P A R T - II

C H A P T E R - 1

OCCURRENCE OF CYCLOSATIVENE IN INDIAN TURPENTINE OIL (*Pinus roxburghii*), ISOLATION OF (+)-α-LONGIPINENE, (+)-LONGICYCLENE AND (+)-CYCLOSATIVENE

Abstract

For the present work, rather large quantities of (+)-α-longipinene were needed. Careful fractionation of a prefraction from industrial production of (+)-longifolene (Camphor and Allied Products Ltd., Bareilly) lead to isolation not only of (+)-longipinene and (+)-longicyclene, but also a new tetracyclic sesquiterpene component, identified as (+)-cyclosativene. This is the first report of its occurrence in essential oil from Pinus roxburghii.

INTRODUCTION

Pinus longifolia, Roxb. (Family, Pinaceae; natural order, Pinales) is a 100-110 ft tall tree, more or less deciduous, growing on the Himalayan slopes, at the hight of 2,000-7,000 ft. The trunk is usually naked with a girth of 12 ft. The bark is reddish brown in colour. The branches are symmetrically whorled, forming a rounded head of light foliage. The leaves are 9"-12" in length and slender. The male catkins are cylindrical and 0.33-0.5" long. The female cones are on short stiff stalks, spreading solitarily in whorls of 2-5. The seeds are unequally sides, 0.5-1" in length, oblong in shape, with a thin membranous wings.[1]

The plant is of immense economic importance, as its oleoresin (an exudate obtained by wounding the tree) furnished the turpentine oil (14-20%) and rosin, an distillation. This is the only worked out source for these products in our country.[2]

The essential oil being of high economic importance has been the subject of various investigations, which date back to 1905. Results of earlier investigations are briefly summarized below.

The presence of pinenes was soon established by earlier workers[3-6] and one of them[6] further suspected the presence of another terpene which on treatment with HCl gas gave sylvestrene dihydrochloride, investigated later on by Simonsen[7a] and was found to be a bicyclic terpenoid, named Δ^3-carene; these workers also established the presence of a new sesquiterpene hydrocarbon, longifolene[7a-b](4). It is present in this turpentine to the extent of 5-10% and is the main sesquiterpene constituent, co-occurring with minor amounts of other sesquiterpenes; longipinene, longicyclene, caryophyllene, humulene and β-bisabolene. The isolation of the first tetracyclic sesquiterpenoid-longicyclene- was reported by Nayak and Sukh Dev[8] from this oil in 1963. With the establishment of structure of himachalenes[9,10] it became apparent that himachalenes and longifolene, in all probability, stem from a common biogenetic precursor[10], however, complete absence of himachalene-type sesquiterpenes occurring in Pinus roxburghii has been demonstrated.[11]

PRESENT WORK

Sesquiterpenoids constitute only about 10% of the Indian turpentine oil of P. roxburghii and occurrance of (+)-α-longipinene (1)[12] was reported[11] many years ago from the same (+)-α-longipinene has also been shown to be present in,

1) various generas of Bryophytes[13],

2) Artemisia[14] and Dodonaea[14] of Angiosperms, Dicot,

and 3) various members of family Pinaceae.[15]

For the present work rather large quantities of (+)-α-longipinene (1) were needed and for this purpose, a 'prefraction' from industrial production of (+)-longifolene (4) (Camphor and Allied Products, Bareilly) was utilyzed. GLC analysis of the 'prefraction' showed presence of considerable amount of both (+)-α-longipinene (1) and (+)-longicyclene (1) along with (+)-longifolene (4) as the major component.

The 'prefraction' was fractionated repeatedly into various fractions which were pooled into five groups, depending on their b.p. and GC analysis. Based on GC analysis, pool-2 and 3 were selected for the isolation of (+)-α-longipinene (1) and (+)-longicyclene (2). Pool No. 2 (+)-α-longipinene and polar impurities). This material was

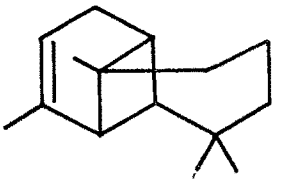
found to be contaminated with some polar impurities (5-10%) which were separated by a preliminary chromatography over Al_2O_3 to give (+)-α-longipinene (1), of reasonable purity (95-96% purity).

