove any organic acid formed during oxidation. The ether solution was dried over sodium sulphate. On evaporation, costal (0.75 g) was obtained. It was distilled b.p. 130° (bath)/0.8 mm., n_D^{27} 1.4990; $\lambda_{max.}$ 216 mμ; ε 17,240.

Analysis

Found: C, 82.96; H, 10.53.

$C_{15}H_{22}O$ requires: C, 82.51; H, 10.16%.

Its IR spectrum (Fig.5) showed bands at: 2703, 1689, 1639, 1621 and 886 cm^{-1}.

Semicarbazone of costal

Costal (0.5 g) dissolved in ethanol (5 ml) was added to an aqueous solution of semicarbazide hydrochloride (0.25 g) and sodium acetate (0.3 g). The semicarbazone (0.3 g) after three crystallisations from ethyl alcohol melted at 223°, $\lambda_{max.}$ 262 mμ; ε 26,530.

Analysis

Found: C, 69.78; H, 9.15; N, 15.26.

$C_{16}H_{25}N_3O$ requires: C, 70.04; H, 9.19; N, 15.31%.

Conversion of costol (II) into β-selinene (V)

Reduction of costol with lithium in liquid ammonia

Metallic lithium (0.5 g) was added in small lots and under stirring to liquid ammonia (500 ml) collected in a well insulated three-necked flask fitted with a mechanical stirrer and a reflux condenser. Costol (0.64 g) dissolved in dry tetrahydrofuran (150 ml) was then added dropwise during 30 minutes to the lithium-liquid ammonia complex. Stirring was continued for three hours after the addition was complete. The reaction mixture was allowed to stand at room temperature overnight when most of the ammonia escaped. The residue was carefully diluted with water and extracted with ether. The ether solution was washed twice with water and dried over anhydrous sodium sulphate. The product obtained after evaporation of ether, was passed through a column of neutral alumina (grade I, 10 g) and eluted with petroleum ether (40-60°). On removal of the solvent, the residue was distilled (0.4 g), b.p. 140° (bath)/7 mm., n_D^{29} 1.4900; $(\alpha)_D^{30}$ + 89.51° ($CHCl_3$, c, 2.05).

Analysis

Found: C, 88.3; H, 11.76.

$C_{15}H_{24}$ requires: C, 88.16; H, 11.84%.

It showed IR bands at (Fig.6) 3086, 1637, 1395, 1368, 1253, 1140, 1042, 980, 952, 885 and 854 cm^{-1}.

Conversion of costol (II) into selinane (VI)

Costol (0.26 g) dissolved in ethanol (15 ml) was hydrogenated in presence of palladium charcoal(5%, 0.25 g) catalyst, until no further absorption of hydrogen took place (80 ml H_2 at N.T.P.). The product was isolated as usual and passed through a column of neutral alumina (grade II; 15 g) and eluted with petroleum ether (40-60°) and ether. Petroleum ether fraction gave a hydrocarbon (0.15 g) on removal of solvent. It was distilled over metallic sodium, b.p. 150°(bath)/7 mm., n_D^{28} 1.4793; $(\alpha)_D^{27}$ + 8.32° ($CHCl_3$; c, 3.1).

Analysis

Found: C, 86.32; H, 13.7.

$C_{15}H_{28}$ requires: C, 86.50; H, 13.50%.

IR spectrum (Fig.7) was identical with that of the authentic sample of selinane (VI).

The product eluted with ether was identified as tetrahydrocostol, b.p. 140°(bath)/0.5 mm., n_D^{28} 1.4932.

Analysis

Found: C, 79.81; H, 12.62.

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

Ozonization of methyl costate

Methyl costate (0.78 g) was dissolved in glacial acetic acid (20 ml) and ozonised oxygen was passed through it at room temperature for three hours. The reaction

mixture was left at room temperature for two hours. Hydrochloric acid (2N, 20 ml) was then added to it. Glacial acetic acid (20 ml) was also added to make a clear, homogeneous solution. The solution was allowed to stand overnight at room temperature. The solvent was evaporated under reduced pressure and the residue taken up in ether. The ether solution was washed with water and then extracted with sodium carbonate (10%, 25 ml) solution. The aqueous alkaline solution was acidified with dilute hydrochloric acid. The bulk was saturated with ammonium chloride and thoroughly extracted with ether. Removal of ether gave the keto acid (VII). It was esterified with diazomethane (prepardd from 1 g. nitrosomethyl urea) and the methyl ester (0.3 g) distilled, b.p. 145° (bath)/0.08 mm. IR bands at: 1730, 1706, 1250 cm^{-1}.

Semicarbazone of the above methyl ester (0.3 g) was prepared by adding an aqueous solution of semicarbazide hydrochloride (0.17 g) and sodium acetate (0.2 g) to the alcoholic solution (5 ml) of the methyl ester. The semicarbazone was crystallised three times from ethanol, m.p. 236°.

Analysis

Found: C, 60.01; H, 7.69; N, 15.22.

$C_{14}H_{23}O_3N_3$ requires: C, 59.76; H, 8.24; N, 14.94%.

Tetrahydrocostic acid (VIII)

Costic acid (0.1987 g) dissolved in ethanol (8 ml) was hydrogenated in presence of palladised carbon (5%; 0.05 g). Total hydrogen uptake was 38.77 ml at N.T.P. corresponding to two double bonds. Reaction mixture was filtered and the alcohol was evaporated from the filtrate. The tetrahydrocostic acid thus obtained was a thick liquid.

The methyl ester of VIII

Tetrahydrocostic acid (0.19 g) was esterified with the ethereal solution of diazomethane and the methyl ester (0.15 g) was distilled, b.p.135° (bath)/ 0.7 mm., n_D^{28} 1.4849; $(\alpha)_D^{27}$ + 24.88° ($CHCl_3$; c, 2.7).

Analysis

Found: C, 76.35; H, 11.28.

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

Its UV spectrum showed no characteristic absorption (λ max. 210 mμ; ε 718). It exhibited IR bands (Fig. 8) at : 1727, 1250 cm^{-1}. There was no absorption in the double bond region.

Conversion of costic acid into dihydroeudesmol (XIII)

Tetrahydrocostic acid (1.2 g) dissolved in dry ether (10 ml) was added at 0° to a stirred solution of

methyl lithium (prepared from 0.5 g. lithium and 5 g. methyl iodide) in dry ether, during 10 minutes. The reaction mixture was stirred at room temperature for 30 minutes after the addition. Excess reagent was carefully destroyed by addition of moist ether, followed by water. Aqueous and ethereal layers were separated. The ether layer was washed with water and dried over anhydrous sodium sulphate. On removal of ether an oil was obtained (1 g). It was chromatographed on neutral alumina (grade II, 20 g) and eluted with petroleum ether (40-60°). Removal of solvent gave the methyl ketone (IX; 0.86 g), b.p. 135° (bath)/ 0.4 mm., n_D^{27} 1.4850.

Analysis

Found: C, 80.87; H, 12.01.

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

It showed IR bands at: 1704, 1381, 1366, 1220, 1155, 1109, 975, 943 and 866 cm^{-1}.

The methyl ketone (IX, 0.7 g) was dissolved in chloroform (20 ml) and 0.2 N perbenzoic acid (20 ml) and toluene-p-sulphonic acid (0.05 g) were added to it. The mixture was stirred at room temperature for 40 hours. The chloroform solution was washed with water, sodium carbonate solution and again with water. It was dried over sodium sulphate. The product obtained after removal of

chloroform was passed through a column of alumina(grade II; 10 g) and eluted with petroleum ether (40-60°). Removal of solvent gave the acetate (X; 0.63 g).

The crude acetate was saponified with 10% alcoholic potash (20 ml) for 3 hours. The reaction product was worked up as usual and the neutral product was isolated as a thick liquid. It was chromatographed on neutral alumina (grade II; 13 g) and eluted with pet.ether and ether. The ether fraction on evaporation yielded the alcohol (XI;0.5 g), b.p.140°(bath)/0.4 mm., n_D^{27} 1.4923.

Analysis

Found: C, 79.68; H, 12.30.

$C_{14}H_{26}O$ requires: C, 79.93; H, 12.46 .

The alcohol (XI; 0.4 g) was dissolved in acetone (10 ml) and oxidised with Jone's chromic acid reagent. The product was isolated as usual and chromatographed on neutral alumina (grade II, 10 g). The petroleum ether eluate on removal of solvent gave the C_{14}-ketone (XII; 0.35 g). It was distilled, b.p.140°(bath)/1 mm., n_D^{27} 1.4870; $(\alpha)_D^{27}$ - 2.0° ($CHCl_3$; c, 4.7).

Analysis

Found: C, 80.67; H, 11.68%.

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

IR spectrum (Fig.9) exhibited bands at: 1709, 1449, 1379, 1351, 1238, 1156, 1018 and 975 cm^{-1}.

Semicarbazone of XII

The C_{14}-ketone (0.1 g) dissolved in ethyl alcohol (4 ml) was added to a solution of semicarbazide hydrochloride (0.05 g) and sodium acetate (0.055 g) in water (8 ml). The solid thus obtained was crystallised twice from ethanol, m.p. 206-207°.

Analysis

Found: N, 15.64.

$C_{15}H_{27}ON_3$ requires: N, 15.80%.

Dihydroeudesmol (XIII) from the methyl ketone (XII)

The ketone (0.2 g) dissolved in dry ether (10 ml) was added at 0° under stirring to a solution of methyl magnesium iodide (prepared from 0.2 g magnesium wire and 3 g. methyl iodide) in dry ether (30 ml). The reaction mixture was stirred at 0° for one hour and was then poured into an ice-cold saturated solution (25 ml) of ammonium chloride. The mixture was allowed to warm to room temperature and the organic layer separated. The aqueous layer was extracted twice with ether and the ether extract mixed with the original ether solution. It was dried over sodium sulphate and the ether distilled when a solid (0.2 g) was obtained. It was sublimed at 75°/0.6 mm. The sublimate, m.p. 70-73° was crystallised from petroleum ether (60-80°)

to give pure dihydroeudesmol (XIII), m.p. 83-85°; mixed m.p. with authentic sample was undepressed, $(\alpha)_D^{27}$ + 15.09° ($CHCl_3$; c, 2.7). Authentic sample of dihydroeudesmol had $(\alpha)_D^{27}$ + 17.18° ($CHCl_3$; c, 1.9).

Analysis

Found: C, 80.24; H, 12.60.

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

Its IR spectrum (Fig.10) showed bands at: 3390, 1466, 1379, 1205, 1175, 985, 961, 909 and 854 cm^{-1}.

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P A R T IIb

INVESTIGATIONS ON THE ALCOHOLIC FRACTION OF COSTUS ROOT OIL

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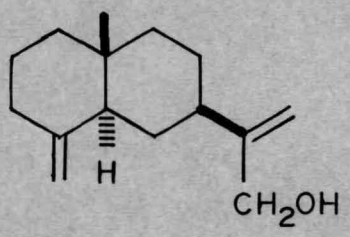
SUMMARY

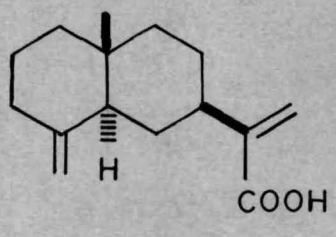
Costus root oil contains a substantial amount of alcoholic constituents, of which costol is one. Sorm and co-workers determined the structure of costol on the basis of spectral and chemical evidences.

It has now been found that costol examined by Sorm was not a single, homogeneous compound, but a mixture of at least three components, one of which possesses structure assigned by Sorm. This complex alcoholic mixture has been oxidised, first with chromic acid and then with silver oxide, to a mixture of acids, from which crystalline costic acid has been obtained. Pure costol has been obtained, via the reduction of methyl costate.

Amongst the other components, elemol has been identified from its physical properties and IR spectrum.



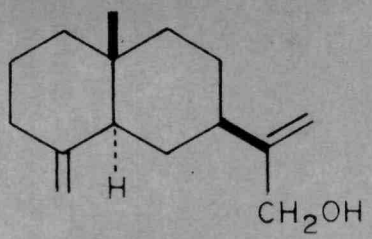
COSTOL



COSTIC ACID

Costus root oil is known to contain a substantial amount of alcohols as was first mentioned by Semmler and Feldstein.[1] They obtained a sesquiterpenic primary alcohol, costol, from the fraction boiling at 175-190°/11 mm. They oxidised this alcohol to the corresponding aldehyde from which they obtained a semicarbazone, m.p. 217-18°. They also suggested a structural relationship between costol and a sesquiterpenic acid, costus acid, $C_{15}H_{22}O_2$, which they found to be present in costus root oil. They, however, did not arrive at any conclusions regarding the structure of the alcohol or the corresponding acid.

Subsequently, Sorm and coworkers[2] examined costus root oil more critically and obtained a primary alcohol, costol, from the neutral portion of the oil. They assigned structure (I) to this compound on the basis of spectral and chemical evidences.[3] Sorm found that it did not form any solid derivative. It was purified by elaborate chromatography and through its non-crystalline phthalic half-ester. The only solid derivative was the semicarbazone of the aldehyde obtained from this alcohol. The catalytic hydrogenation data indicated that it was allylic in nature. A saturated hydrocarbon, selinane (II) presumably formed due to hydrogenolysis, was obtained together with tetrahydrocostol (III), during hydrogenation.



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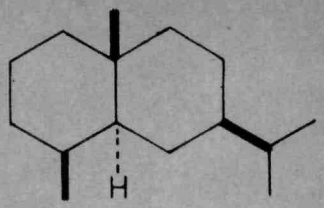

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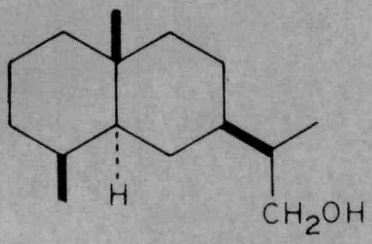

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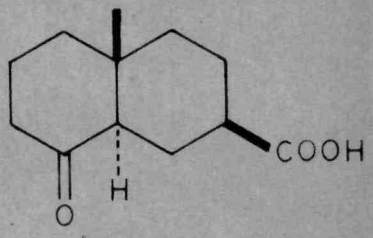

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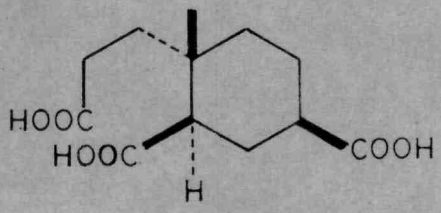

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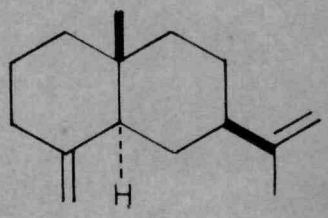
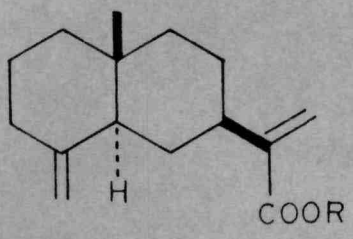
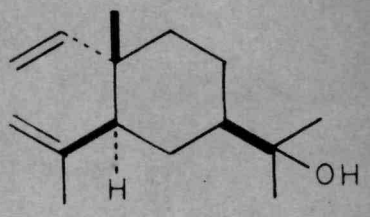


VI



VII a) R = H

b) R = CH_3



VIII

The final proof of the structure and absolute configuration of costol was obtained by the ozonolysis of costol to the corresponding ketocarboxylic acid (IV), from which on further degradation, a tricarboxylic acid (V) was obtained. This acid (V) has also been obtained by Semmler and Risse[4] and also by Sorm and collaborators[3] from β-selinene (VI). In view of the fact that the absolute configuration of β-selinene[5] is known, the two tricarboxylic acids obtained from β-selinene and costol, could be correlated; and hence costol was assigned the stereostructure (I).

In our laboratory, we had a substantial amount of costus root oil at our disposal and as a part of the project to characterise as many of its constituents as possible, we undertook the investigation of the alcoholic fraction of the oil.

The partially delactonised, acid-free, costus root oil[6] was distilled under reduced pressure and collected into two fractions. The first fraction, b.p.90-150°/9 mm. contained mostly hydrocarbons as indicated by its physical properties and spectral data. The second fraction, b.p. 100-130°/0.2 mm. exhibited in the IR spectrum a strong band in the hydroxyl region and also bands in the carbonyl region for ketones and lactones. The higher boiling residue was not investigated.

The second fraction, mentioned above, was initially saponified to remove most of the lactones and then chromatographed on neutral alumina (grade II) and eluted successively with petroleum ether (40-60°), benzene and ether. The petroleum ether fraction was found to be free of alcoholic impurities and contained mostly ketonic compounds as indicated by the IR spectrum. This fraction was not examined further by the present author. The benzene fraction afforded a product which was evidently a mixture of ketones and alcohols. The last, the ether fraction, was rich in alcoholic compounds.

The IR spectrum (Fig.1a) of this fraction showed bands at 3350, 1020, 1640 and 887 cm^{-1}, indicating the presence of a primary hydroxyl group as well as exocyclic methylenic double bond. On distillation, the product showed physical properties closely similar to those reported by Sorm[3] for costol. GLC (Fig.2) and TLC analyses at this stage, however, indicated that the product was not homogeneous but a mixture of atleast three components, two of which were in almost equal proportion. This led us to suspect that costol examined by Sorm was also an impure product; and hence we undertook a thorough investigation of the same.

The product was chromatographed twice over neutral, grade II alumina using a ratio of 1:50 and 1:100 and eluted successively with petroleum ether (40-60°),

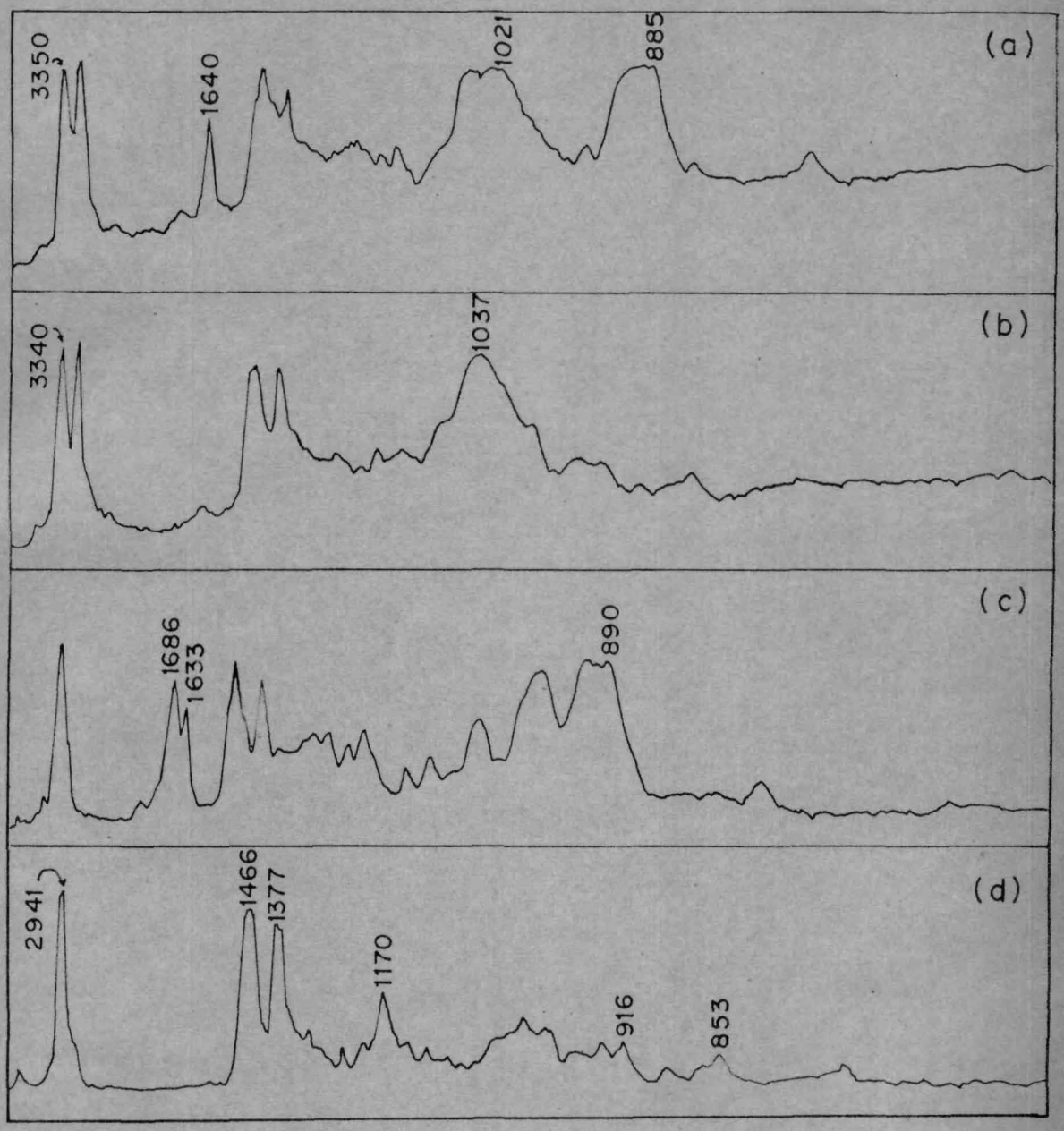


FIG. 1.

(a) IR SPECTRUM OF "COSTOL" (LIQUID FILM).

(b) IR SPECTRUM OF TETRAHYDROCOSTOL (LIQUID FILM).

(c) IR SPECTRUM OF COSTAL (LIQUID FILM).