Pool No. 3 (+)-longicyclene and (+)-α-longipinene). This material was found to be a mixture of 1 and 2 in a ratio of 30:70 respectively. The mixture was treated with per acetic acid in chloroform, which preferentially reacted with olefinic contaminant, thereby furnishing (+)-longicyclene on careful distillation. It was observed (GLC) that the product always contained 12-14% of a hydrocarbon, different from (+)-longicyclene (2). It was surmised that this may be a fully saturated hydrocarbon, which may be present in the starting prefraction, not detectable by GLC, as it is having same retention time as (+)-α-longipinene (1). The two compounds were separated by preparative GLC. The hitherto undetected compound was readily, recognized from its spectral characteristics and other physical properties as (+)-cyclosativene (3),[16], which is known to occur in the essential oil of several Pinus[17] species. Also its occurrence in Cascarilla essential oil, Hymenaea courbaril and Scapania robusta Horik are well-documented in literature[18]. However, this is the first report of its occurrence in the

essential oil from Pinus roxburghii. (+)-Cyclosativene (3) is related to sativene (5) in the same way as tricyclene is related to camphene or longicyclene (2) to longifolene (4). The identity of (+)-cyclosativene (3) was established by comparison of the physical constants (b.p., $[\alpha]_D$, n_D) and spectral data (IR, NMR)[47].

The biogenesis and biosynthesis of sesquiterpenoids has been thoroughly reviewed[19,20a] and the differentiation into individual compounds has been proposed to begin with the cyclization of trans/trans and trans/cis farnesyl pyrophosphoates. The 1/10 and 1/11 cyclization of trans/trans and 1/6, 1/7, 1/10 and 1/11 cyclization of the trans/cis compound lead to the six intermediate monocyclic carbonium ions[20b] (Fig. 2), further transformation of which lead to the known sesquiterpenoids.

On the basis of these biogenetic considerations, our results, though based only on the hydrocarbons portion of sesquiterpenoids, indicate that in P. roxburghii trans/cis farnesol via 1/11 (±)-α-longipinene (1), (+)-longicyclene (2), (+)-longifolene (4) cyclization amounts for major of the materials, chiefly through the formation of large amounts of (+)-longifolene (4). On the other hand,



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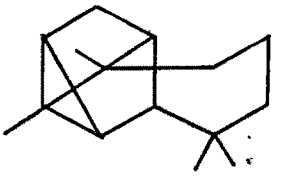

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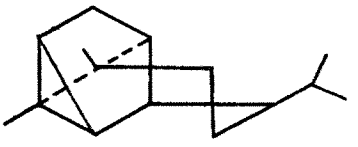

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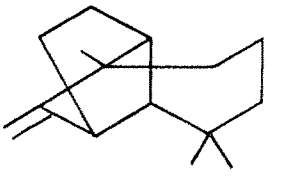

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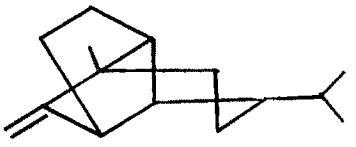

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Fig. 1. Sesquiterpenes isolated from Pinus roxburghii.