(d) IR SPECTRUM OF SELINANE (LIQUID FILM).

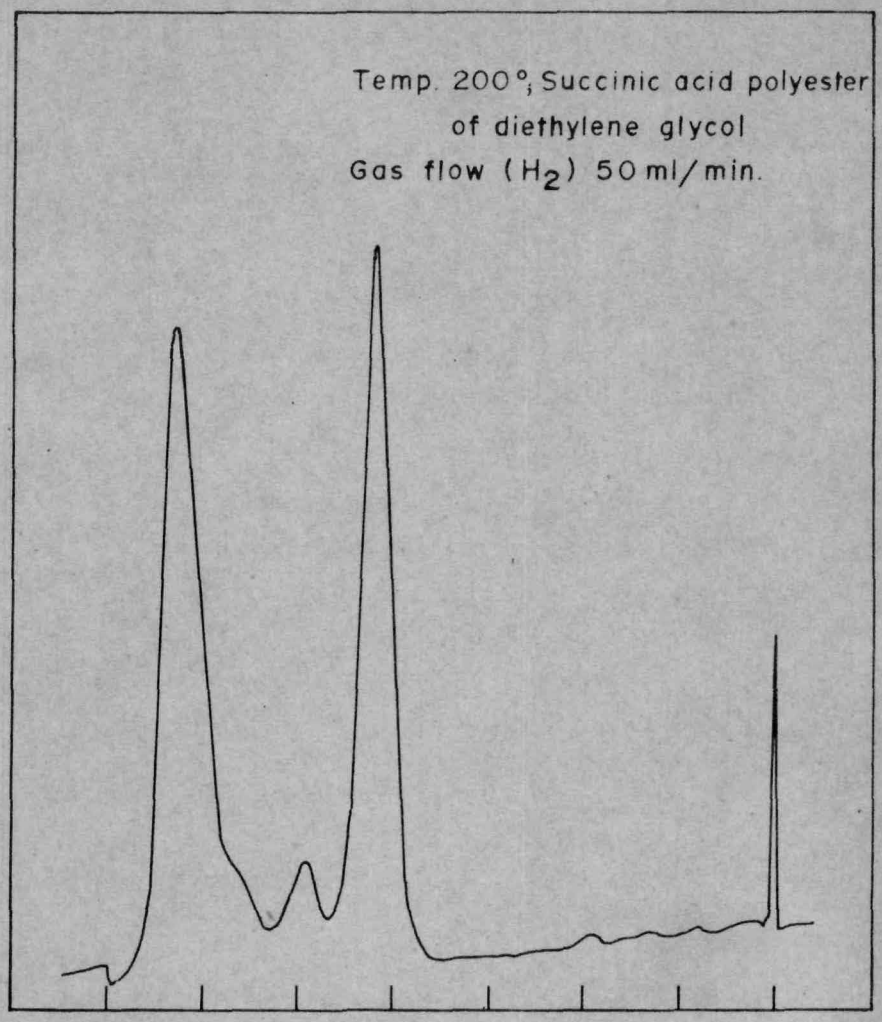


FIG. 2. GAS–LIQUID CHROMATOGRAM OF "COSTOL".

pet.ether benzene (1:1), benzene, ether and methanol. About fifty small fractions of 50 ml each were collected. The pet.ether and pet.ether-benzene fractions did not yield any alcohol-containing product. Alcohol was eluted only with benzene, ether and methanol. Each of these fractions was treated separately. GLC examination of almost every alternate fraction revealed nearly identical pattern not particularly different from the starting material before chromatography. The IR spectra of all the fractions were nearly identical. This proved that chromatography was of little value for the separation of the components of this alcohol mixture, which will be called "costol" hereafter for the sake of convenience.

The 'costol' did not form any of the conventional solid derivatives such as phenyl urethane, p-nitro- and 2,4-dinitrobenzoate and phthalic acid half ester.

'Costol' was then oxidised with Jones' chromic acid reagent to form the corresponding aldehyde in good yield. The aldehyde readily formed a crystalline semicarbazone, m.p. 218°. The distilled aldehyde on GLC analysis exhibited two peaks of almost equal intensity, similar to those of the parent alcohol.

It was then thought that 'costol' could best be purified through the semicarbazone of its aldehyde. The aldehyde was regenerated from the semicarbazone with

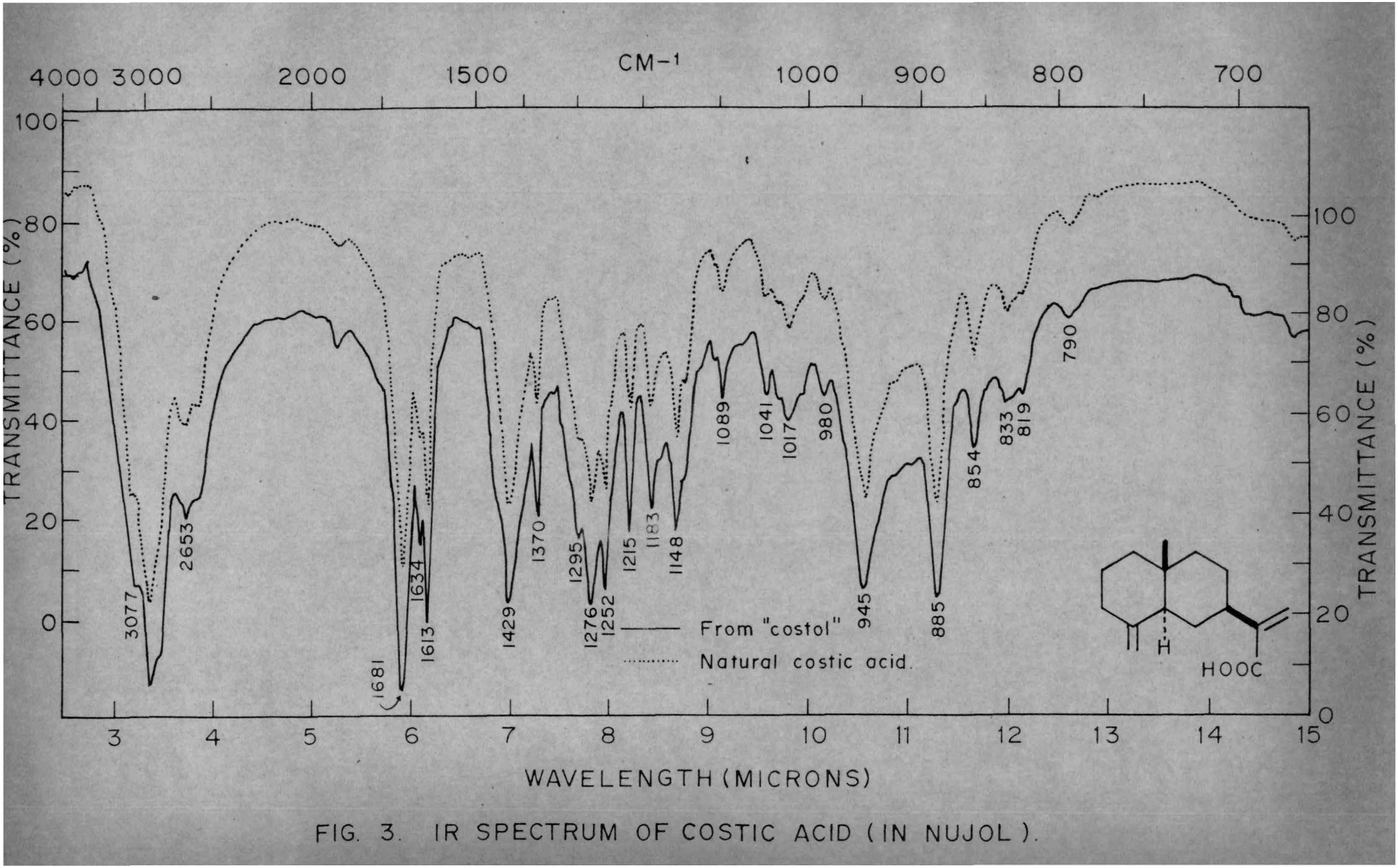


FIG. 3. IR SPECTRUM OF COSTIC ACID (IN NUJOL).

oxalic acid[7] and also with levulinic acid[8] under mild conditions. But, surprisingly, the regenerated aldehyde thus obtained also exhibited the same pattern as for the original aldehyde, on GLC analysis.

At this stage we were simultaneously engaged in the structure elucidation of the new crystalline, sesquiterpenic acid, costic acid[9] (VIIa), described in Part IIa of this thesis. The structure and absolute configuration of this acid was shown to be as in VIIa which obviously suggested a structural relationship with costol (I) of Sorm.

As is mentioned in Part IIa of this thesis, methyl ester of costic acid (VIIb) on reduction with aluminium hydride gives an allylic alcohol which is a single, homogeneous compound. Comparative GLC analyses of this compound with the natural 'costol' indicated that the latter contained one component corresponding to the alcohol obtained from crystalline costic acid.

The aldehyde obtained from the allylic alcohol, derived from pure, crystalline costic acid gave a semicarbazone, m.p. 223°. On admixture with a sample of semicarbazone (m.p. 218°) from the natural 'costol' series, a slight depression (215°) was observed. It was evident from this fact that the semicarbazone obtained via the natural source was contaminated with closely similar compounds. The UV spectral characteristics of the two

aldehydes and the corresponding semicarbazones were in close agreement and indicated the α,β-unsaturated nature of the aldehydes and the allylic nature of the parent alcohols.

It was also found that the hydrocarbon obtained as a result of hydrogenolysis during catalytic hydrogenation of natural 'costol' did not agree in properties with the pure selinane (II) obtained from similar experiments in the costic acid series.

All these evidences suggested that the natural 'costol' is not homogeneous but consists of a mixture of several alcohols, one of which possesses the structure and stereochemistry as in I. This was further confirmed by converting natural 'costol' into costic acid.

Natural 'costol' was oxidised with Jones' chromic acid reagent,[10] as well as with activated manganese dioxide[11] to give a good yield of the corresponding aldehydes. The distilled aldehydes were further oxidised to the corresponding acids by means of silver oxide[12] at room temperature. A sample of the methyl ester prepared from the mixture of acids exhibited mainly two peaks in the GLC analysis.

The crude acid mixture obtained above was then treated with cyclohexylamine followed by dicyclohexylamine as in the case of isolation of pure costic acid. The

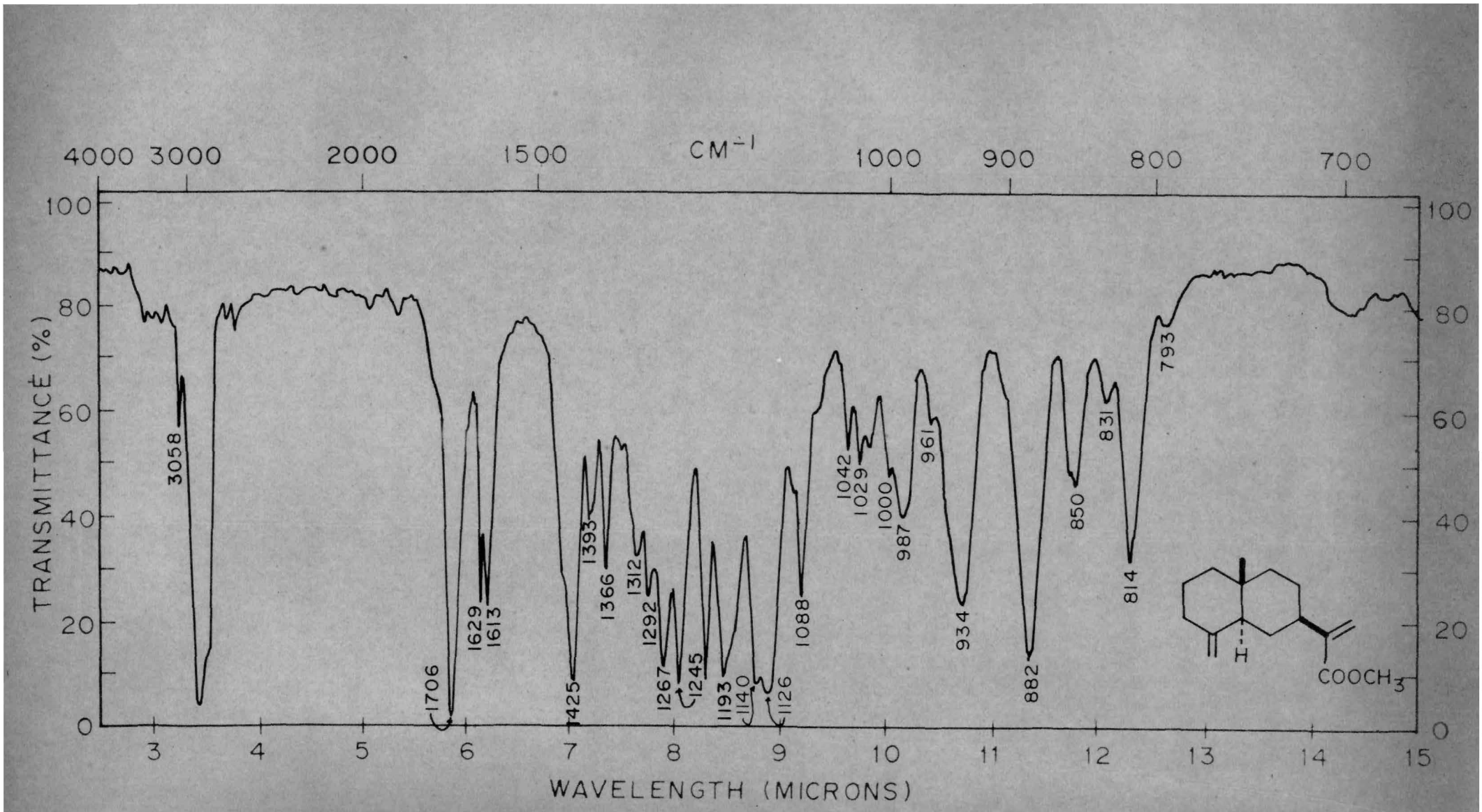


FIG. 4. IR SPECTRUM OF METHYL COSTATE (LIQUID FILM).

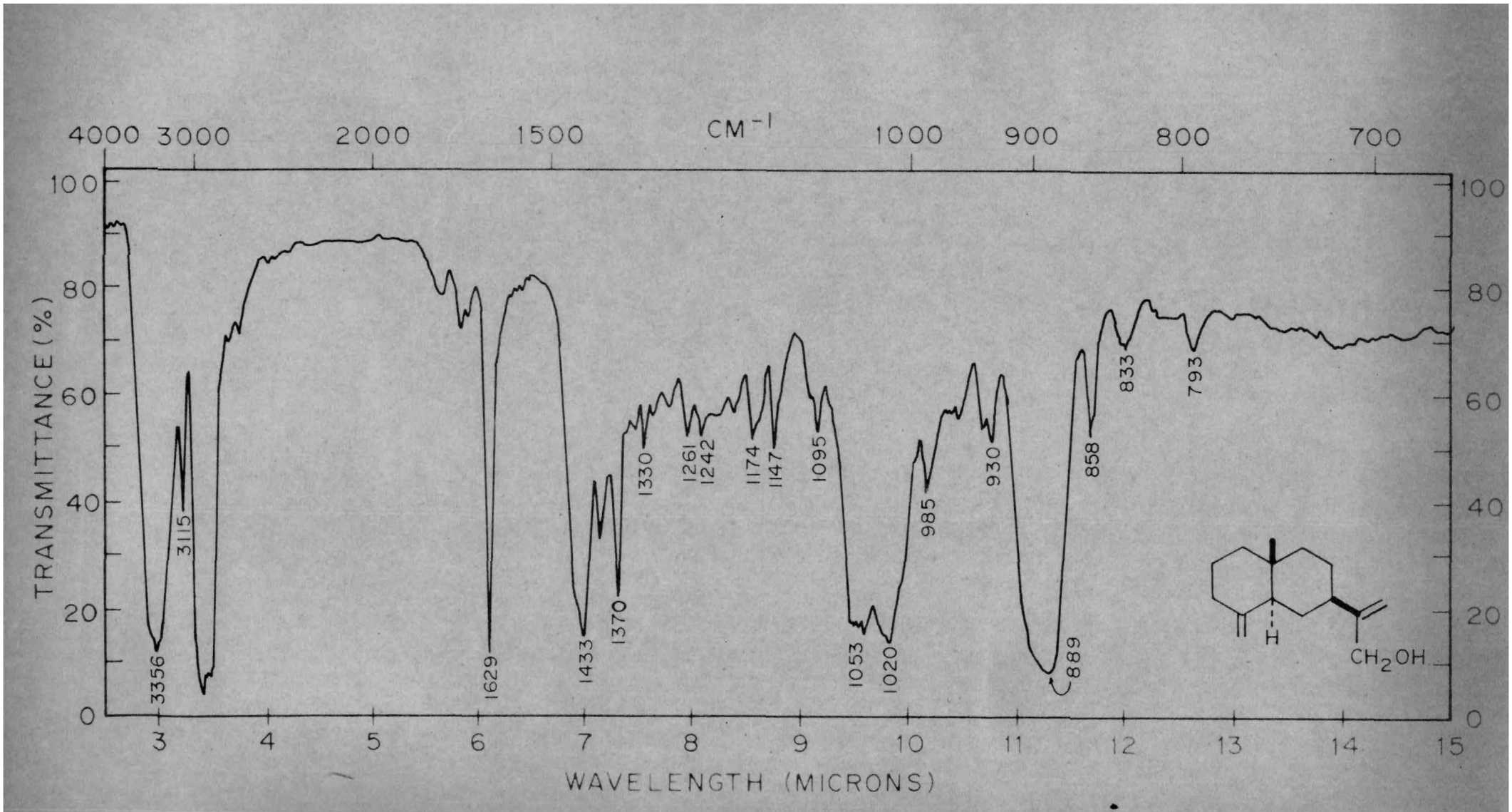


FIG. 5. IR SPECTRUM OF COSTOL (LIQUID FILM).

regeneration of the corresponding acid from the amine salt yielded a solid acid. It was crystallised from dilute alcohol and was found to be identical in all respects with the natural costic acid, from its m.p., IR, UV, NMR spectra and physical properties.

The methyl ester of this acid was reduced with aluminium hydride to give an alcohol which was a homogeneous product and identical in all respects with the allylic alcohol obtained from methyl costate (VIIb).

The mother liquors from the crystallisation of the amine salts, were processed to obtain an acid in a liquid form. This acid was found to be a mixture of two components from the GLC analysis of its methyl ester, one of which was evidently the costic acid. The separation of the other constituent is in progress and is not reported in this thesis.

During the oxidation of the 'costol' fraction with chromic acid, a substantial amount of alcoholic products remained unoxidised. This was separated from the resultant aldehyde via semicarbazone formation and also by treatment with Girard's reagent. The alcoholic fraction, thus obtained, was also found from GLC and TLC analyses to be a mixture of at least three components. This fraction was carefully examined and its elaborate chromatography yielded one crystalline alcohol which was identified as

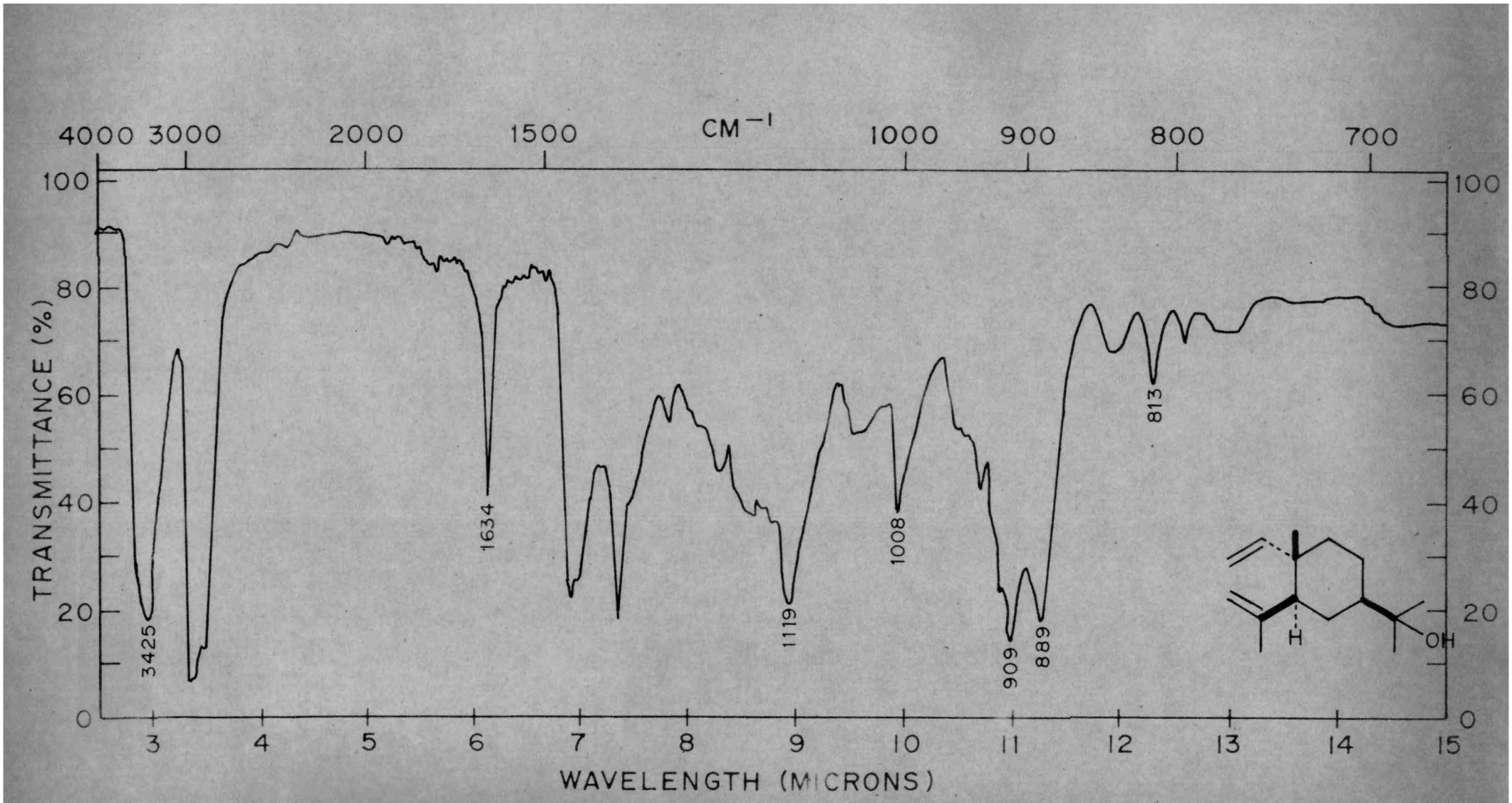


FIG. 6. IR SPECTRUM OF ELEMOL (IN NUJOL).

elemol (VIII). It was not possible to identify the other components. Thus it is clear that the 'costol' of Sorm is a mixture of several alcoholic constituents, one of which (not exceeding 40% of the total alcohol) is the true costol corresponding to the stereoformula I. Its corresponding acid is the crystalline costic acid.