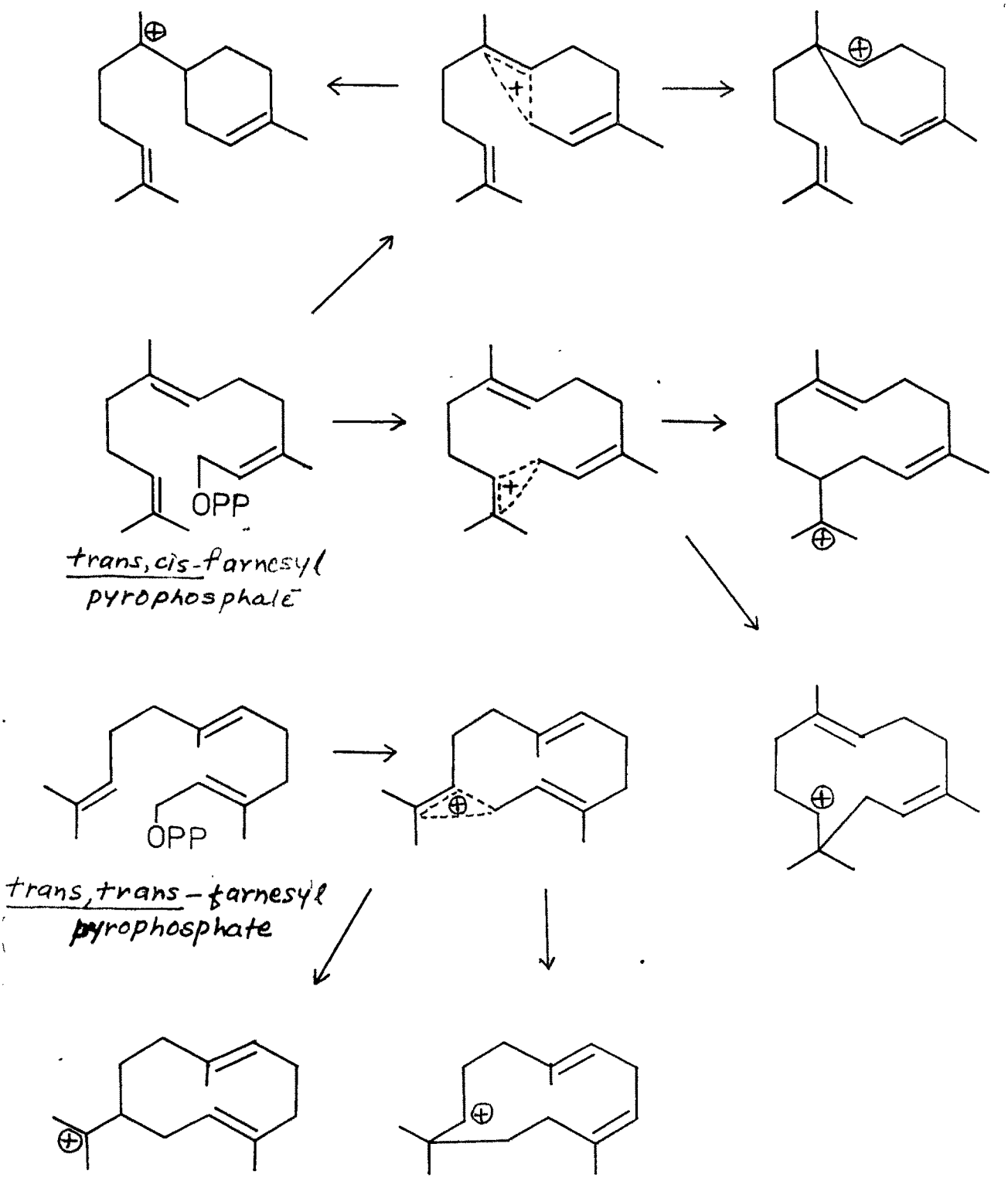

Fig. 2 Biogenesis and biosynthesis of sesquiterpenoids

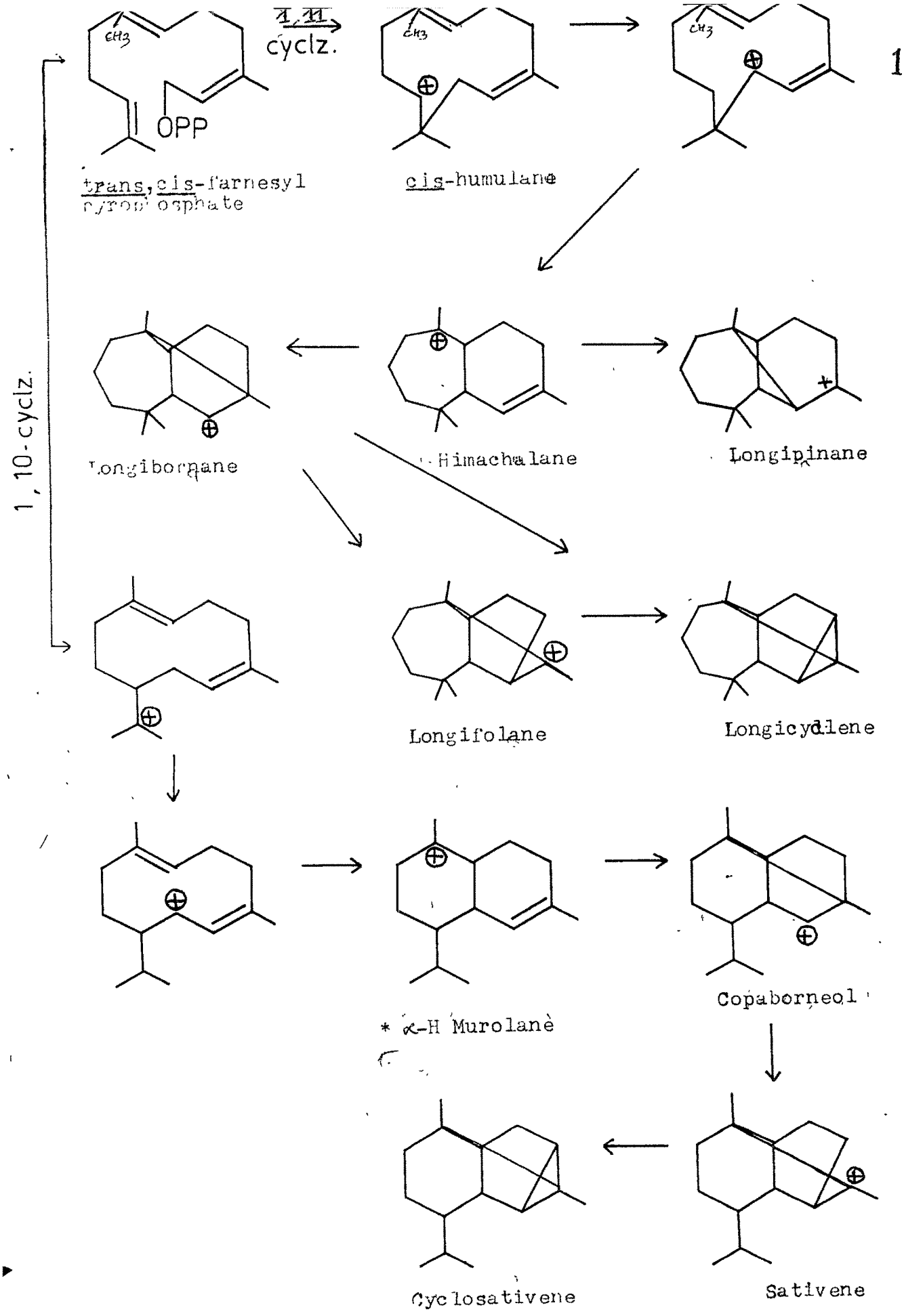

Fig. 3. 1/11 and 1/10 cyclization of trans/cis farnesyl pyrophosphate

(+)-cyclosativene (3) is a sesquiterpene derived from the same precursor via 1/10 cyclization (Fig. 3).

EXPERIMENTAL

For general remarks see page No.71

Isolation of (+)-α-longipinene and (+)-longicyclene

(+)-α-Longipinene and (+)-longicyclene were isolated by repeated fractionations of 'prefraction' 3.34 kg, GLC (Fig. 5) composition: (+)-α-longipinene (22.5%), (+)-longicyclene (19%), (+)-longifolene (36.5%) and low boilers (22%) on a 6' x 1" fractionating column (theoretical plates 35), packed with glass helices (4-6 mm diameter) with a reflux ratio 20: 1 to give different fractions which were pooled into five groups. The fractionation data is given in Table 1 (Fig. 4).

Pool No. 1 (low boilers)

The combined fraction (305 gr, b.p. 55-90°/10-7 mm) from its GLC analysis, was mainly a mixture of low boilers,

having same RRT to that of (+)-longifolene on 10% CW column.

Pool No. 2: (+)-α-longipinene and polar impurities

The combined fractions (446 gm, b.p. 55-57°/2 mm) constituted pool No. 2 and was essentially more than 90% pure (+)-α-longipinene (1) along with 8-10% of polar impurities not separable by fractionation (GLC, Fig. 6).

(+)-α-Longipinene: This mixture (130 gm) was chromatographed over Al_2O_3/I (650 gm, 70 x 3.6 cms) to furnish pure (+)-α-longipinene 1, 116 gm, eluted with light pet. 100 ml x 5, GLC, (Fig. 6) as colourless liquid, b.p. 78-80°/1.5 mm, n_D^{25} 1.4959, $[\alpha]_D$ + 40 ($CHCl_3$, c 1.5%). (Lit.[15] n_D 1.4924, $[\alpha]_D$ + 36.9 in $CHCl_3$, c 2.2%). IR (liq.) Fig. 7): 2920, 1649, 1430, 1370, 1139 and 781 cm^{-1}. ^{1}H-NMR (CCl_4) (Fig. 8): Me-C (6H, s, 0.883 ppm), Me-C (3H, s, 0.9 ppm), Me-C=CH (3H, dd, 1.65 ppm), Me-C-CH (1H, m, 5.14 ppm). (Lit.[15] IR, ^{1}H-NMR). Mass: m/z 204 (M^+, 21.5%), 161 (16%), 136 (10.7%), 133 (45%), 119 (100%), 105 (54%), 107 (32%), 91 (36.5%), 93 (42%), 55 (36.5%).

Pool No. 3: (+)-α-Longipinene and (+)-longicyclene

The combined fractions (180 gm, b.p. 57-58°/2 mm) constituted pool No. 3 and was mainly a mixture of (+)-α-longipinene (1) and (+)-longicyclene (2) 73 and 27% respectively as shown by GLC (Fig. 9).

Pool No. 5 [(+)-longicyclene along with (+)-longipinene and (+)-longifolene]

The combined fractions (140 gm, b.p. 58-59°/2.3 mm) constituted pool No. 5 and was mainly a mixture of (+)-longicyclene (2, 60%), (+)-longipinene (1, 22%) and (+)-longifolene (4, 18%), as shown by GLC (Fig. 9).

Pool No. 4 (+)-Longicyclene and (+)-α-longipinene

The combined fractions (441 gm, b.p. 58°/2 mm) constituted pool No. 4 and was mainly a mixture of (+)-longicyclene (2) and (+)-α-longipinene (1) (73.5 and 26% respectively) along with traces of (+)-longifolene (4), GLC (Fig. 10).

(+)-Longicyclene. It was isolated by treating above mixture (180 gm, 0.265 mole of (+)-α-longipinene) with azeotropic peracetic acid* (40%, 70 ml, 0.368 moles) in

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* Azeotropic peracetic acid was added slowly maintaining 0-10° temperature in pot within 2 hr while stirring.

chloroform (250 ml), buffered with $NaHCO_3$ (60 gm, 0.714 moles). The contents were stirred further for 1.5 hr after addition of peracetic acid maintaining the same temperature. It was then treated with water (150 ml), and the aqueous layer was extracted with $CHCl_3$ (50 ml x 3). The combined organic layers were treated with water (100 ml), 5% $Na_2S_2O_3$ (100 ml), 20% Na_2CO_3 (100 ml) and brine (75 ml x 2). Drying and removal of the solvent furnished a crude product (184 gm) which was carefully distilled (using 6' Vigreux column) to yield (+)-longicyclene (2, 144 gm, b.p. 94-96°/4-3 mm, 88% pure by GLC) along with 12% of saturated hydrocarbon, (+)-cyclosativene (3) as shown by GLC (Fig. 10).

(+)-Longicyclene and (+)-cyclosativene. These were further purified by preparative GLC (Al. column, 20% CW on Chromosorb W NAW 45-60 mesh, 3/8" x 12', 140°). The pure samples had the following characteristics. (+)-Longicyclene (2): n_D^{25} 1.4910, $[\alpha]_D$ + 37.6 ($CHCl_3$, c 2.1%). (Lit.[18] n_D^{30} 1.4888, $[\alpha]_D$ + 33.6°, neat). The identity of (+)-longicyclene (2) was also established by comparison of spectral data. (Lit.[8] ^{1}H-NMR, IR). (+)-Cyclosativene: n_D^{25} 1.4845, $[\alpha]_D$ + 92.4 ($CHCl_3$, c 1.8%) (Lit.[17] $[\alpha]_D$ + 94.1 in $CHCl_3$, c 0.5%).