EXPERIMENTAL

Isolation of 'costol' (I)

Partially delactonised costus root oil was treated with alkali to remove the free acids (see Part IIa of this Thesis). The acid-free oil (355 g), thus obtained was fractionated under reduced pressure using an 8" Vigreaux column.

Fraction No.	b. p.	Yield
1	90-150°/9 mm.	80 g.
2	100-130°/0.2 mm.	130 g.

The high boiling residue (135 g) was preserved.

Fraction 2 (130 g) was saponified with alcoholic potash (200 ml; 10%) for 8 hrs. The reaction mixture was worked up as usual and separated into neutral (95 g) and lactonic constituents.

The neutral product (350 g) from several experiments was chromatographed on neutral alumina (grade II, 5.0 kg). The results are shown in Table 1.

Table 1

Fr.	Solvent	Volume (L)	Weight (g)
1	Pet.ether (40-60°)	3.0	185
2	Benzene	1.0	20
3	Ether (1)	1.5	65
4	Ether (2)	1.5	50
5	Methanol	1.0	20

Fractions 3, 4 and 5 were combined (135 g) and fractionated under reduced pressure.

Fr.No.	b. p.	Yield
1	85-110°/0.3 mm.	17 g.
2	110-25°/0.3 mm.	80 g.

The high boiling residue (33 g) was preserved.

Fraction 2 (25 g) was chromatographed on neutral alumina (grade II, 500 g) and eluted with pet.ether, pet. ether-benzene (1:1), benzene, ether and methanol. The results are shown in Table 2. (Page 98)

IR spectra of fractions 1,2 and 5 revealed the presence of a weak band at 1755 cm^{-1} due to the lactone and a strong peak at 1708 cm-1 due to a keto carbonyl group. Fraction 5 showed, in addition to the above peaks, a band at 3350 cm^{-1} indicating the presence of a hydroxyl group. Evidently, they were mixtures consisting mostly of ketones with traces of lactones and alcohols. GLC analyses of the distilled products obtained from each of these fractions showed at least six peaks.

IR spectra of fractions 12, 16 and 19 indicated that the lactone impurity was totally eliminated, but the ketone still persisted in fraction 12, though only to a small extent. Fractions 16 and 19 were almost pure alcohols. The GLC analysis of the distilled samples revealed three major peaks with two other minor peaks. Partial separation was thus achieved.

Table 2

Fr.No.	Solvent	Volume(ml)	Weight (g)
1	Pet.ether	500	3.3
2	Pet.ether-benzene	750	5.8
3	Benzene	100	2.1
4	"	"	2.5
5	"	"	1.7
6	"	"	1.0
7	"	"	0.9
8	"	"	0.8
9	"	"	0.7
10	"	"	0.4
11	"	"	0.4
12	Ether	"	0.4
13	"	"	0.7
14	"	"	0.8
15	"	"	0.5
16	"	"	0.4
17	"	"	0.2
18	"	"	0.3
19	Methanol	1000	0.4

Alcohol-rich fractions were then collected (22 g) and chromatographed on neutral alumina (grade II; 2.2 kg) and eluted with pet.ether, pet.ether-benzene (1:1), benzene, benzene-ether (1:1), ether, ether-methanol (1:1) and methanol. Fifty fractions of 50 ml each were collected. Fractions 1, 8, 15, 24, 38 and 50 were examined by GLC to indicate that the pattern was the same for each fraction, showing two prominent peaks and two minor ones. Fractions 24-50 were mixed together and distilled under reduced pressure, b.p. 140° (bath)/0.5 mm. Yield 10.5 g. n_D^{25} 1.5132; $(\alpha)_D$ + 6.7° ($CHCl_3$; c, 4.2).

Analysis

Found: C, 81.8; H, 11.4.

$C_{15}H_{24}O$ requires: C, 81.76; H, 11.98%.

IR spectrum (Fig.1a) shows bands at 3350, 1020 cm^{-1} (primary hydroxyl group) and 1640 and 887 cm^{-1} (exocyclic methylene group).

Preparation of phthalate ester of 'costol'

Freshly sublimed phthalic anhydride (12.0 g) was added to a solution of 'costol' (10.0 g) in dry benzene (150 ml). The mixture was refluxed for six hours. Most of the benzene was distilled and the residue was diluted with petroleum ether. The precipitated phthalic anhydride was filtered. The mother liquor was extracted thoroughly with sodium carbonate solution (10%) to extract the acid.

The sodium carbonate solution did not yield any acid corresponding to the phthalic acid half ester. The unreacted 'costol' (9.0 g) was recovered from the pet.ether solution.

Oxidation of 'costol'

(a) With activated manganese dioxide

A mixture of 'costol(2 g) dissolved in chloroform (200 ml) and freshly prepared activated manganese dioxide (20 g) was mechanically shaken at room temperature for 24 hrs. Manganese dioxide was filtered and the filtrate was evaporated, when a residue (1.9 g) was obtained as an oil. It was distilled under reduced pressure, b.p. 180° (bath)/5 mm., n_D^{27} 1.4990.

(b) With Jones' chromic acid reagent

'Costol' (2 g) dissolved in acetone (15 ml) was oxidised with Jones' chromic acid reagent. Excess reagent was decomposed by addition of methanol and the mixture was diluted with water (25 ml) and extracted thoroughly with ether. The ether solution was washed with water to remove mineral acid and then with sodium carbonate solution (5%) to extract the acid formed during oxidation. The residual ether solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave the aldehyde (1.7 g) as a crude product. The sodium carbonate solution, on acidification,

gave an acid (0.2 g) as a thick liquid. The aldehyde was distilled, b.p. 185° (bath)/5 mm., n_D^{26} 1.4989. The aldehydes (costal), obtained by the two methods (a) and (b) above, were found to be identical.

Analysis

Found: C, 81.83; H, 9.9.

$C_{15}H_{22}O$ requires: C, 82.51; H, 10.16%.

IR spectrum (Fig. 1c) exhibited bands at 1686, 1633, 890 cm^{-1}. UV spectrum showed λmax. 218 mμ, ε 10,430. GLC analysis revealed two peaks of almost equal intensity, and a small hump accounting for about 10% impurity.

Semicarbazone of costal

A solution of semicarbazide hydrochloride (0.5 g) and sodium acetate (0.6 g) in water (5 ml) was added to a solution of costal (1 g) in ethanol (10 ml). A solid immediately separated. It was filtered and crystallised three times from alcohol, m.p. 218°; $(\alpha)_D$ + 35.37° (AcOH; c, 0.8). The mother liquor was preserved.

Analysis

Found: C,70.04; H,9.19; N, 15.31.

$C_{16}H_{25}N_3O$ requires: C,69.78; H,9.15; N, 15.26%.

UV spectrum showed λmax. 263 mμ; ε 25,680.

Regeneration of costal from the semicarbazone:

(a) With oxalic acid:

A mixture of the semicarbazone (1 g), alcohol (15 ml), oxalic acid (2 g) dissolved in water (50 ml), 40% aqueous formaldehyde (8 ml) and petroleum ether (40-60°; 100 ml) was refluxed for six hours. The aqueous and organic layers were separated. The pet.ether solution was washed twice with water and dried over anhydrous sodium sulphate. On evaporation of the solvent, the aldehyde, costal (0.4 g) was obtained. It was distilled, b.p. 175°(bath)/4 mm., n_D^{27} 1.4990.

It yielded the semicarbazone m.p. 218°, which was identical with the starting compound.

(b) With levulinic acid

A mixture of the semicarbazone (1 g), levulinic acid (50 ml) and 1N hydrochloric acid (10 ml) was shaken mechanically at room temperature for 24 hours. The clear solution thus obtained was diluted with water and neutralised with solid sodium bicarbonate. The solution was extracted thoroughly with ether; the ether solution washed with water and dried. Evaporation of ether gave the aldehyde, costal (0.7 g). It was distilled, b.p.160-65°(bath)/3 mm., n_D^{27} 1.4988.

Analysis

Found: C, 82.32; H, 10.0.

$C_{15}H_{22}O$ requires: C, 82.51; H, 10.16%.

Hydrogenation of 'costol'

'Costol' (2 g) in methanol (25 ml) was hydrogenated in the presence of Adams' platinum oxide catalyst (0.05 g). The total hydrogen uptake was 468 ml at N.T.P. corresponding to 2.3 double bonds. The catalyst was filtered and the filtrate evaporated. The residue (1.9 g) was obtained as a thick liquid.

Isolation of selinane (II) and tetrahydrocostol (III)

The hydrogenated product (1.9 g) was chromatographed on neutral alumina (grade II; 38 g) and eluted with pet.ether and ether.

The petroleum ether eluate (100 ml) on evaporation of the solvent gave selinane (II) as a mobile liquid(0.5 g). It was distilled over metallic sodium, b.p. 130°(bath)/ 7 mm., n_D^{23} 1.4731; d_{24}^{24} 0.8654; $(\alpha)_D$ + 9.65° ($CHCl_3$; c, 4.3).

Analysis

Found: C, 86.4; H, 13.8.

$C_{15}H_{28}$ requires: C, 86.45; H, 13.55%.

The properties of selinane were somewhat different from those of selinane obtained from crystalline costic acid as shown below.

Properties of selinane

	From costic acid	Present product	Sorm's product
Density	d_{28}^{28} 0.8871	d_{24}^{24} 0.8654	d_{4}^{20} 0.8732
Refractive index	n_D^{28} 1.4793	n_D^{23} 1.4731	n_D^{20} 1.4721
Specific rotation	$(\alpha)_D$ + 8.32°	$(\alpha)_D$ + 9.65°	$(\alpha)_D$ + 11.9°

Selinane, obtained above, was found to be a mixture of atleast two components, as revealed by GLC analysis. Its IR spectrum (Fig.1d) showed bands at: 1466, 1449, 1377, 1369, 1170, 1109, 1001, 982, 932, 916, 853, 790 cm^{-1}.

The ether eluate yielded tetrahydrocostol (III; 1.1 g) b.p. 145°(bath)/0.7 mm., n_D^{26} 1.4923; $(\alpha)_D$ + 11.28° ($CHCl_3$; c, 2.3).

Analysis

Found: C, 79.90; H, 12.69.

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

Its IR spectrum (Fig.1b) showed bands at: 3340, 1467, 1381, 1037 cm^{-1}.

Conversion of 'costol' (I) to costic acid (VIIa):

Oxidation of costal with silver oxide:

A mixture of freshly distilled costal (5 g), dissolved in alcohol (25 ml) and silver nitrate (8 g)

dissolved in distilled water (20 ml) was cooled in ice-salt bath to -10°. A solution of potassium hydroxide (8 g) in water (20 ml) was added under stirring to the chilled mixture at -10°. The whole mixture was stirred at -10° for 30 minutes and then allowed to warm to room temperature. Alcohol (50 ml) was added and the mixture stirred at room temperature for 48 hours. The reaction mixture was filtered, the silver oxide slurry was washed thoroughly with alcohol and the combined filtrate evaporated under reduced pressure. The residue was diluted with water and extracted with ether. The clear, aqueous alkaline solution was carefully acidified in cold, and the liberated acid was extracted with ether. The ether solution was washed free of mineral acid and dried over anhydrous sodium sulphate. Ether was removed by distillation when the acid (4.1 g) was obtained.

A small quantity of the acid was converted into its methyl ester (diazomethane). It was distilled, b.p. 130°(bath)/0.2 mm. Its GLC analysis revealed the presence of two components.

The acid (4.0 g) dissolved in methyl ethyl ketone (15 ml) was treated with cyclohexylamine (1.8 g). The mixture was stirred vigorously when a solid, cyclohexylamine salt separated. It was filtered and recrystallised from methyl ethyl ketone, m.p. 182-184°.

The cyclohexylamine salt (4.5 g) was suspended in ether and an aqueous solution of sodium hydroxide (5 %) was added until all the salt dissolved. The ether layer containing the amine was removed and the aqueous alkaline solution further extracted with ether to remove the residual amine. The clear alkaline solution was acidified and the liberated acid taken up in ether. The ether solution was washed free of mineral acid and dried. Evaporation of ether gave the acid (2.2 g).

The above acid (2.2 g) was dissolved in acetone (8 ml) and dicyclohexylamine (1.6 g) was added to it. The dicyclohexylamine salt (3.0 g), thus formed, was filtered and crystallised from acetone, m.p. 90-91°.

The acid regenerated from the salt (m.p.90-91°) as described above, was obtained as a solid (1.2 g). It was crystallised from aqueous ethanol, m.p.87-88°; $(\alpha)_D^{27}$ + 21.2° ($CHCl_3$; c, 1.8). Mixed m.p. with an authentic sample of costic acid was undepressed.

Analysis

Found: C, 76.61; H,9.70%; Eq.wt.232.6.

$C_{15}H_{22}O_2$ requires: C, 76.88; H,9.46%; Eq.wt. 234.33.

Its IR spectrum (Fig.3) exhibited bands at: 3077, 1681, 1634, 1613, 885 cm^{-1} and was superimposable on that of costic acid.

The UV spectrum showed end absorption at 210 mμ; ε5280.

Methyl costate (VIIb)

The acid (m.p. 87-88°) was converted into its methyl ester (diazomethane). The ester was distilled under reduced pressure; b.p.140° (bath)/0.4 mm., n_D^{27} 1.5055; $(\alpha)_D^{27}$ + 29.63° ($CHCl_3$; c, 2.2).

Analysis

Found: C, 77.10; H, 9.69.

$C_{16}H_{24}O_2$ requires: C, 77.37; H, 9.74%.

The GLC analysis showed the presence of a single component.

Its IR spectrum (Fig.4) showed bands at: 1706, 1629, 1613, 1245, 885 and 814 cm^{-1}.

The UV spectrum showed λmax. 210 mμ, ε 5700.

Pure costol (I) from methyl costate (VIIb)

Methyl costate (1 g), dissolved in dry ether (25 ml) was added to a stirred suspension of aluminium hydride (prepared from 0.42 g anhydrous aluminium chloride and 0.46 g lithium aluminium hydride) in dry ether at room temperature. The stirring was continued for 45 minutes. Excess reagent was destroyed by careful addition of moist ether and the product was isolated in the customary manner. The alcohol (0.8 g) was distilled, b.p. 145° (bath)/0.5 mm., n_D^{26} 1.5177; $(\alpha)_D^{26}$ + 32.8° ($CHCl_3$; c, 4.3).

Analysis

Found: C, 81.59; H, 11.1.

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

GLC analysis showed a single peak.

Its IR spectrum (Fig.5) showed bands at: 3356, 1639, 889 cm^{-1}.

Isolation of elemol (VIII)

The mother liquor from the semicarbazone of costal (prepared by Jones' reagent oxidation of 10 g. 'costol') was concentrated in vacuum, and the residue diluted with water and extracted with ether. The ether solution was washed thoroughly with water and dried over anhydrous sodium sulphate. On evaporation of ether, a thick liquid (3.8 g) was obtained.

The liquid (3.8 g) was dissolved in absolute alcohol (30 ml) and glacial acetic acid (5 ml) was added to it. To this, Girard's reagent T (2.1 g) was added and the solution was refluxed on a water bath for 4 hours. The reaction mixture was diluted with water containing sodium bicarbonate (3.5 g) and thoroughly extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulphate. The ether solution, containing the non-carbonyl compounds, was evaporated, when a residue (3.1 g) was obtained. It showed a strong hydroxyl band at 3509 cm^{-1}

in the IR spectrum. It was distilled, b.p.140° (bath)/0.4 mm. It revealed the presence of atleast three components on GLC analysis.

The distilled alcohol mixture (2.8 g) was chromatographed on neutral alumina (grade II; 84 g) and eluted with pet.ether, pet.ether-benzene (1:1), benzene and ether. The pet.ether-benzene (1:1) eluate, on concentration, afforded a viscous liquid (1.0 g), which was crystallised from pet.ether, m.p.51-52°; $(\alpha)_D$ - 4.13° ($CHCl_3$; c, 2.8). The mixed m.p. with an authentic sample of elemol (m.p.53°) was undepressed.

Analysis

Found: C, 80.91; H, 11.60.

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

Its IR spectrum (Fig.6) showed bands at: 3425, 1634, 1119, 1008, 909, 889, 813 cm^{-1}, and it was identical with that of an authentic sample of elemol.

Other fractions of the chromatography were not examined.

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PART II (APPENDIX)

BIOGENETIC RELATIONSHIP OF THE CONSTITUENTS OF COSTUS ROOT OIL

***

SUMMARY

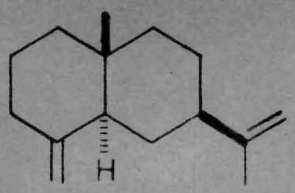
Attempt has been made to bring forth the close biogenetic relationship of the various constituents of costus root oil. The well-known hypotheses on the biogenesis of polyisoprenoids have been logically used to show that all the compounds present in this oil could arise from a single common precursor, *trans*-farnesyl pyrophosphate.

Costus root oil mainly contains sesquiterpenic compounds, some of which are described in this Thesis. Amongst the hydrocarbons, β-selinene (I) and β-elemene (II) are present together with the major acyclic, straight-chain hydrocarbon, aplotaxene (III). Costol (IV), elemol (V), costic acid (VI), costunolide (VII) and dehydrocostus lactone (VIII) are the major and important oxygenated compounds. β-Selinene, costol and costic acid form a series, which may be assumed to arise from a common precursor. These compounds can be looked upon as the different stages of oxidation (or reduction) of the common precursor.

By the same analogy, β-elemene and elemol also must arise from a common precursor.

The biogenesis of aplotaxene is of great interest. According to Sorm[1] the reasonable postulate is that aplotaxene is formed by decarboxylation of an unsaturated C_{18}- monocarboxylic acid.

Costunolide (VII) and dihydrocostunolide (IX) are the ten-membered carbocyclic lactones present in this oil. Saussurea lactone (X), which is not a natural product, but an artefact arising from dihydrocostunolide, has also been isolated from this oil. This conversion has been achieved experimentally in the laboratory.[2]



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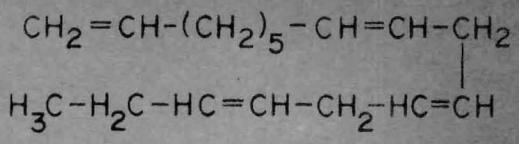

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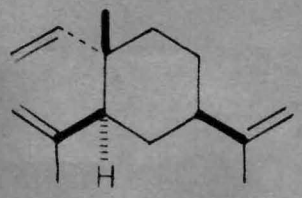

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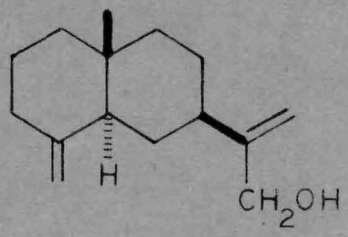

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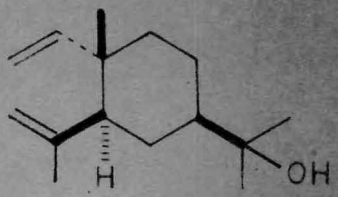

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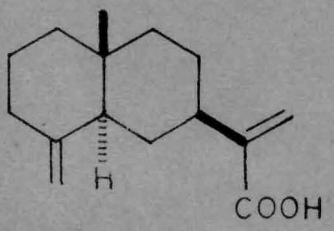

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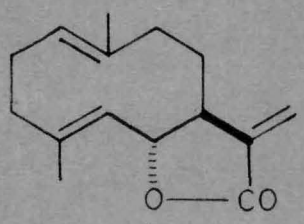

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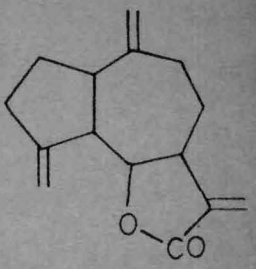

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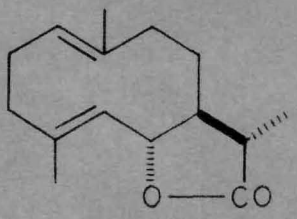
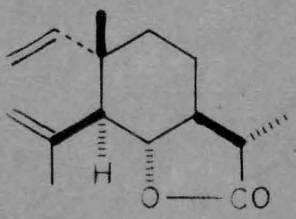
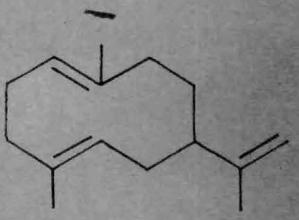


IX



X



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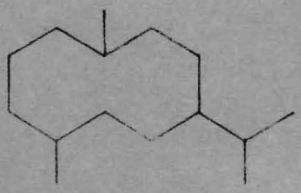
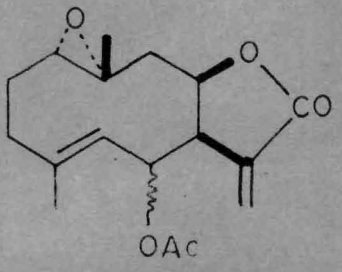


XII



XIII

On similar considerations, the hydrocarbon β-elemene may be considered to be an artefact arising from a hydrocarbon (XI), which is a ten-membered carbocyclic hydrocarbon having three double bonds. Considering the presence of large amount of costunolide in costus root oil, the presence of hydrocarbon XI, cannot be ruled out. Such a hydrocarbon, obviously, will be very unstable and under the experimental conditions, is most likely to yield β-elemene according to the same mechanism as saussurea lactone is formed from dihydrocostunolide.[2] A search for this hydrocarbon in costus root oil, however, proved fruitless. The importance of such a hydrocarbon may well be imagined when one considers the variety of ways in which transannular reactions can take place on such a molecule. This hypothetical hydrocarbon would constitute the parent compound in the germacrane (XII) series to which costunolide and dihydrocostunolide belong.

According to Sorm,[3] " the readiness with which most of the unsaturated derivatives of germacrane type undergo transannular cyclisation to give compounds with a selinane skeleton (in the case of lactones, relatives of santonin) suggests a biogenetic significance of such reactions. It may be assumed that the cyclodecane-type lactones are the primary products in nature, and that

lactones of the santonin and guaianolide series are formed from them by secondary processes, analoguous to well-known stereospecific transannular cyclisations observed in the laboratory. This hypothesis is supported by the frequent occurrence of lactones of the germacrane type in the Compositae family, which is also the source of the other two types of sesquiterpenic lactones. However, direct evidence for this biogenetical relationship is still lacking."

The importance of the germacrane skeleton is further stressed by Barton[4] in connection with his work on the ten-membered ring lactone, pyrethrosin(XIII). It is possible, by establishing different bonds across the germacrane ring, to construct the carbon skeletons of most of the bicyclic sesquiterpenoids. This observation is experimentally illustrated by the cyclisation reactions of pyrethrosin (loc.cit).

The work carried out in our laboratory on costunolide[5,6] and dihydrocostunolide lends additional support to the above observations.

Ruzicka's[7] illuminating hypothesis on the biogenesis of terpenoids, considers an acyclic precursor of the farnesane type for mono- and bicyclic sesquiterpenes.

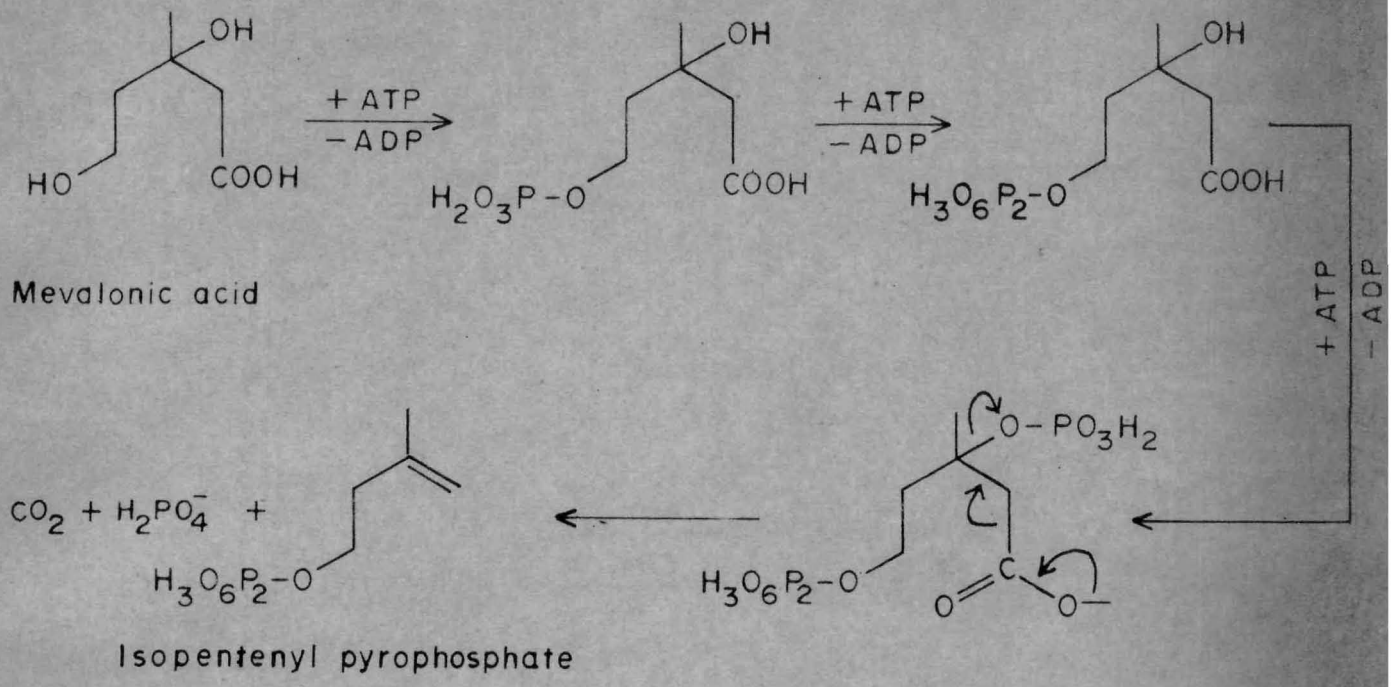
It is further supposed that the displacement of the allylic hydroxyl group of farnesol (XIV) by either of the isolated double bonds gives rise to 6-, 10- or 11-membered ring intermediates.[8] The medium ring intermediates possess unique conformations which dictate the structure as well as the stereochemistry of the sesquiterpenes derived from them by a variety of simple further reactions, notably double bond cyclisations.

It is now generally believed that the actual isoprenoid unit involved in terpene biogenesis is mevalonic acid (XV).[9] Mevalonic acid, under the influence of certain enzymes, is converted into isopentenyl pyrophosphate (XVI);[10-12] which can further isomerise to dimethyl allyl pyrophosphate (XVII).[13,14] One molecule of dimethyl allyl pyrophosphate can condense with two molecules of isopentenyl pyrophosphate to form exclusively the trans-trans farnesyl pyrophosphate (XVIII).[11,15,16] It was shown that nerolidyl pyrophosphate (XIX) also accompanies farnesyl pyrophosphate in an equilibrium mixture.[17,18] Nerolidol and farnesol were thus shown to be the simplest acyclic sesquiterpenoids (see Schemes 1 and 2).

The biogenesis of sesquiterpenes from farnesyl pyrophosphate (XVIII) involves an ionisation of the allylic

Scheme 1

Enzymic conversion of mevalonate to iso-pentenyl pyrophosphate

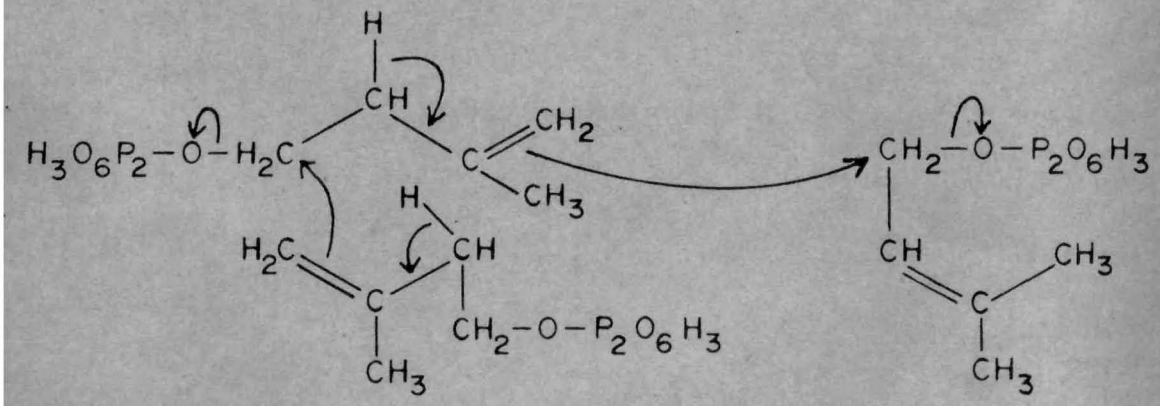


Mevalonic acid

Isopentenyl pyrophosphate

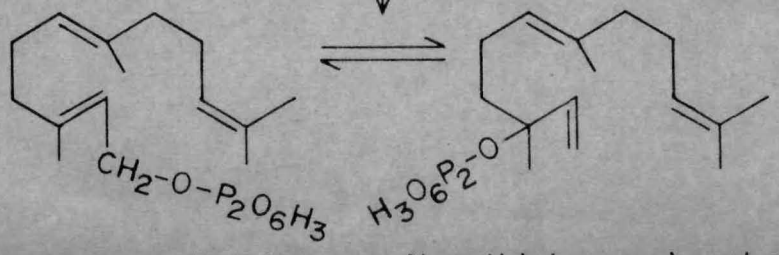
Scheme 2

Formation of farnesyl pyrophosphate



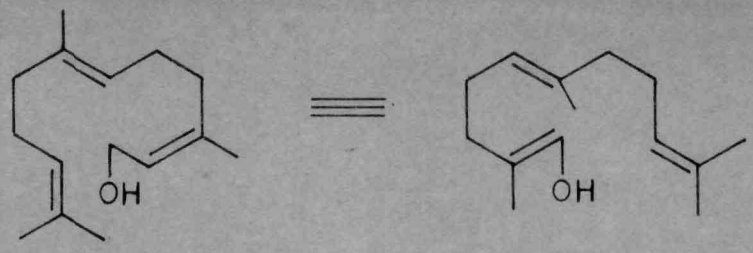
Isopentenyl pyrophosphate (2 moles)

Dimethylallyl pyrophosphate (1 mole)



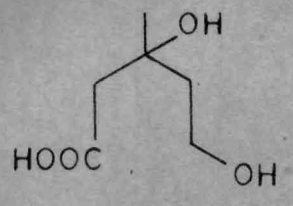
Farnesyl pyrophosphate

Nerolidyl pyrophosphate

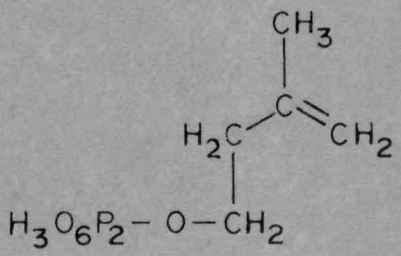


cis– trans–

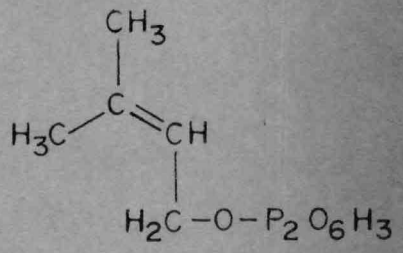
XIV



XV



XVI



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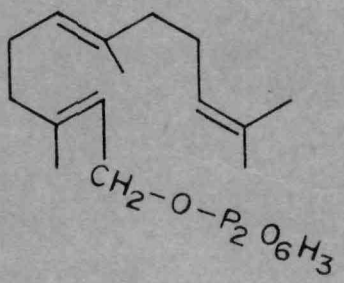

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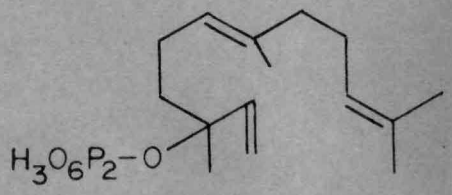


XIX

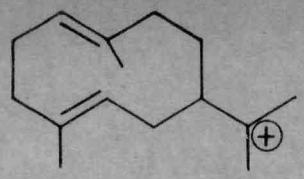
diphosphorylated hydroxyl group and cyclisation of one of the other double bonds to form a series of cations.[8] The cis- and trans-farnesol derivatives can furnish various cations but the present discussion will be restricted to the cation (XX) formed from trans-farnesol.

Models of the trans-cation (XX) reveal that the two double bonds are extremely close to each other. The cationic center resides out in the isopropyl side chain, where it may be hydrated or it may suffer further extensive oxidation yielding the sesquiterpenic lactones, all of which can be shown to be derived from XX.

The simplest way in which this cation can be neutralised is by elimination of a proton to create a double bond in the isopropyl side chain, thus giving rise to the hypothetical hydrocarbon (XI).

Simple hydration of this cation results in alcohol (XXIa & b) which has two double bonds uniquely situated for concerted cyclisation with complete trans-anti-parallel additions to the double bonds to yield the bicyclic products (XXII a & b).

Alcohol (XXIIa) is a direct precursor of eudesmol (XXIII) and other sesquiterpenes of this skeleton (santonin, cyperone, costol) and is in complete accord with the



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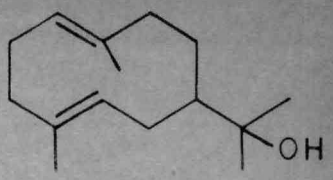
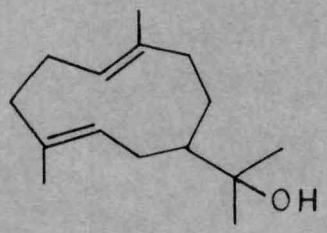


XXI a



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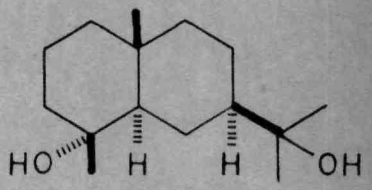
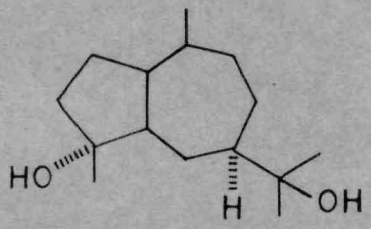
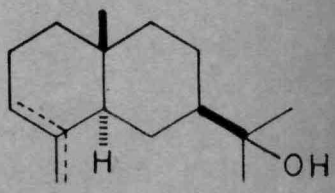


XXII a



XXII b



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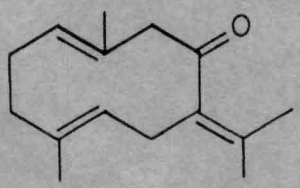
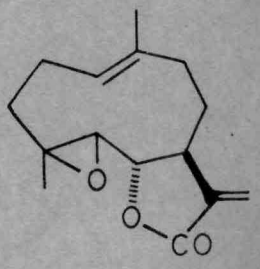


XXIV



XXV

stereochemistry of this group. Furthermore, a direct six-centred rearrangement of the electrons in (XXIa) yields elemol (V). Thus, cation XX is shown to be the precursor of β-selinene (I), costol (IV), costic acid(VI), β-elemene (II) and elemol (V).

Product XXIIb is the direct precursor of the large body of sesquiterpenes with the guaiazulene skeleton and predicts their stereochemistry. This may include dehydrocostus lactone (VIII) present in costus oil.

Finally, there is a possibility of finding compounds related to uncyclised XX from natural sources. This expectation has recently been realised by the isolation and identification of products like germacrone[19] (XXIV), pyrethrosin(XIII),[4] parthenolide (XXV)[20] and costunolide (VII).[5] All these compounds undergo facile cyclisations to the corresponding bicyclic products, as postulated above, in the biogenetic scheme.

Thus, it is readily seen that under this biogenetic hypothesis, compounds belonging to the selinane, germacrane, elemane and guaiane series, may arise from the same common precursor (XX) obtainable from trans-farnesyl pyrophosphate. The occurrence of all the four types of compounds in costus root oil lends further support to this hypothesis and brings forth the close biogenetic relationship of the products isolated from costus root oil.

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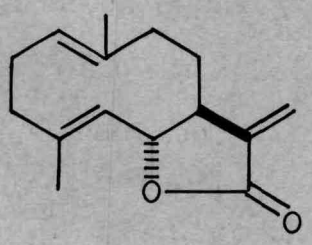
P A R T III

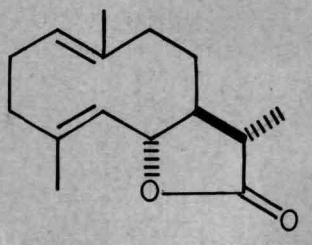
STRUCTURE AND ABSOLUTE CONFIGURATION OF PARTHENOLIDE

SUMMARY

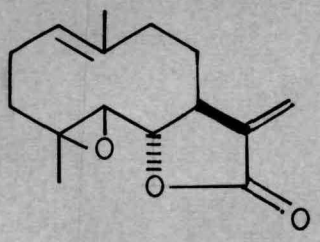
Epoxidation of costunolide and dihydrocostunolide has been studied with a view to correlate their epoxides with compounds from the parthenolide series. The diepoxide of dihydrocostunolide has been obtained as a crystalline solid. It has been found to be identical with epoxy dihydroparthenolide. This proves the stereochemistry of the lactone moiety in parthenolide and also the stereochemistry at C_{11} of dihydroparthenolide, as shown below.



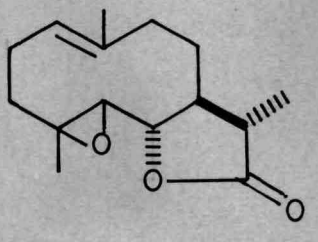
COSTUNOLIDE



DIHYDROCOSTUNOLIDE



PARTHENOLIDE



DIHYDROPARTHENOLIDE

Costunolide is an important sesquiterpenic lactone occurring in costus root oil. Its structure and absolute configuration (XXVII) have been determined in our Laboratory[1] and by Sorm and collaborators.[2] It possesses a ten-membered carbocyclic ring system with a unique distribution of two trisubstituted double bonds. The molecule contains three double bonds, as determined by catalytic hydrogenation. The double bond estimation by means of perbenzoic acid, however, indicated the presence of only two double bonds. These results taken with IR and UV data of costunolide conclusively proved that one of the double bonds in costunolide was in conjugation with the carbonyl group of the lactone moiety.

The treatment of costunolide with perbenzoic acid has been previously used only as an analytical procedure for the estimation of the number of double bonds, and the epoxides, thus formed, were not isolated. In view of the occurence of several epoxy compounds in essential oils and particularly of the sesquiterpenic epoxy lactones, pyrethrosin and parthenolide, it was considered that preparation of epoxy compounds from costunolide and its derivatives would be interesting.

Before going into the details of preparation of epoxides of costunolide and dihydrocostunolide, a very brief survey of naturally occurring epoxy terpenoids will not be out of place.

(1) Linaloöl monoxide (I) (2,3-epoxy-2,6-dimethyl-7-octen-6-ol)

Schimmel and Co.[3] first observed this monoxide in Mexican linaloe oil where it is probably formed by aerial oxidation of linaloöl while still in the plant. Naves[4] found that epoxylinaloöl occurs free or as an ester in oil of lavendin, in the oil of 'shiu', the content ranging from 0.5 to 4.2%. Its structure was determined by Naves and Bachmann.[4]

Linaloöl monoxide is an oil possessing a musty odour reminiscent of camphor and fenchyl alcohol. This oxide is not used as such in the perfumery industry.

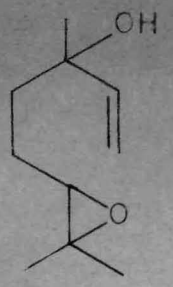
(2) l-Δ^3-Caren-5,6-epoxide (epoxycarene) (II).

The l-modification of this oxide was identified by Simonsen and coworkers[5] as the main constituent in the oil of Zieria smithii. Blanchard[6] recently reexamined its structure and found it to be chrysanthenone[7] (III).

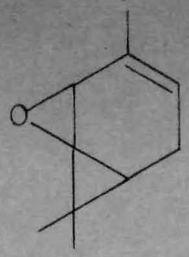
(3) Piperitone oxide (IV) and piperitenone oxide, (lippione) (V) occur in several mentha species.[8]

(4) Isolaserpitin (VI) has been isolated by Sorm and co-workers[9] from the roots of Laserpitin latifolium L.