IR (liq.) (Fig. 11): 3040, 1382, 1367, 1315, 1280, 1260, 1081, 973, 860, 841 and 820 cm^{-1}. ^1H-NMR (CCl_4)(Fig. 12): Me-C (3H, singlets at 0.966 and 0.744 ppm), isopropyl (3H doublets at 0.90 and 0.86 ppm, J = 6Hz), and a partly unresolved proton at 0.76 ppm).

Mass: m/z 204 (M^+ 76%), 189 (25%), 161 (66%), 133 (46%), 119 (100%), 105 (97%), 94 (84%), 91 (62%), 79 (34%), 55(43%), (Lit.[17] IR, ^1H-NMR and Mass).

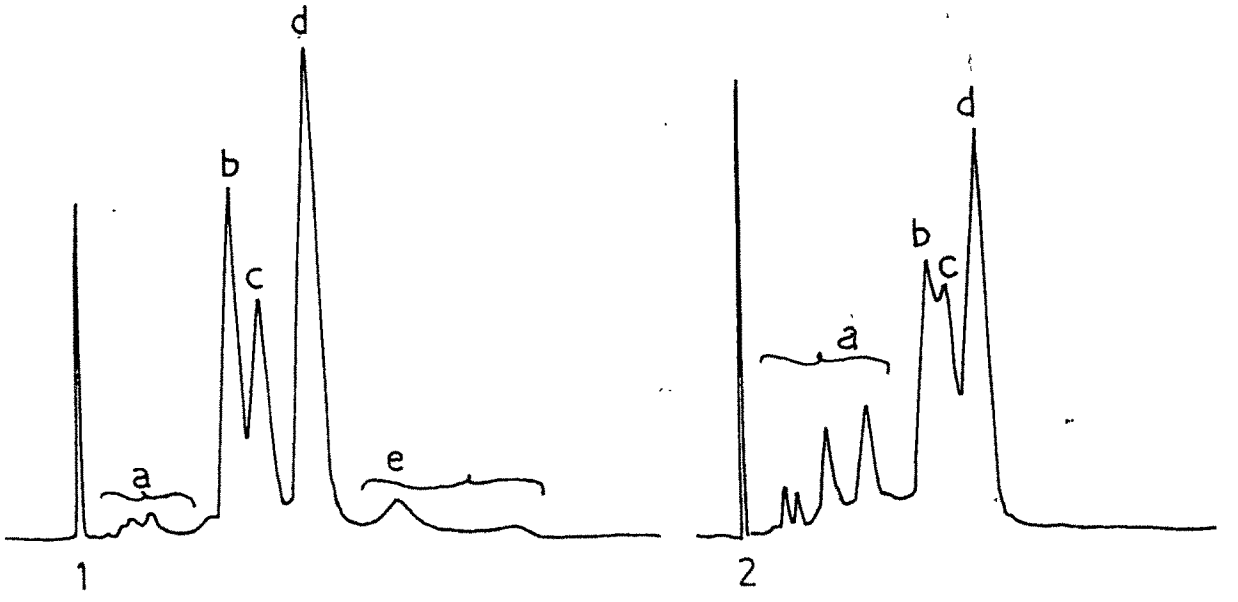
Table 1

Prefraction (P. roxburghii)

B.p. 55-90°/3340 µm 10-4 mm

Pool No.	Type	B.p.	Vacc. mm	Reflux ratio	Wt. gms.	GLC analysis			
						Low boilers %	(+)-α-Longi-cinene (%)	(+)-longi-cyclene (%)	(+)-longi-folene (%)
1	Low boilers	53-91	15-7	20:1	805	major	traces	-	-
2	(+)-α-Longicinene	55-57	2-3	20:1	446	10	90	-	-
3	Intermediate-1	57-58	2-3	20:1	180	-	73	27	-
4	(+)-Longicyclene	58	2-3	20:1	441	-	26	73.5	trace
5	Intermediate-2	58-59	2-3	20:1	140	-	22	60	18
	POT MATERIAL	-	-	-	1290	-	-	7	93

Fig. 4.