(5) Picrotoxinin is one of the components of the bitter principle of 'picrotoxinin'. Its structure (VII) was brilliantly proved by Conroy.[10]



I



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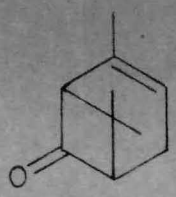

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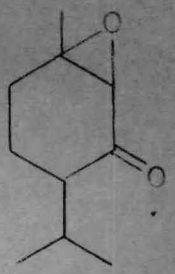

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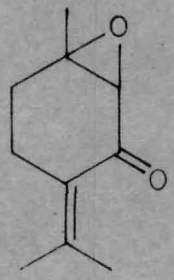

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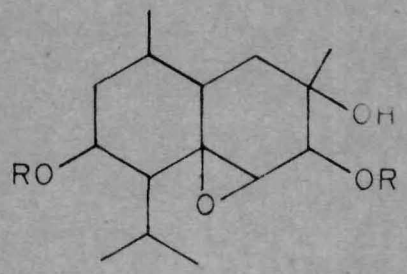
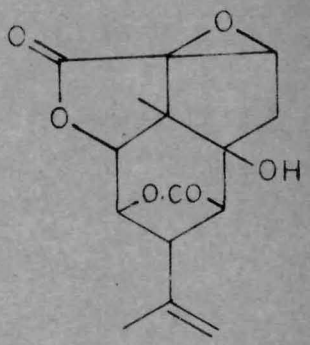
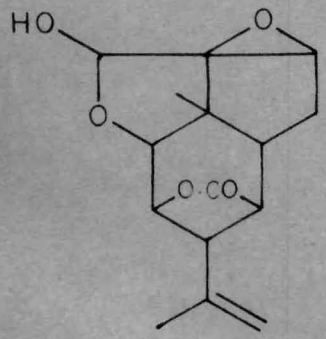


R= ·CO·CMe:CHMe

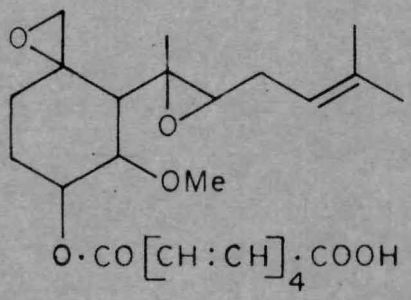
VI



VII

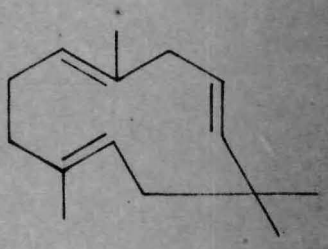


VIII

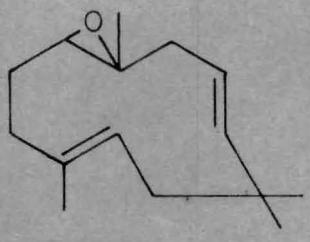


O·CO[CH:CH]$_4$·COOH

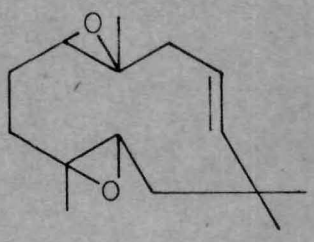
IX



X



XI



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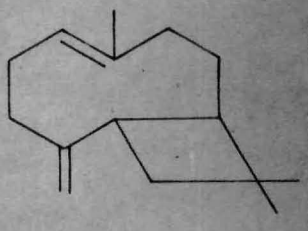

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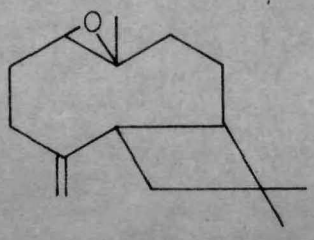


XIV

(6) Coriamyrtione (VIII) occurs in several Coriaria species. Its constitution has been determined by the Japanese workers.[11]

(7) Fumagillin, a potent antibiotic isolable from the mould Aspergillus fumigatus was assigned a non-isoprenoid structure (IX) by Tarbell and coworkers.[12] The structure was later confirmed by NMR spectral data.

(8) Humulene monoxide (XI) and humulene dioxide (XII). Ramaswami and Bhattacharyya[13] isolated the mono- and diepoxides of humulene (X) from the essential oil of wild ginger, (Zingiber zerumbet, Smith). The optically active fraction of the oil on repeated fractionation followed by chromatography, yielded two pure compounds. The major component was identified as the monoepoxide (XI) of humulene on the basis of chemical and spectroscopic evidences.

The second, a minor component was similarly identified as the diepoxide (XII) of humulene.

The occurence of humulene dioxide is the first instance of a terpenoid diepoxide in nature.

In this connection it may be pointed out that a closely related sesquiterpene, caryophyllene (XIII) also occurs as its epoxide (XIV) in nature.[14]

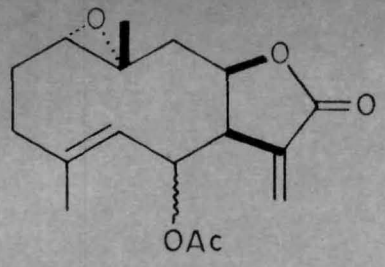
Treibs and co-workers[14] isolated caryophyllene oxide from the oil of cloves and it was later shown to be identical with a crystalline oxide isolated by Seidel, Muller and Schinz[15] from the oil of lavender.

Epoxy lactones

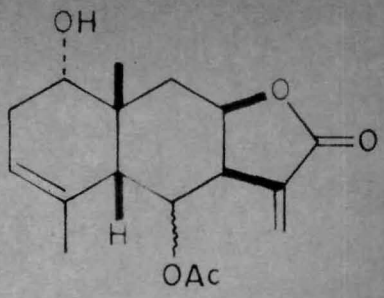
(1) Pyrethrosin (XV)

Barton and deMayo[16] isolated pyrethrosin from Chrysanthemum cinerariaefolium, a wellknown member of the compositae family. This compound, $C_{17}H_{22}O_5$, contains a lactone ring, an acetoxy group, two double bonds and an oxygen function which is neither a hydroxyl nor a keto group and was, therefore, assigned oxide character. The molecule was thus monocarbocyclic. One of the double bonds was shown to be conjugated with the carbonyl group of the γ-lactone. The key experiment in the structure determination was an attempt to acetylate pyrethrosin in acid solution, which led to the opening of the oxide ring and formation of cyclopyrethrosin (XVI), a bicyclic product of the selinane series.

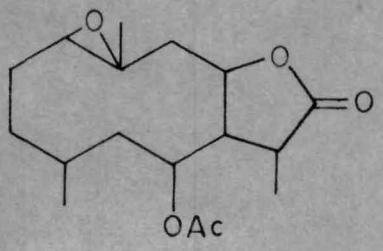
Pyrethrosin was transformed into a derivative of the santonin series by an elegant sequence of reactions, which leaves no doubt about the ten-membered ring structure. Quite recently Barton, Bockman and deMayo[17] proved the presence of the 1,2-epoxide in the molecule by isomerisation of tetrahydropyrethrosin (XVII) to the ketone (XVIII), by means of borontrifluoride.



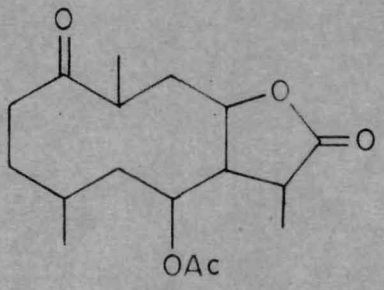
XV



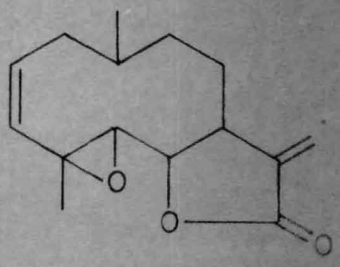
XVI



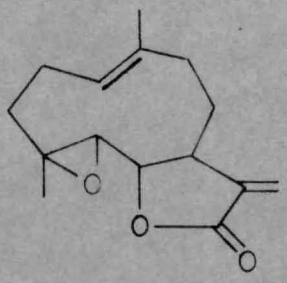
XVII



XVIII



XIX



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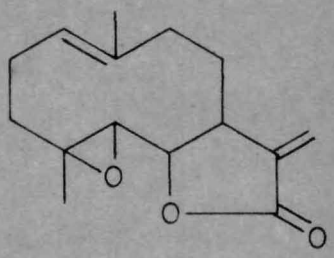

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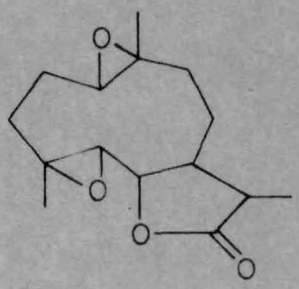


XXI

Barton and coworkers (loc.cit) were able to propose the absolute configuration of pyrethrosin at most of the asymmetric centres as in XV, by a study of the various chemical, physical and spectral properties of the molecule and its derivatives.

(2) Parthenolide (XIX)

Sorm and coworkers[18] isolated parthenolide, $C_{15}H_{20}O_3$ from the extractives of Chrysanthemum parthenium(L) Bernh.

On the basis of various chemical degradative experiments, IR and UV spectra they assigned to it the structure XIX.

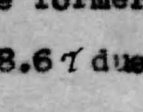
Govindachari and co-workers[19] who isolated parthenolide from the trunk bark of Michelia champaca arrived at structure XX for it as follows:

Parthenolide, m.p.115°; $(\alpha)_D$ - 78°, possesses a γ-lactone ring, two double bonds and an oxygen function. One of the double bonds is exocyclic and conjugated with the lactone carbonyl group. The oxygen function was found to be present as a 1,2-epoxide. These results prove the ten-membered carbocyclic structure of parthenolide. The NMR spectrum of parthenolide shows a singlet (3H) at 8.72 τ (methyl on carbon carrying oxygen), a singlet (3H) at 8.28 τ (methyl on a double bond), two sets of doublets (2H)

at 4.4 and 3.7 τ (J = 3.5 cps) indicative of an exocyclic methylene group, whose presence is further confirmed by the formation of formaldehyde on ozonolysis and the presence of an IR absorption band at 880 cm^{-1}. A broad signal (1H) at 4.7 τ is ascribed to a vinyl proton.

Hydrogenation of parthenolide in the presence of palladised carbon gives dihydroparthenolide. The latter shows NMR signals at 8.29 τ (3H) due to methyl group on a double bond, 8.74 τ (3H) due to methyl group on carbon carrying oxygen and a doublet at 8.72 τ (3H, J= 7 cps) due to the new methyl group created by reduction of the exocyclic double bond. The doublets at 4.4 and 3.7 τ present in parthenolide have disappeared.

The study of the NMR spectra of parthenolide and dihydroparthenolide clearly shows that the isolated double bond in parthenolide is trisubstituted and hence Sorm's structure XIX for parthenolide is untenable.

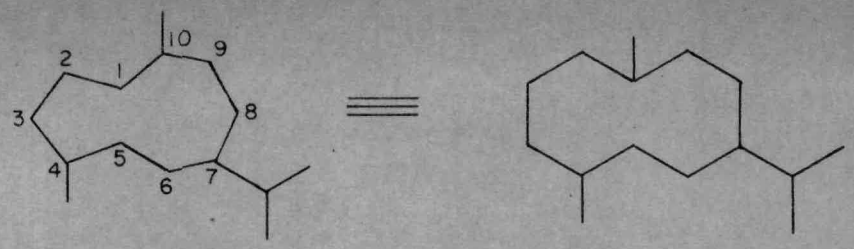
Dihydroparthenolide on reaction with perbenzoic acid gives the epoxy derivative (XXI), $C_{15}H_{22}O_4$, m.p.172-73°. The signals for the vinyl proton at 4.4 τ and vinyl methyl at 8.28 τ in the former disappear and a sharp singlet (3H) appears at 8.6 τ due to a methyl on the system  .

Parthenolide is monocarbocyclic and since dihydroparthenolide gives chamazulene on dehydrogenation it must possess the germacrane skeleton (XXII).

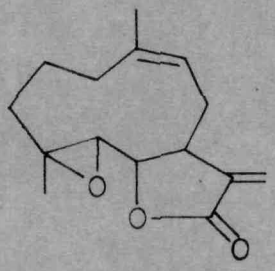
The position of the epoxy ring on carbon atoms 4 and 5 was conclusively proved by chemical evidences as follows. Tetrahydroparthenolide on treatment with boron trifluoride gives an allylic alcohol, which on reduction yields a glassy triol. The latter consumes periodic acid indicating the presence of 1,2-diol. This is possible only if the epoxide is on carbon atoms 4 and 5 in the germacrane frame work. NMR data supports this conclusion. Thus, only two structures XX and XXIII are possible.

That parthenolide is represented by structure XX was confirmed by carrying out a series of degradative reactions on dihydroparthenolide, which ultimately led to the formation of levulinic aldehyde. The levulinic aldehyde can only arise from structure XX with the tri-substituted double bond between C_1 and C_{10}.

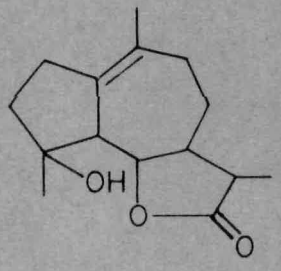
Dihydroparthenolide readily rearranges to a tertiary alcohol (XXIV) on treatment with borontrifluoride etherate. The compound gives chamazulene with great facility on heating with palladium charcoal. On the basis of its molecular weight, determined by mass spectroscopy, it should be bicyclic. The compound was assigned structure XXIV on the basis of its NMR spectrum whose principal features are a doublet (3H, J= 7 cps) at 8.78 τ (methyl at C_{11}), a sharp singlet (3H) at 8.72 τ (methyl at C_4 on carbon bearing hydroxyl), a doublet



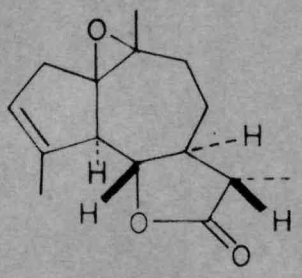
XXII



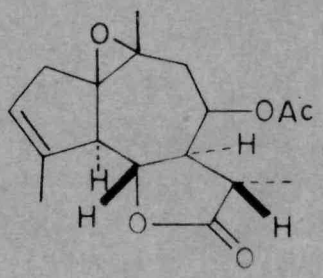
XXIII



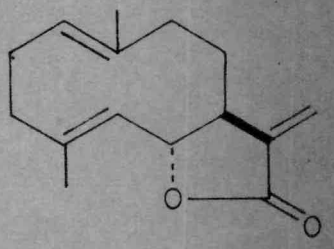
XXIV



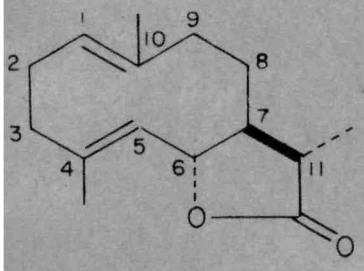
XXV



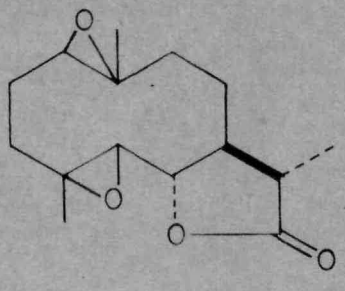
XXVI



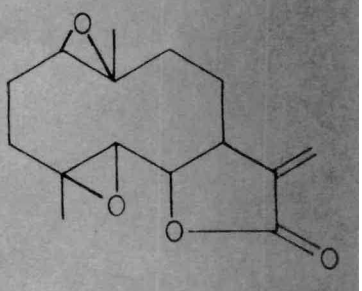
XXVII



XXVIII



XXIX



XXX

(3H; J= 1.5 cps) at 8.32 τ (methyl on double bond) and no signals in the 5 τ region. The formation of perhydroazulenic compound from dihydroparthenolide should have stereochemical significance since in all previously recorded instances in the case of ten-membered sesquiterpenes only perhydronaphthalenes have been reported.[20]

(3) Arborescin (XXV)

An epoxy guaianolide, arborescin, obtained from Artemisia arborescens and Matricaria globifera belonging to the family compositae, has recently been assigned structure XXV by Bates et al,[21] on the basis of NMR spectroscopy.

(4) Globicin (XXVI)

Another closely related guaianolide, globicin (XXVI) isolated from Matricaria globifera was recently found to be an epoxide by Bates and coworkers.[22]

Considering the occurrence of these epoxy lactones in the plants of the compositae family and the important structural features they exhibit, it was felt that a versatile compound like costunolide be converted into its mono- and di-epoxides. Costunolide (XXVII), which is a ten-membered ring lactone has a characteristic distribution of double bonds in the molecule and can rightly be considered the parent compound of the germacranolides. The importance

of ten-membered carbocyclic compounds in terpene biogenesis has been discussed in literature.[16,20,23,24] Extensive work has been carried out in our laboratory on costunolide,[25] but its epoxides were not isolated and studied, though, by the action of perbenzoic acid the double bonds were estimated. As the knowledge about the structure and different reactions of costunolide accumulated, the aspect of the chemistry of its epoxides could not be ignored. The occurrence of pyrethrosin and parthenolide in nature provided further impetus to this idea.

The revised structure of parthenolide (XX), discussed earlier, immediately disclosed its relationship with the structure of costunolide. Though the structure of parthenolide was firmly established, the problem of its absolute configuration had remained unsettled. It appeared that valuable information about this point may be obtained by studying the epoxides of costunolide as Govindachari's structure of parthenolide was nothing but the 4,5-epoxide of costunolide. If a monoepoxide of costunolide could be prepared and characterised, the structure of parthenolide could be further confirmed and the stereochemistry could also follow, since both these aspects have been rigorously established for costunolide.[1]

Dihydrocostunolide, m.p. 77-78°, was selected as the starting compound for studying epoxidation, as it is more stable and simpler than costunolide.

It was observed early in the present investigation that the product obtained by the prolonged (48 hours) action of perbenzoic acid on dihydrocostunolide exhibited a sharp band in the IR spectrum at 3448 cm^{-1} indicating the presence of a hydroxy compound. The hydroxy band was found to be absent in products of reactions of shorter duration (12 hours).

Experiments were then carried out with dihydrocostunolide (XXVIII) using one mole and two moles of perbenzoic acid in chloroform solution at 0° for 12 hours. The products, however, showed a considerable range in the melting point. Repeated crystallisations using different solvents and solvent mixtures were of no avail.

For example, when dihydrocostunolide was treated with one mole of perbenzoic acid in chloroform solution at 0° for 12 hours, the product was found to have m.p. 75-110°, even after several crystallisations.

It was evident from this, that the time factor was important. A kinetic study of the epoxidation reaction using one and two moles of perbenzoic acid in chloroform on dihydrocostunolide was initiated. It was observed that one mole of perbenzoic acid was consumed within 15 minutes after which the absorption virtually stopped. The second mole was consumed at the end of

96 hours. This observation was found true for costunolide as well.

Dihydrocostunolide was therefore treated with one mole of perbenzoic acid in chloroform solution and the reaction mixture was worked up after 15 minutes. The product, even after several crystallisations, was, however, found to be nonhomogeneous, m.p. 80-115°.

Comparable results were obtained when costunolide was employed under similar conditions.

Dihydrocostunolide on treatment with 2.2 moles of perbenzoic acid in chloroform solution at 0° for 96 hours, furnished a crystalline compound, m.p. 160-65°. It was crystallised twice from benzene-petroleum ether, m.p. 172-173°; $(\alpha)_D^{27}$ - 66.43°. Its IR spectrum (Fig. 1) exhibited bands at 1773, 1379, 1342, 1309, 1300, 1258, 1242, 1183, 1136, 1093, 1064, 1047, 1026, 995, 962, 934, 900, 874, 836, 809, 783, 733 and 714 cm^{-1}.

Its NMR spectrum (Fig.2) showed signals at 8.78, 8.65 and 8.58 τ (3H each) due to the three methyl groups at C_4, C_{10} and C_{11}, at 7.23, 7.08 τ (1H, each) due to the two protons at C_1 and C_5 and a triplet at 6.19, 6.04 and 5.90 τ (1H) due to the proton at C_6 on carbon carrying an oxygen, in agreement with structure XXIX for diepoxide of dihydrocostunolide.

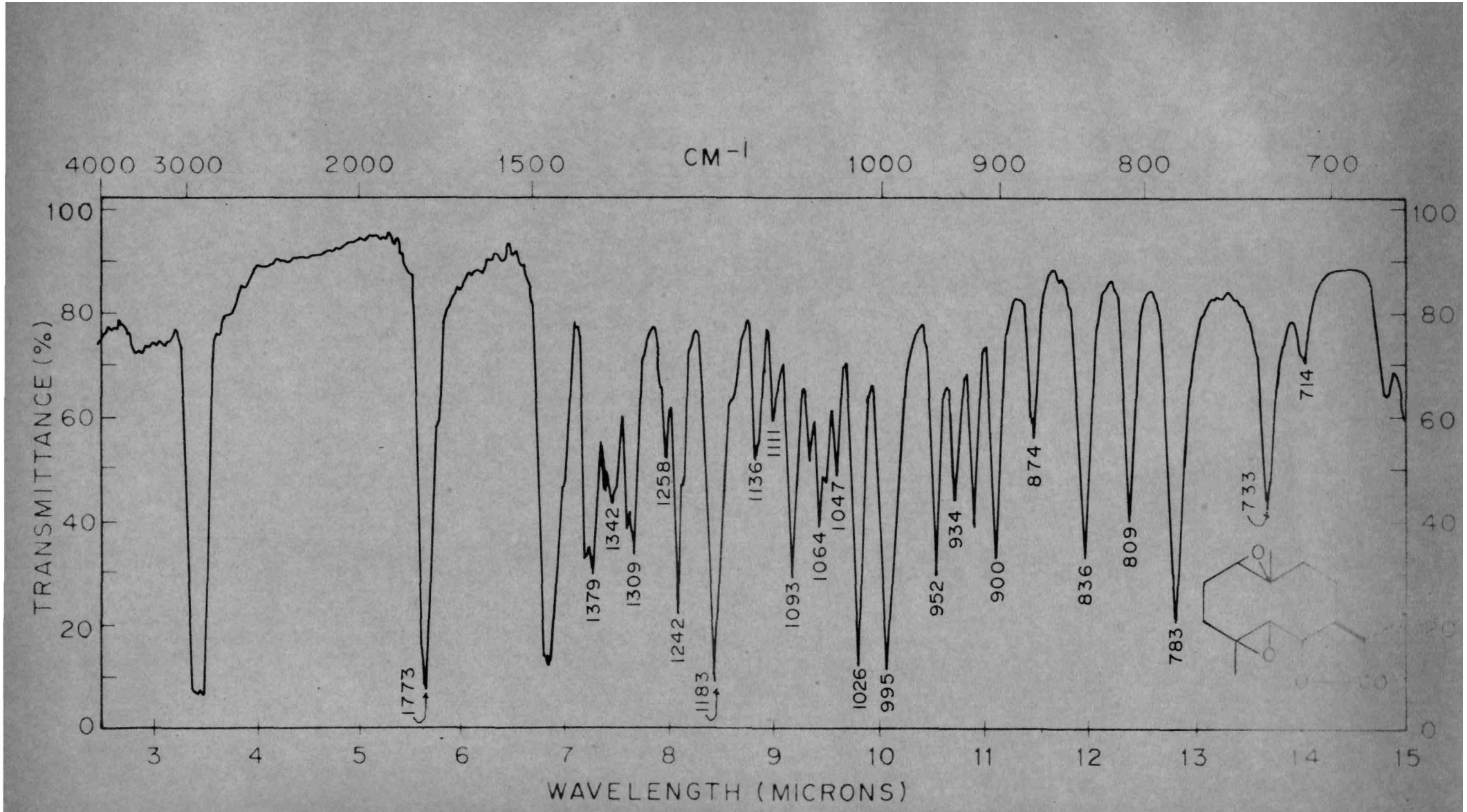


FIG. 1. IR SPECTRUM OF DIEPOXIDE OF DIHYDROCOSTUNOLIDE (IN NUJOL).

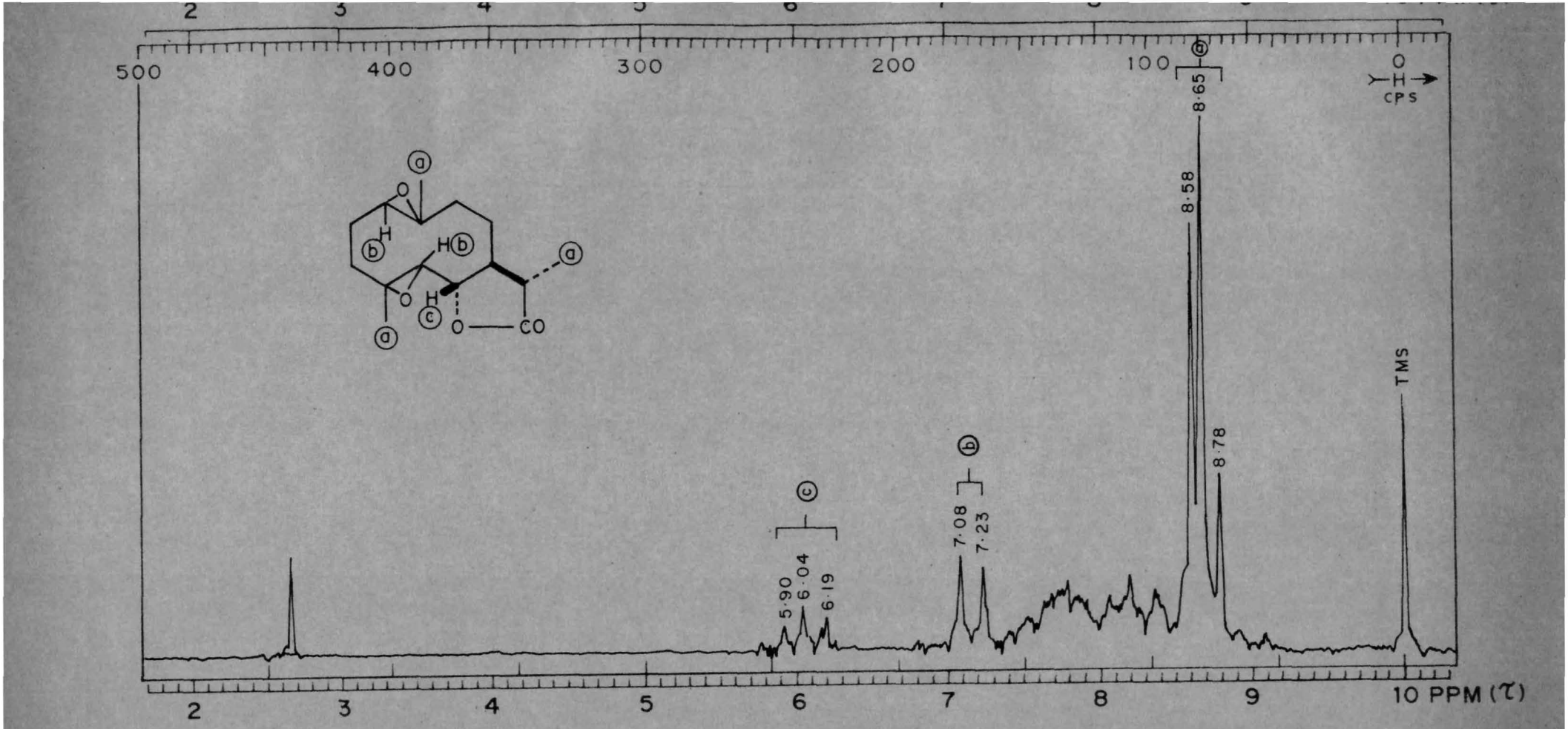


FIG. 2. NMR SPECTRUM OF DIEPOXIDE OF DIHYDROCOSTUNOLIDE.

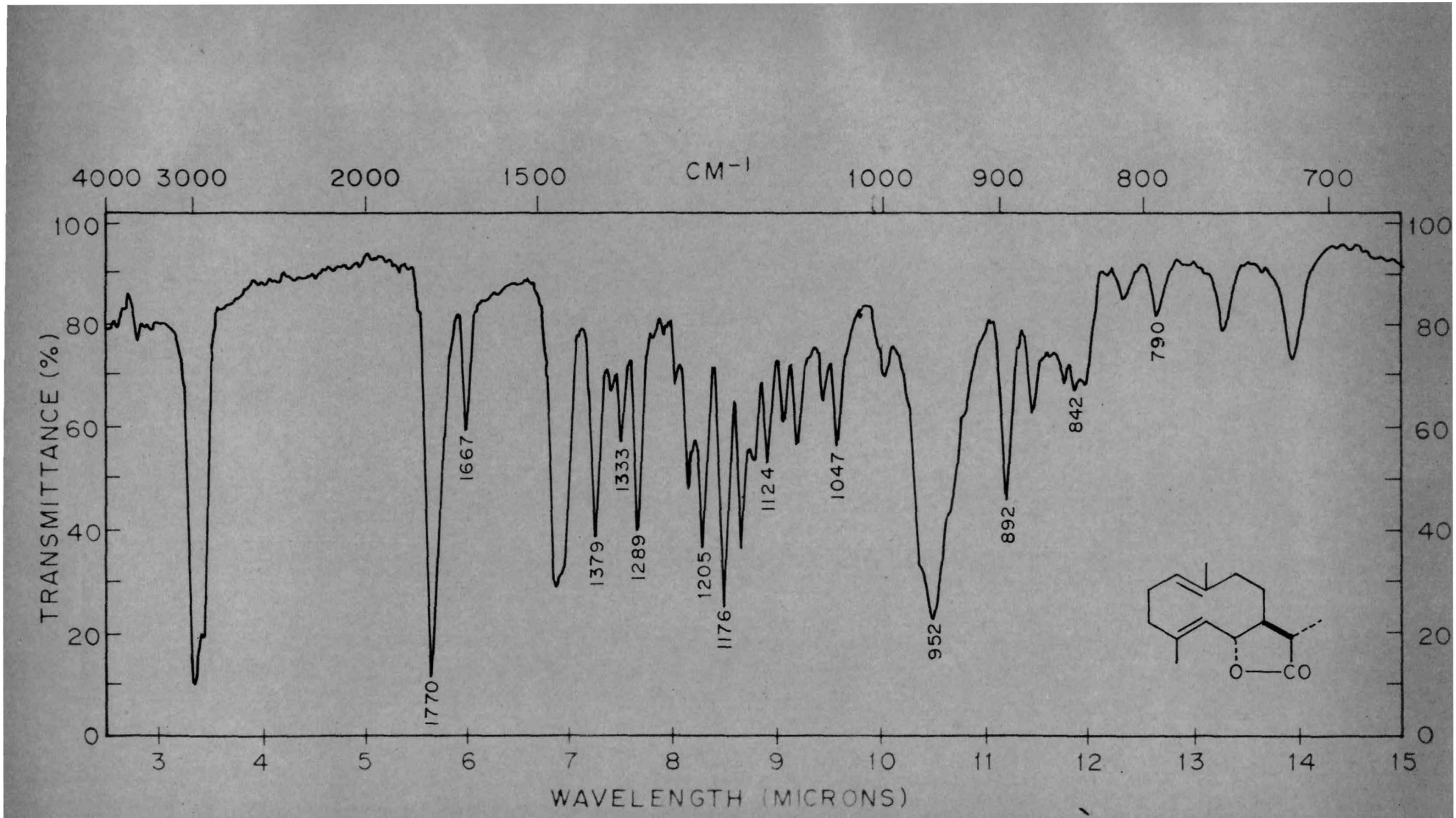


FIG. 1. IR SPECTRUM OF DIHYDROCOSTUNOLIDE (IN NUJOL).

The melting point and the specific rotation of the diepoxide of dihydrocostunolide agreed with those of epoxy dihydroparthenolide (XXI).[19] The identity was confirmed by taking the mixed melting point with an authentic sample of epoxydihydroparthenolide (kindly supplied by Professor Govindachari). IR spectra of both the samples were superimposable. This identity conclusively proved that epoxydihydroparthenolide (XXI) had the same stereochemistry as dihydrocostunolide (XXVIII) at C_6, C_7 and C_{11}, viz. the γ-lactone ring is transfused and the C_{11}- methyl is α-oriented. The stereochemistry of parthenolide itself at the lactone ring juncture thus follows.

Attempts to prepare the diepoxide (XXX) of costunolide under the same conditions were fruitless, as the resulting product was always a polymerised material even under mild temperature conditions.

From the kinetic data it was observed that the first mole of perbenzoic acid was absorbed within fifteen minutes while the other required 96 hours for completion. It was thus reasonable to suppose that in such a case a monoepoxide could be easily isolated. But this idea did not materialise and even chromatography did not yield a pure compound. Apparently, both the trisubstituted double bonds in costunolide were not equivalent in their reactivity

towards perbenzoic acid and the first mole preferentially attacked the more reactive centre. But it may also react with the other site of unsaturation simultaneously, though to a limited extent. Thus the resultant product was a mixture of isomeric monoepoxides, a small quantity of diepoxide as well as some unreacted starting material. Due to close similarity in the polarity of these compounds, it was not possible to effect a separation and to isolate the products in a state of purity.

EXPERIMENTAL

Monoepoxide of costunolide

A solution of perbenzoic acid in chloroform (18 ml of 0.53 N; 0.65 g., 0.0047 mole)was added to costunolide (1 g; 0.0043 mole) at 0°. The reaction mixture was worked up after 15 minutes. The chloroform solution was washed thoroughly with sodium bicarbonate solution (10%) and then with water. It was dried over anhydrous sodium sulphate and the chloroform removed under reduced pressure at 40 ± 2°. The crude product, m.p. 75-120°, was crystallised six times from benzene-petroleum ether, m.p . 80-120°.

The product (0.8 g) was then chromatographed on neutral alumina (grade II; 25 g) and eluted with pet.ether,= benzene and chloroform. A solid was obtained from the chloroform eluate, m.p. 90-115°. Attempts to purify this product by crystallisation did not yield any dependable results.

Diepoxide of costunolide

A solution of perbenzoic acid in chloroform (28 ml of 0.7 N; 1.34 g., 0.0096 mole) was added to costunolide (1 g., 0.0043 mole) at 0°. The reaction mixture was kept in the refrigerator for 96 hours. The product was isolated as described above, the temperature being maintained at 40 ± 2°, at all stages. It was a polymerised material from which no crystalline product could be isolated.

Monoepoxide of dihydrocostunolide

A chloroform solution of perbenzoic acid (14 ml of 0.7 N; 0.67 g., 0.0048 mole) was added to dihydrocostunolide (1 g., 0.0042 mole) at 0°. The product was isolated after 15 minutes, m.p. 70-110°. It was crystallised six times from benzene-petroleum ether mixture, m.p. 90-120°. Chromatography on neutral alumina was not helpful in purifying it, $(\alpha)_D^{27}$ - 12.39° ($CHCl_3$; c, 1.6).

Analysis

Found: C, 71.91; H, 8.83.

$C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%.

Diepoxide of dihydrocostunolide (XXIX)

A chloroform solution of perbenzoic acid (28 ml of 0.7 N; 1.34 g., 0.0096 mole) was added to dihydrocostunolide (1 g., 0.0042 mole) at 0°. The reaction mixture was kept in the refrigerator for 96 hours. The product was isolated as described earlier; m.p. 160-165°. It was crystallised twice from benzene-petroleum ether, m.p. and mixed m.p. 172-173°; $(\alpha)_D^{27}$ - 66.43° ($CHCl_3$; c, 5.7).

Analysis

Found: C, 67.89; H, 8.45.

$C_{15}H_{22}O_4$ requires: C, 67.64; H, 8.33%.

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P A R T IV

STUDIES

ON

THE STEREOCHEMISTRY OF DEHYDROCOSTUS LACTONE

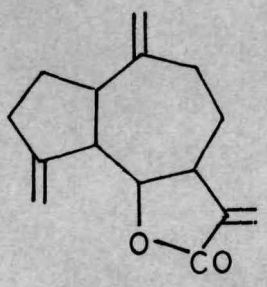
***

SUMMARY

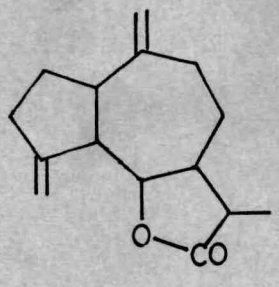
The structure of dehydrocostus lactone has now been firmly established. The problem of its stereochemistry has two aspects, namely, the stereochemistry of the ring juncture and the stereochemistry of the lactone moiety. In this part, an attempt has been made to examine the latter. No conclusive results have been obtained so far, but it is felt, that continuation of this study may ultimately lead to the solution of this problem.

Dehydrocostus lactone possesses a γ- lactone function as in the case of costunolide. It was thought interesting to examine the possibility of obtaining a common component from both the series.

Dihydrocostunolide on ozonisation gives a keto lactone carboxylic acid. It forms suitable solid derivatives. A similar approach was followed in the case of



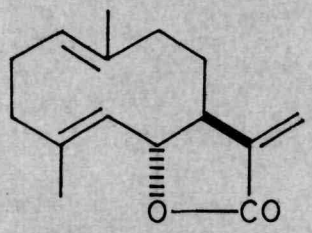
DEHYDROCOSTUS LACTONE



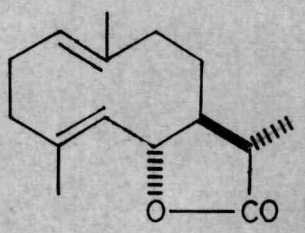
DIHYDRODEHYDROCOSTUS LACTONE

dihydrodehydro costus lactone. An attempt has been made to obtain a diene lactone, which on ozonolysis would give the same keto lactone carboxylic acid, if the stereochemistry of the lactonic moiety in both the compounds be the same.

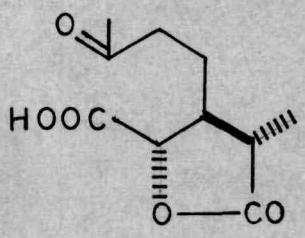
Various experiments leading to the formation of the diene lactone have been described.



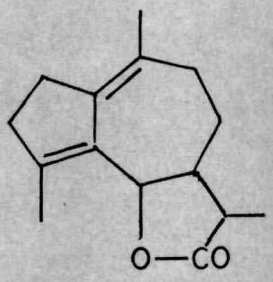
COSTUNOLIDE



DIHYDROCOSTUNOLIDE



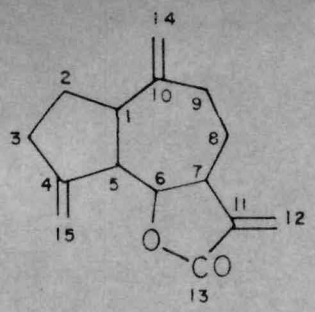
KETO—LACTONE—CARBOXYLIC ACID



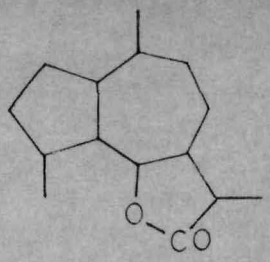
THE DIENE LACTONE

In this chapter are described some experiments which have been carried out for determining the stereochemistry of dehydrocostus lactone (I) and correlating it with costunolide. It has not been possible to come to any final conclusion as yet, but it is felt that continuation of work on this line will eventually lead to a solution of this problem. For this reason, it seems desirable to put on records the experiments which have been carried out so far.

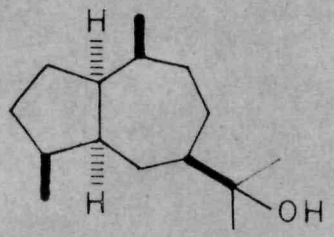
Dehydrocostus lactone is one of the main lactonic constituents of costus root oil, obtained by the low-temperature solvent extraction procedure developed in in our laboratory.[1] It was first isolated by Ukito[2] and later by Carabalona[3] from costus root oil. Its constitution was initially studied by Naves,[4] who, on the basis of its quantitative ozonolysis and dehydrogenation of the saturated lactone concluded that it contains two exocyclic double bonds and possesses a guaiane skeleton. Sorm and co-workers[5] subsequently established the presence of three exocyclic methylene groups, as a result of quantitative ozonolysis. They also found, on critical examination of its IR spectrum, that dehydrocostus lactone does not contain any methyl groups. Hexahydrodehydrocostus lactone (II) on selenium dehydrogenation gives a mixture of four azulenes in very



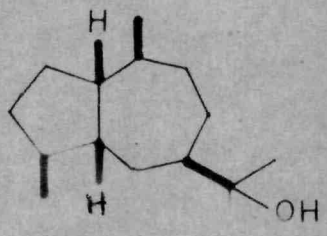
I



II



III



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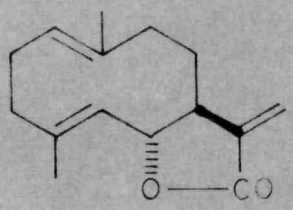

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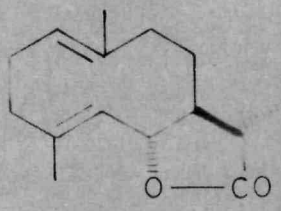

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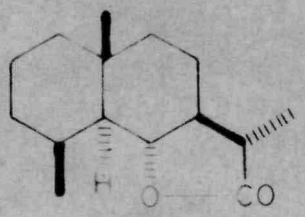

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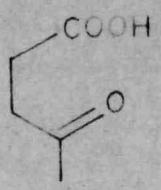
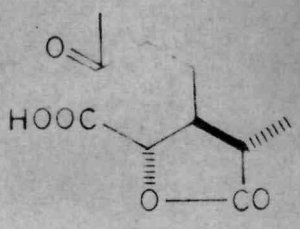


VIII



IX

low yields. From all these evidences, the Czech workers assigned structure I to dehydrocostus lactone.

In one of the recent communications from our Laboratory,[6] we have reported chemical evidences, based on pure crystalline derivatives, which would support the structure I for dehydrocostus lactone. It is but natural that the attempts to solve the stereochemistry should follow the structure determination. There are two aspects of this problem: (i) stereochemistry of the ring juncture, and (ii) stereochemistry of the lactone ring.

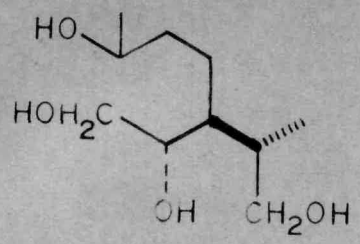
From certain experiments which have been carried out in our Laboratory[7] on dihydroguaiol epimers (III, IV) a solution of this problem seems to be in sight. In the present case attention has been concentrated on the stereochemistry of the lactone moiety.

A similar γ-lactonic unit occurs in costunolide (V) and dihydrocostunolide (VI), the stereochemistry of which has been rigorously established by converting them to santanolide 'c' (VII) of known absolute configuration.[8] The spectral properties would suggest that the lactone moiety in dehydrocostus lactone (I) is also possibly a trans-lactone. However, other physical tools

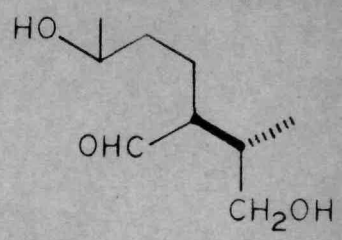
such as molecular rotation difference between the lactone and the diol could not be used with certainty, specially because the γ-lactone moiety is fused to a 7-membered ring and not to a 6-membered ring as in santanolide 'c' and vis-a-vis V and VI. For this reason it was felt desirable to try to prepare a common component from dehydrocostus lactone (I) and costunolide (V).

When costunolide (V) is preferentially hydrogenated in ethanol in the presence of 5% palladised carbon, it gives solid dihydrocostunolide (VI) as the major product. The latter, on ozonisation, is cleaved into two components, one of which is levulinic acid (VIII) and the other, a ketolactone carboxylic acid (IX).[8] As the compound (IX) is obtained directly by ozonolysis, it necessarily retains the original stereochemistry of the lactone moiety. Its methylester forms suitable solid derivatives such as 2:4-dinitrophenyl hydrazone and thiosemicarbazone, both of which can be used as reference materials for comparison purposes.

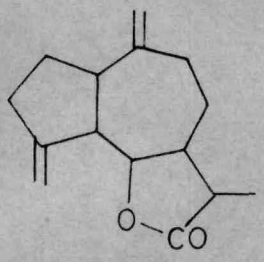
The methylester of compound IX on reduction with lithium aluminium hydride forms the tetrol (X) which on reaction with sodium metaperiodate affords the aldehydo diol (XI). Its semicarbazone and other derivatives can also be used as reference compounds. Since these various products are readily available, efforts were made to obtain



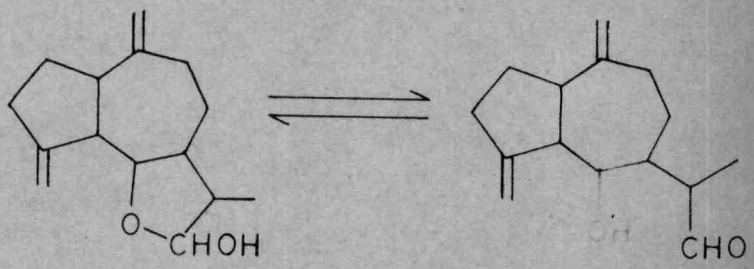
X



XI



XII



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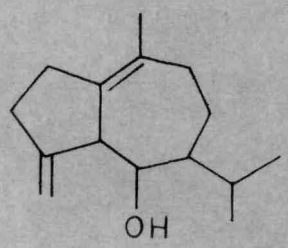
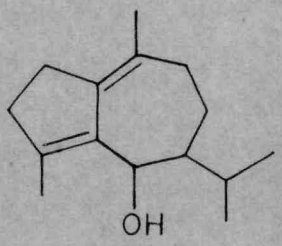
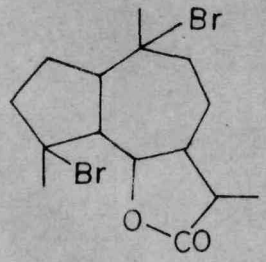


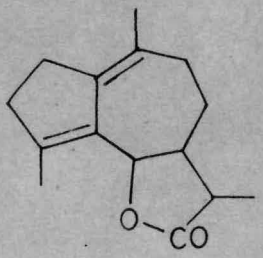
XIV



XV



XVI



XVII

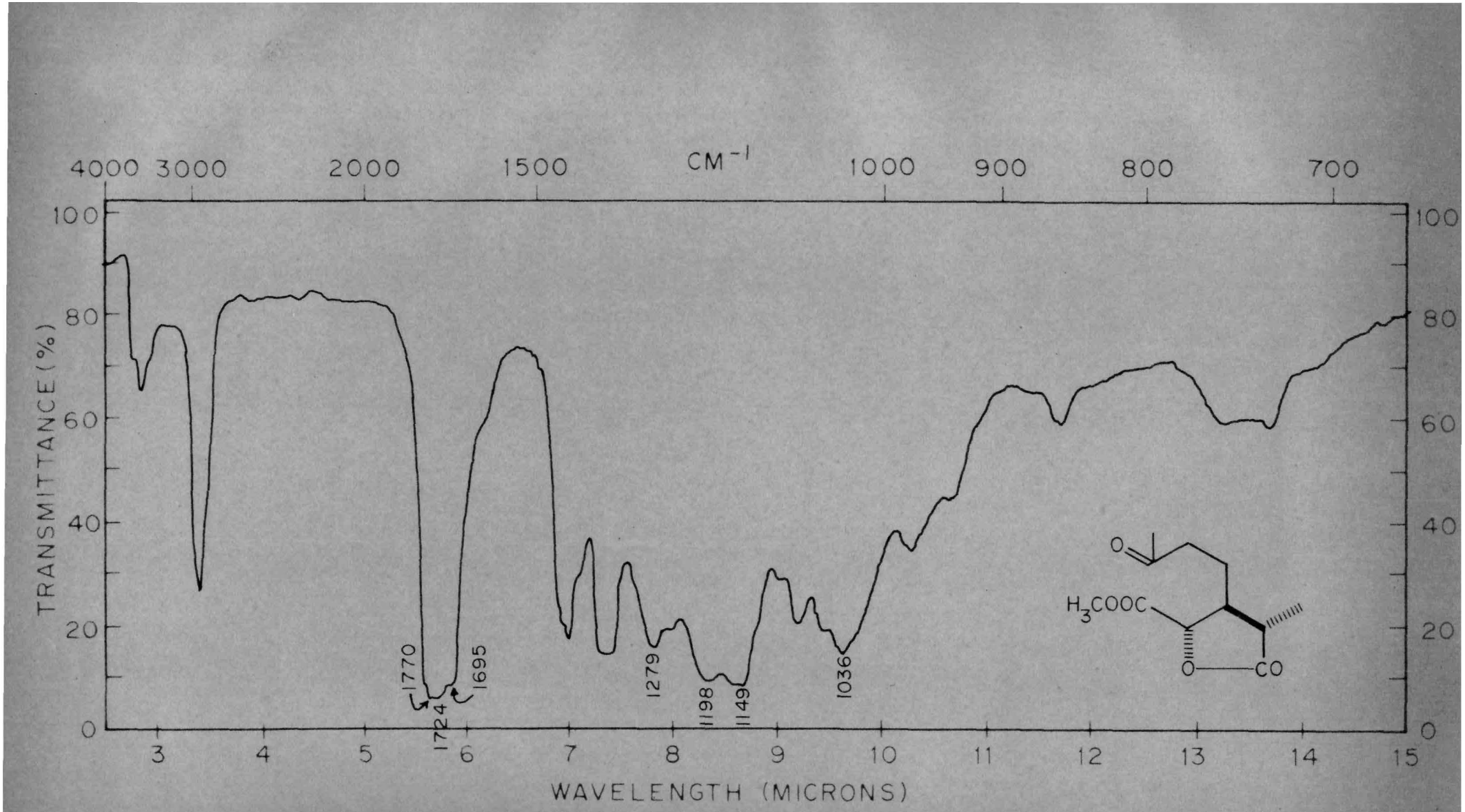


FIG. 2. IR SPECTRUM OF THE METHYL ESTER OF IX (LIQUID FILM).

same or similar products from dihydrodehydrocostus lactone (XII).

Attention was then directed to the preparation of pure dihydrodehydrocostus lactone (XII) by stereospecific reduction of the methylene group in conjugation with the 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 etal.[4]

However, this method was inadequate to obtain an epimerically pure compound. Reductions, using sodium and aqueous ammonia[9] also failed to give pure XII. Finally, the recent method reported by the Japanese workers[10] to get the dihydrolactone via oxidation of the pure crystalline lactol (XIII) was found to be the most suitable. The lactol was prepared by prolonged reduction of dehydrocostus lactone with sodium borohydride. The dihydroproduct obtained by this method was found to be a single epimer from its GLC and TLC analyses. Direct reduction of dehydrocostus lactone with sodium borohydride gave 80% pure (GLC) dihydrodehydrocostus lactone. It was also observed that it is more convenient to prepare the lactol by controlled reduction of dehydrocostus lactone with lithium aluminium hydride.

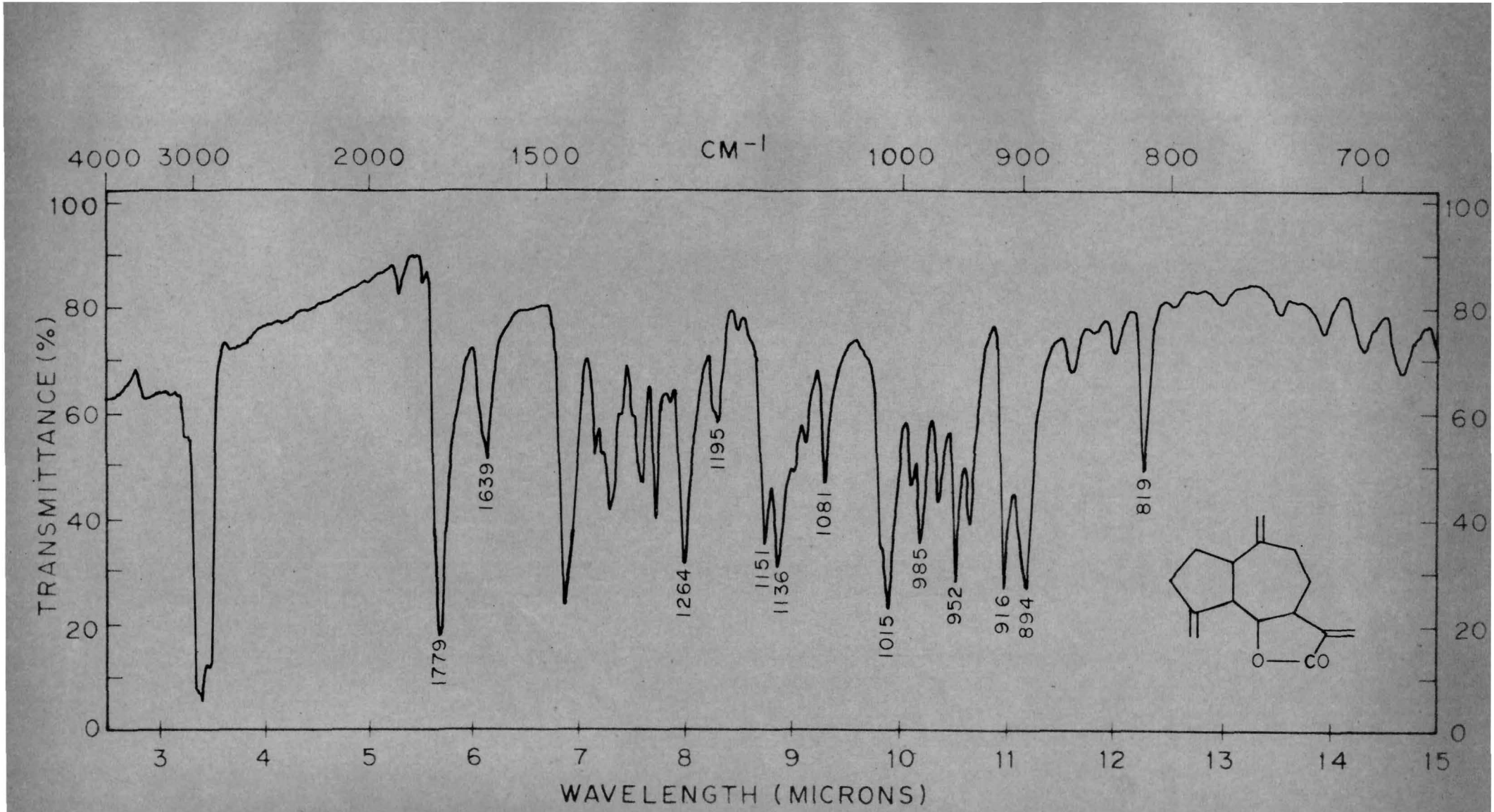


FIG. 3. IR SPECTRUM OF DEHYDROCOSTUS LACTONE (IN NUJOL).

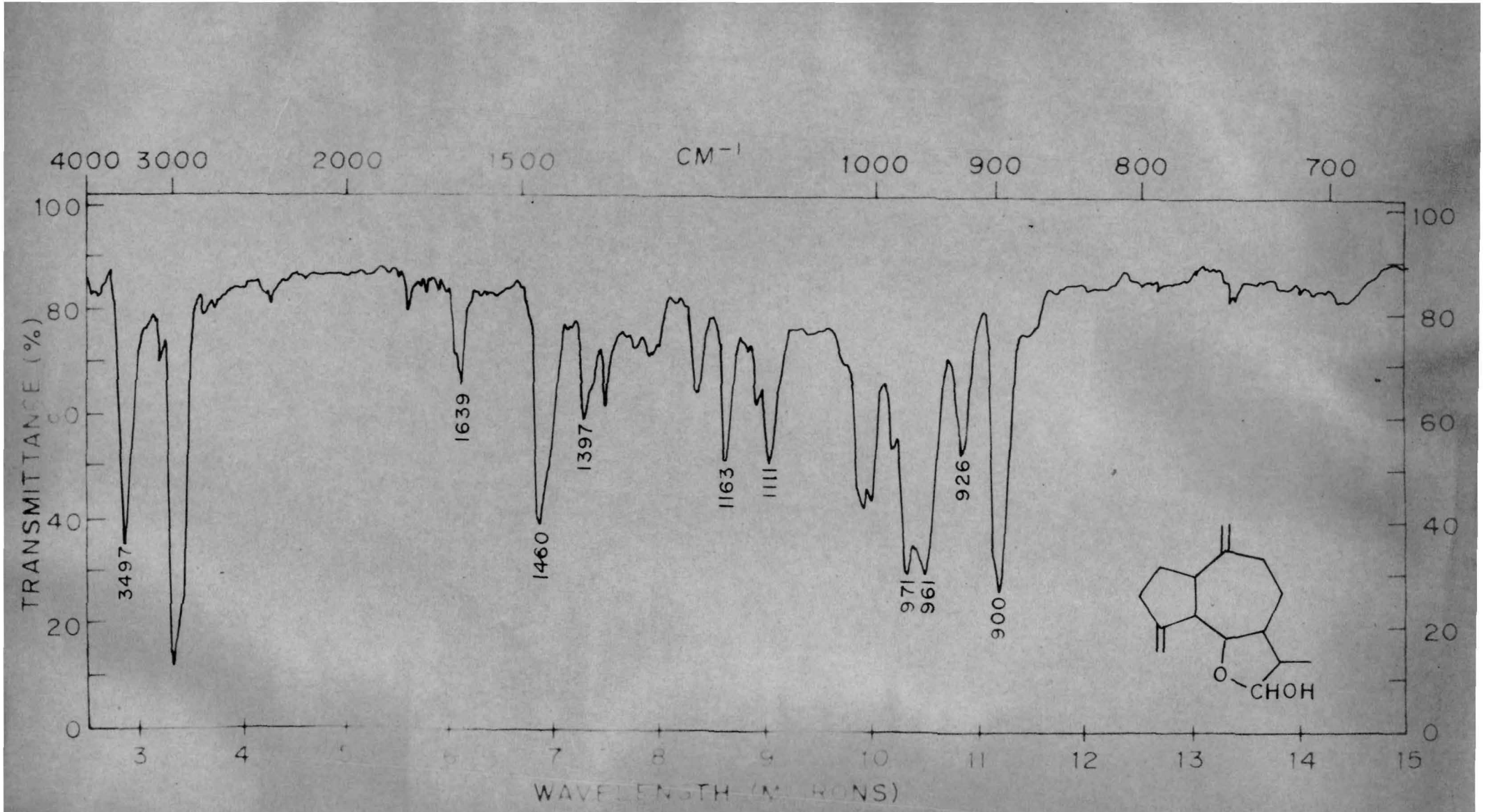


FIG 4. IR SPECTRUM OF THE LACTOL (IN NUJOL).

It has further been found that controlled lithium aluminium hydride or sodium borohydride reduction of the dihydrolactone followed by Huang-Minlon reduction, at different temperatures results in the formation of the diene-monols (XIV) and (XV). In the monol XV the two originally present methylenic double bonds have migrated inside the ring to a conjugated position.*

Efforts of the present author were directed to obtain a similar conjugated lactone (XVII) from dihydrodehydrocostus lactone (XII). Such a conjugated diene would show characteristic UV absorption and its formation could be conveniently detected by adopting this method. Ozonisation of this lactone will result in the formation of a keto-lactone-carboxylic acid which would be identical with IX, if the stereochemistry of the lactone moiety is the same as in costunolide.

For this purpose two general approaches were adopted: (i) isomerisation of double bonds by heating with acids and bases, and (ii) hydrobromination of dihydrodehydrocostus lactone and subsequent dehydrobromination.

The results of isomerisation experiments on the dihydrodehydrocostus lactone with acidic and basic reagents are shown in Table 1.

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* These results have been collected by Mr. S.B. Mathur of our laboratory and will form a part of his Ph.D. thesis.

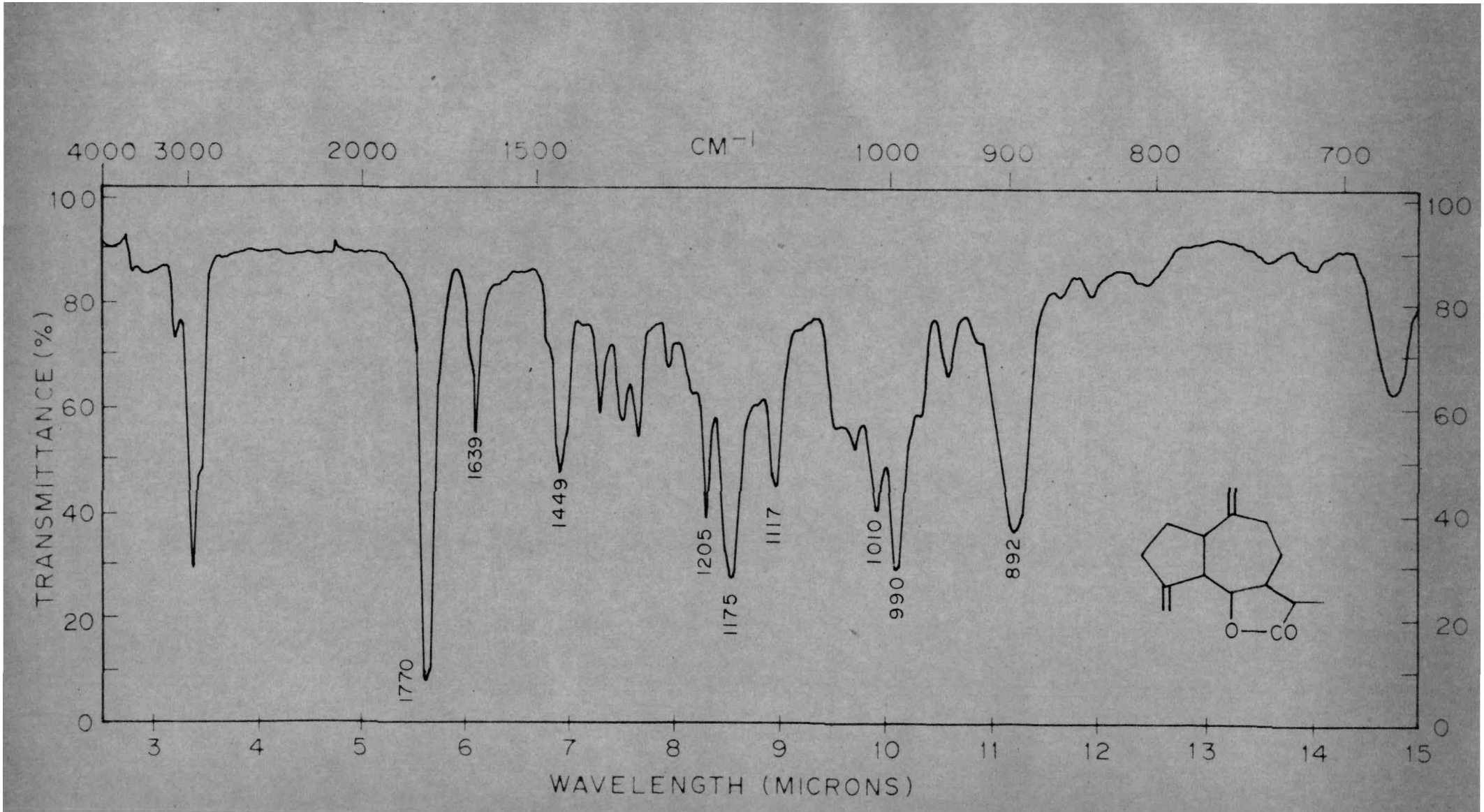

FIG. 5. IR SPECTRUM OF DIHYDRO DEHYDROCOSTUS LACTONE (LIQUID FILM).

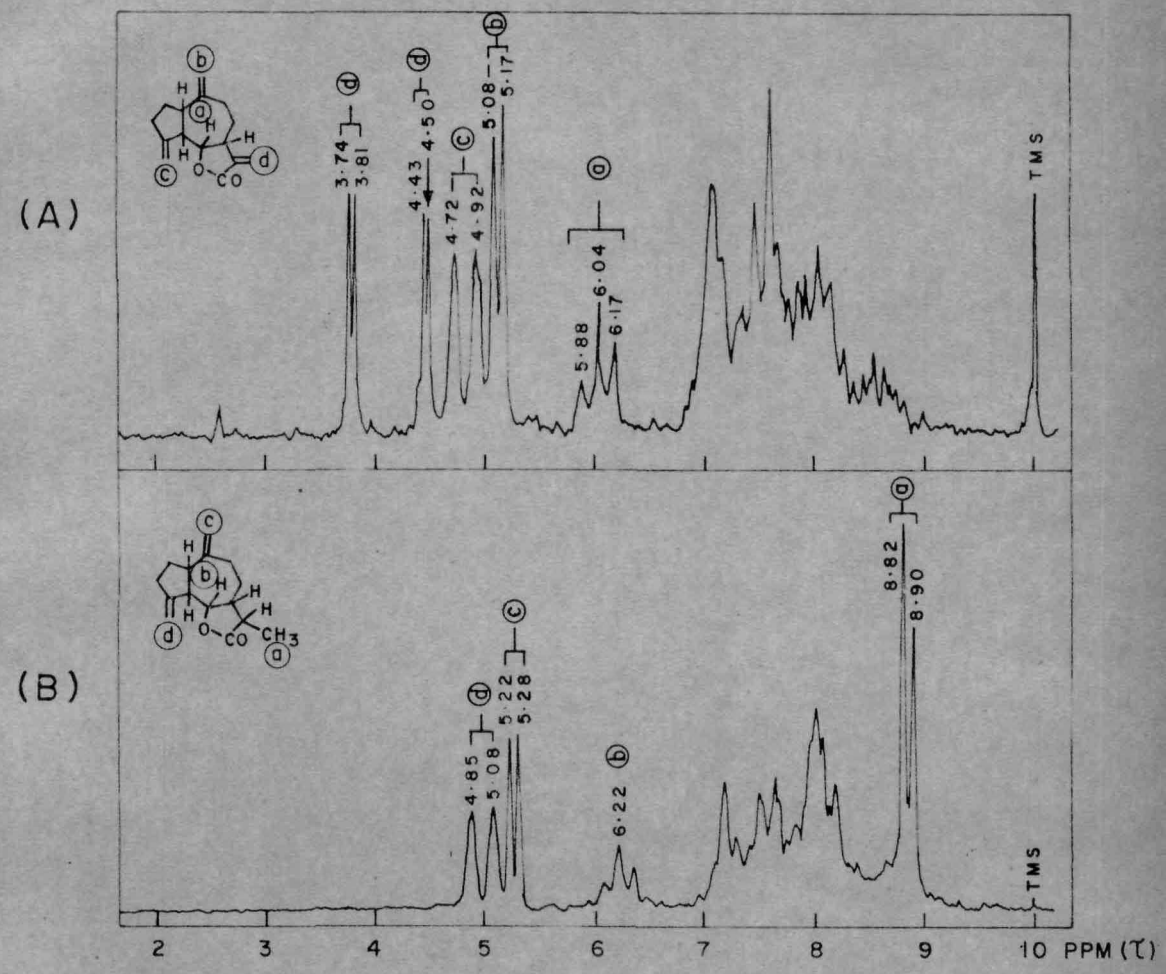

FIG 5

NMR SPECTRA OF (A) DEHYDROCOSTUSLACTONE
(B) DIHYDRODEHYDROCOSTUSLACTONE

TABLE 1

).	Solvent	Acid/Base	Temp./Time	U. V. Data
	Diethylene glycol	KOH	170-80°; 6 hrs.	λmax. 243 mμ; ε 2678.
	"	Aniline & KOH	170-80°; 4 hrs.	λmax. 242 mμ; ε 1995.
	Benzene + diethylene glycol	KOH	refluxed; 5 hrs.	λmax. 244 mμ; ε 2085.
	Benzene	BF_3	room temp., overnight	λmax. 247 mμ; ε 3,588.
	Acetic acid	AcOH	100°; 4 hrs & room temp., overnight	No reaction.
	Acetic acid	$HClO_4$	room temp., 48 hrs.	λmax. 247 mμ; ε 1114.
	Benzene	Toluene-p-sulphonic acid	refluxed; 4 hrs.	No reaction.

It is evident from Table 1, that the results of isomerisation of dihydrodehydrocostus lactone itself have not been very encouraging. It seems that extensive trials will be required before the objective is achieved. This aspect is being scrutinised.

Hydrobromination and dehydrobromination of dihydrodehydrocostus lactone

Hydrobromination of dihydrodehydrocostus lactone was easily affected by passing dry hydrogen bromide gas into a solution of the lactone in anhydrous ether. The dibromo derivative (XVI), thus obtained, was then dehydrobrominated under a variety of conditions. The course and extent of dehydrobromination was followed by quantitative UV absorption measurements of the resulting dienes.

The results of the dehydrobromination experiments tried under various conditions are shown in Table 2. In several cases, the neutral product was chromatographed on neutral alumina (grade III) and UV spectral characteristics of suitable fractions were determined as indicated in the Table.

TABLE 2

No.	Solvent	Base	Temp./Time	U. V. DATA	
				Neutral product.	Acid
1	CCl_4	Dimethyl aniline	room temp. overnight and refluxed 1 hr.	Pet.ether frn: λmax. 247 mμ; ε 5335. λmax. 290 mμ; ε 4980. Pet.ether + Benzene frn. λmax. 247 mμ; ε 3190. λmax. 290 mμ; ε 4206. Benzene frn: λmax. 247 mμ; ε 2642. λmax. 290 mμ; ε 3953.	λmax.244 mμ, ε 6132. λmax.290 mμ, ε 6132.
2	EtOH	Alcoholic KOH	room temp. 96 hrs.	λmax. 247 mμ; ε 5335. λmax. 290 mμ; ε 4409.	λmax.247 mμ, ε 3944. λmax.290 mμ; ε 3944.
3	C_6H_6	Pyridine	refluxed 6 hrs.	λmax. 244 mμ; ε 4930. λmax. 290 mμ; ε 4639.	
4	Pyridine	Pyridine	refluxed 1.5 hr.	λmax. 244 mμ; ε 7508. λmax. 290 mμ; ε 7508.	
5	CCl_4	Aniline	room temp. overnight & refluxed 2 hrs.	λmax. 250 mμ; ε 4796. λmax. 290 mμ; ε 4143.	

Table 2 contd.

No.	Solvent	Base	Temp/Time	UV Data
				(Neutral product)
6	Pyridine	Pyridine	refluxed 3 hrs(in N_2 atmosphere).	Pet.ether-benzene frn. λmax. 247 mμ; ε 3149. λmax. 290 mμ; ε 5139. Benzene frn. λmax. 247 mμ; ε 4419. λmax. 290 mμ; ε 3535. Chloroform frn. λmax. 247 mμ; ε 4640. λmax. 290 mμ; ε 3148.

Experiment No.4, above, appeared promising and was repeated on a larger scale in an atmosphere of nitrogen (experiment No.6) with the hope of isolating a pure product showing the characteristic UV absorption, but chromatography of the product was of no help.

As can be seen from Tables 1 and 2, though a variety of conditions was tried it was difficult to obtain a conjugated diene lactone because of the formation of by-products. Such a conjugated diene lactone would be expected to show a UV absorption maximum around 250-254 mμ with ε value of about 10,000 to 12,000 units. In all the cases studied above, the maxima appeared around 247 mμ, the shift may be explained as due to the presence of impurities. In addition to the maximum at 247 mμ, another maximum at 290 mμ was also observed in almost all the cases studied. The absorption at 290 mμ is characteristic of compounds containing three conjugated double bonds, and as such, cannot be readily explained especially in the case of neutral products.

However, formation of the desired lactone (XVII) was clearly indicated by the characteristic UV absorption at 247 mμ. The ε value was usually of the order of 5000 units. In the case of experiment 4, where pyridine was used as the base, the product showed ε value of 7508,

which indicated a purity of the order of about 70% of the diene lactone. The product is accompanied by several by-products, the complete removal of which has not been possible so far. This problem is being intensively pursued and it is hoped to obtain the desired pure material. Its ozonisation will be carried out with a view to obtain the keto- lactone carboxylic acid (IX) and the dihydroxy aldehyde (XI), or their respective derivatives.

EXPERIMENTAL

Dihydrocostunolide (VI)

It was obtained according to the procedure previously established in this Laboratory.[8]

Costunolide (V; m.p. 106-107°; $(\alpha)_D$ + 128°) (10.0 g) dissolved in ethanol (150 ml) was hydrogenated in presence of palladium-charcoal catalyst (5%; 0.5 g). Absorption of hydrogen virtually stopped when 982 ml (at N.T.P.) were absorbed, corresponding to 1.02 moles of hydrogen. The catalyst was filtered and the ethanol was removed from the filtrate under suction. The residue was taken up in ether, washed with sodium carbonate solution to remove the acid formed due to hydrogenolysis, and then with water. Removal of ether followed by crystallisation of the residue from methanol furnished 7.0 g. of dihydrocostunolide, m.p. 77-78°; $(\alpha)_D$ + 112.5° ($CHCl_3$; c, 2.8).

Analysis

Found: C, 76.62; H, 9.31.

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

Its IR spectrum (Fig.1) showed bands at: 1779, 1667, 1198, 1059, 811 cm^{-1}.

Ozonolysis of dihydrocostunolide[8]

Ozonised oxygen was passed into a solution of dihydrocostunolide (10.0 g) in ethyl acetate (75 ml) at 0° for 12 hrs. The solvent was removed under reduced pressure at 40°, and the residue was mixed with water (50 ml) and heated on a water bath for six hours. The ozonolysis product was soluble in water. The solution was saturated with ammonium sulphate and thoroughly extracted with ether. The ether solution was extracted with sodium carbonate solution to extract the acids. The aqueous alkaline solution was acidified with dilute hydrochloric acid and thoroughly extracted with ether. Removal of ether afforded a mixture of acids (11.5 g). It was esterified with diazomethane.

Methyl esters of levulenic acid (VIII) and keto-lactone carboxylic acid (IX)

The above methylester was fractionated:

Fraction No.	B. P.	Yield
1	70-75°/5 mm.	2.5 g.
2	140-155°/0.5 mm.	7.0 g.

Fraction 1 was identified as methyl levulinate (2,4-dinitrophenylhydrazone, m.p. 140-41°).

Fraction 2 was refractionated and the portion b.p.180-85°/3 mm. was collected, n_D^{26} 1.4620; $(\alpha)_D^{28} \pm 0°$ ($CHCl_3$; c, 4.2).

Analysis

Found: C,58.01; H, 7.18.

$C_{11}H_{16}O_5$ requires: C,57.88; H, 7.07%.

Its IR spectrum (Fig.2) shows bands at: 1770, 1724, 1695, 1279, 1198, 1149 and 1036 cm^{-1}.

2,4-Dinitrophenylhydrazone of methylester of IX

A mixture of the methyl ester (0.2 g), 2,4-dinitrophenyl hydrazine (0.18 g) and ethanol (15 ml) was heated to boiling. It was slightly cooled and two drops of concentrated hydrochloric acid were added when it formed a clear solution. It was allowed to stand overnight when yellow crystals of the derivative were deposited. It was crystallised twice from ethanol, m.p. 94-95°.

Analysis

Found: N, 13.59.

$C_{17}H_{20}O_8N_4$ requires: N, 13.72%.

Thiosemicarbazone of methyl ester of IX

The methyl ester (0.2 g) was added to a boiling solution of thiosemicarbazide (0.09 g) in ethanol (8 ml). The mixture was allowed to cool when crystals of the derivative deposited. They were filtered and crystallised from aqueous ethanol, m.p. 154-155°.

Analysis

Found: N, 13.76.

$C_{12}H_{19}O_4N_3S$ requires: N, 13.95% .

The tetrol (X)[8]

The methyl ester of compound IX (0.5 g) dissolved in dry ether (10 ml) was added to a stirred suspension of lithium aluminium hydride (0.5 g) in dry ether (25 ml) at 0°. The stirring was continued at 0° for one hour and the reaction mixture was then refluxed for 6 hours. Excess reagent was destroyed by adding moist ether and water. Hydrochloric acid (2 N) was carefully added in cold, until all the precipitate dissolved. The ether layer was separated and rejected. The aqueous solution was neutralised with sodium carbonate and filtered. The filtrate was concentrated to a small volume under reduced pressure. It was then continuously extracted with chloroform for 24 hours in a liquid-liquid extractor. Evaporation of chloroform yielded the tetrol (X, 0.4 g) as a thick, viscous liquid.

Its IR spectrum showed broad bands at 3390 and 1031 cm^{-1} and an absence of bands in the carbonyl region.

The aldehydo-diol (XI)

A saturated solution (12%) of sodium metaperiodate (5 ml) was added to a solution of the crude tetrol (X, 0.35 g) in ethanol (8 ml), and the mixture shaken mechanically for 2 hours. The precipitate of sodium iodate was filtered and the filtrate diluted with water and extracted thoroughly

with ether. Ether extract was dried over anhydrous sodium sulphate and the ether evaporated, when the aldehydo-diol (XI, 0.2 g) was obtained as a thick liquid.

Semicarbazone of XI

To an aqueous solution (5 ml) of semicarbazide hydrochloride (0.12 g) and sodium acetate (0.14 g) was added a solution of the aldehydo-diol (0.2 g) in ethanol (3 ml). The mixture was heated and allowed to stand at room temperature for two days, when crystals of the derivative separated. They were collected by filtration and crystallised twice from dilute ethanol, m.p.121-22°.

Analysis

Found: N, 17.89.

$C_{10}H_{21}O_3N_3$ requires: N, 18.18%.

Dehydrocostus lactone (I), m.p.60-61°; $(\alpha)_D$ - 12.8° ($CHCl_3$; c, 3.1) was used for the present work (IR Fig.3; NMR Fig. 6a).

Dihydrodehydrocostus lactone (XII). It was obtained by the procedure previously adopted in this Laboratory.[6]

The lactol (XIII)

(a) Sodium borohydride reduction of dehydrocostus lactone (I)

Sodium borohydride (0.6 g) was added in small lots to a solution of dehydrocostus lactone (10.0 g) in

methanol (50 ml) and the reaction mixture was allowed to stand at room temperature for 24 hours. It was diluted with water, acidified with hydrochloric acid and extracted with ether. The ether layer was washed free of mineral acid and dried. Removal of ether gave a liquid (9.1 g) which was chromatographed on neutral alumina (grade III, 180 g) and eluted with pet.ether, and ether. The pet.ether eluate gave a lactone (6.0 g) and the ether eluate yielded the lactol (XIII) as a thick liquid (2.0 g) which solidified on cooling. It was crystallised three times from pet.ether, m.p.110-111°; $(\alpha)_D$ - 35.7° ($CHCl_3$; c, 2.7).

(b) Controlled lithium aluminium hydride reduction of I

An anhydrous ether solution (100 ml) of lithium aluminium hydride (0.72 g of 75% purity) was added dropwise to a stirred solution of dehydrocostus lactone (10.0 g) in dry ether (100 ml) at -10°. The reaction mixture was stirred at -10° for 3 hours and for a further period of three hours at room temperature. The product was isolated as usual (9.1 g). It was crystallised three times from pet.ether to give the pure lactol (XIII), m.p.110-111°; $(\alpha)_D$ - 33.1° ($CHCl_3$; c, 3.4).

Analysis

Found: C, 76.52; H, 9.60.

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

Its IR spectrum (Fig.4) showed bands at: 3497, 1639, 1460, 971, 961, 926 and 900 cm^{-1}.

Chromic acid oxidation of lactol (XIII)

Jones' chromic acid reagent was added to a solution of lactol (2.0 g) dissolved in acetone (20 ml) until the brown colour persisted. The excess reagent was destroyed by addition of methanol (5 ml). The reaction mixture was diluted with water and extracted with ether. The ether solution was washed free of mineral acid and dried. Removal of ether gave a liquid (1.9 g). It was chromatographed on alumina (grade II; 38 g), and eluted with pet.ether, pet.ether-benzene and ether. The pet.ether-benzene eluate gave the dihydrodehydrocostus lactone (XII), as a liquid, b.p. 155° (bath)/ 0.3 mm., n_D^{26} 1.5210; $(\alpha)_D$ + 18.8° ($CHCl_3$; c, 2.3).

It showed a single peak on GLC analysis.

Analysis

Found: C, 77.28; H, 8.49.

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

Its IR spectrum (Fig.5) showed bands at: 1770, 1639, 1205, 1175, 1117, 1010, 990 and 892 cm^{-1}.

Its NMR spectrum is shown in Fig. 6b.

Isomerisation of dihydrodehydrocostus lactone (XII)

1. A mixture of dihydrodehydrocostus lactone (1.0 g) diethylene glycol (25 ml) and potassium hydroxide (1.0 g)

was heated for 6 hours at 170-180°. The reaction mixture was diluted with water and the neutral product (0.5 g) was extracted with ether. The neutral product showed λmax. 243 mμ; ε 2578.

The aqueous alkaline solution, on acidification and ether extraction furnished an acid (0.3 g), showing λmax. 244 mμ; ε 2515.

2. A mixture of XII (1.0 g), freshly distilled aniline (1.0 g), potassium hydroxide (0.3 g) and diethylene glycol (25 ml) was heated at 170-180° for 4 hours. The reaction product (neutral) was isolated as above: λmax. 242 mμ; ε 1995.

3. A solution of the lactone (XII; 1.0 g) in benzene (10 ml) was mixed with potassium hydroxide (0.5 g) and diethylene glycol (30 ml). The whole mixture was heated under reflux at a bath temperature of 150° for 5 hours. The reaction mixture was diluted with water, and processed as usual to give a product (0.68 g) showing λmax. 244 mμ; ε 2085.

4. The lactone (1.0 g) dissolved in dry benzene (10 ml) was treated with freshly distilled borontrifluoride etherate (5 ml) at room temperature, and the mixture kept overnight. It was poured in a saturated solution of sodium bicarbonate. The neutral product thus obtained showed λmax. 247 mμ; ε3588.

5. A solution of the lactone (1.0 g) in glacial acetic acid (25 ml) was heated on a boiling water bath for four hours and then allowed to stand overnight at room temperature. Starting compound was recovered unchanged (IR spectrum).

6. The lactone (1.0 g) dissolved in a mixture of glacial acetic acid (20 ml) and perchloric acid (5 ml) was kept at room temperature for 48 hours. The reaction mixture was diluted with cold water and extracted with ether. The ether solution was washed with a solution of sodium bicarbonate and water. Removal of ether gave a product showing λmax. 247 mμ; ε 1114.

7. A solution of the lactone (1.0 g) in dry benzene (25 ml) was refluxed in the presence of toluene-p-sulphonic acid (0.2 g) for 4 hours. The product was the unchanged starting material.

Hydrobromination of dihydrodehydrocostus lactone (XII)

Dry hydrogen bromide gas was passed into a solution of dihydrodehydrocostus lactone (10.0 g) in dry ether (30 ml) at 0° until the solution was saturated (found by the increase in weight). The saturated solution was maintained at 0° for four days. The reaction mixture was carefully diluted with ice-cold water and thoroughly extracted with chloroform.

The chloroform solution was washed free of hydrobromic acid and dried over anhydrous sodium sulphate. Chloroform was distilled off and the residue (10.5 g) was obtained as a thick, dark coloured liquid. This dibromolactone (XVI) was used as such for dehydrobromination reactions.

Analysis

Found: Br, 34.78.

$C_{15}H_{22}O_2Br_2$ Requires: Br, 40.61%.

Dehydrobromination of dibromolactone (XVI)

1. Dibromolactone (5 g) dissolved in carbon tetrachloride (50 ml) was mixed with dimethyl aniline (3.0 g) and kept overnight at room temperature. The mixture was then refluxed on a water bath for one hour. The reaction mixture was poured into dilute hydrochloric acid and the organic layer separated. It was washed with water and then with sodium carbonate to extract any acid formed during the reaction. Carbon tetrachloride solution was dried and evaporated to give a neutral product (3.7 g) as a dark coloured liquid. The sodium carbonate solution yielded an acid (0.8 g). The acid showed λmax. 244 mμ; ε 6132 and λmax. 290 mμ; ε 6132.

The neutral product was chromatographed on neutral alumina (grade III; 80 g) and eluted with pet.ether, pet. ether-benzene, benzene and ether.

Pet.ether fraction gave a product, λmax. 247 mμ; ε 5335, and λmax. 290 mμ, ε 4980.

Pet.ether benzene fraction gave a product, λmax. 247 mμ; ε 3190 and λmax. 290 mμ, ε 4206.

Benzene fraction gave a product, λmax. 247 mμ; ε 2642; λmax. 290 mμ; ε 2953.

Ether fraction was not examined.

2. Dibromolactone (1.0 g) was dissolved in alcoholic potash (5%; 20 ml) and kept at room temperature for 96 hours. The neutral and acidic products were isolated as usual. The neutral product showed λmax. 247 mμ; ε 5335; λmax. 290 mμ; ε 4409. The acid showed λmax. 247 mμ; ε 3944 and λmax.290 mμ; ε 3944.

3. A mixture of dibromolactone (1.0 g) dissolved in benzene (25 ml) and pyridine (8 ml) was heated under reflux for 6 hours; The product showed λmax. 244 mμ; ε 4930; and λmax. 290 mμ; ε 4639.

4. A solution of dibromolactone (1.0 g) in dry pyridine (15 ml) was heated under reflux for 90 minutes. The solution was diluted with water containing hydrochloric acid and extracted with ether. The ether solution was washed free of mineral acid and dried. Evaporation of ether gave a product showing λmax. 244 mμ; ε 7508 and λmax. 290 mμ; ε 7508.

5. A mixture of dibromolactone (1.0 g) dissolved in carbon tetrachloride (20 ml) and freshly distilled aniline (3.0 g) was kept overnight at room temperature, and then refluxed for two hours. The product, thus obtained, showed λmax. 250 mμ; ε 4796 and λmax. 290 mμ; ε 4143.

6. Experiment 4, above, was repeated using dibromolactone (10.0 g) and dry pyridine (100 ml). The mixture was refluxed under nitrogen atmosphere for 3 hours. The product (8.2 g) was chromatographed on neutral alumina (grade III; 170 g) and eluted with pet.ether; pet.ether-benzene, benzena and chloroform.

Pet.ether fraction gave a negligible residue.

Pet.ether-benzene eluate gave a product showing λmax. 247 mμ; ε 3149 and λmax. 290 mμ; ε 5139.

Benzene eluate gave a product showing λmax. 247 mμ; ε 4419 and λmax. 290 mμ; ε 3535.

Chloroform eluate gave a product showing λmax. 247 mμ; ε 4640 and λmax. 290 mμ; ε 3148.

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