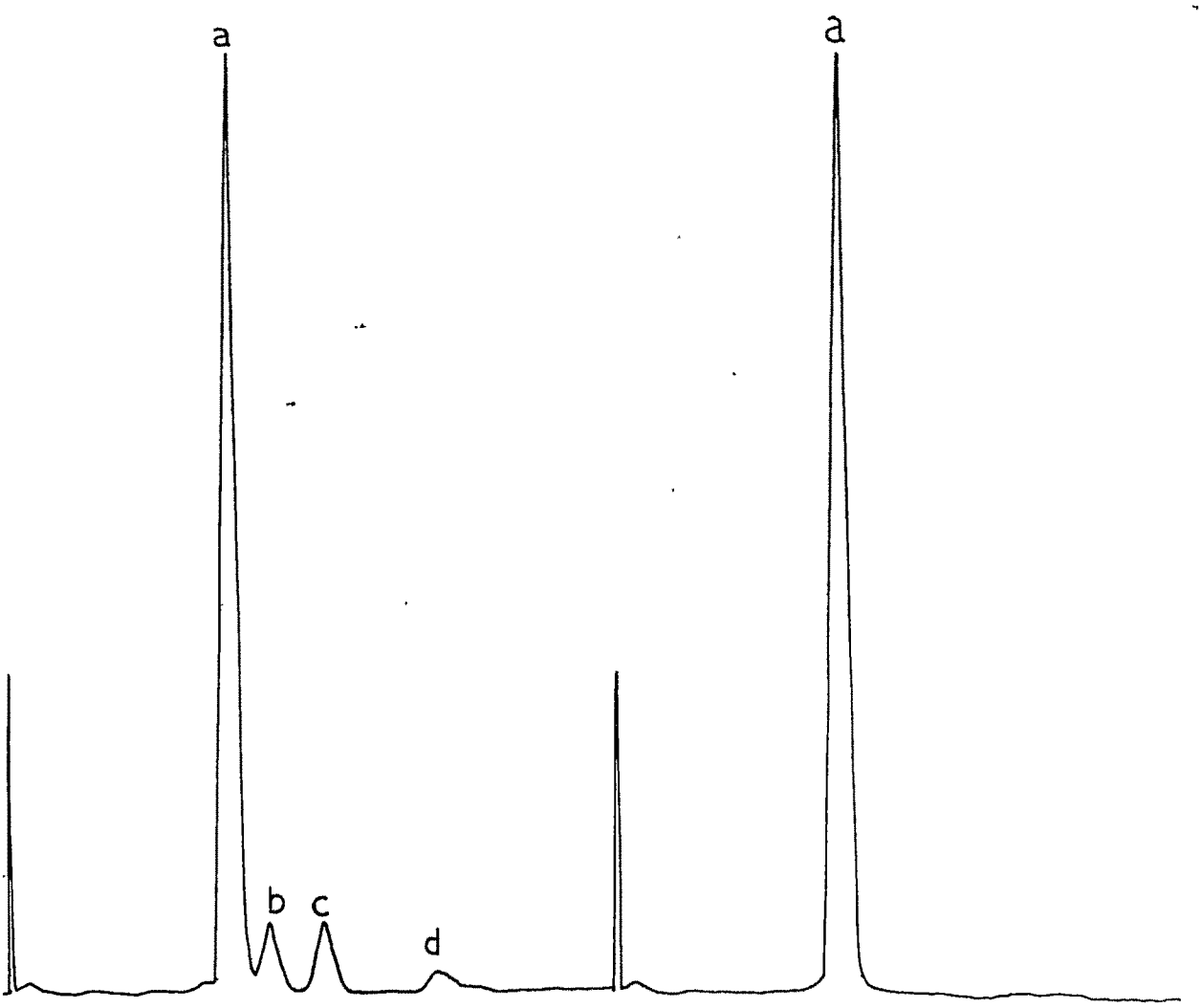


Column. 1) 10% CW 20M (360 cm x 0.6 cm, Al. column) temp. 150°
2) 10% SE30 on chromosorb W, HP 60-80, Temp. 170°

Hydrogen flow: 60 ml/min.

a) Low boilers and polar impurities
b) Longiolnene
c) Longicyclene
d) Longifolene

FIG. 5. GLC OF PREFRACTION



Column: 10% CW 20M (360 cm x 0.6 cm, Al. column)
Hydrogen flow : 60 ml/min
Tem;. 150°

a) Longipinene

b, c and d) Polar impurities

Fig. 6 . GLC OF OOIL No. 2 AND PURE LONGIPINENE

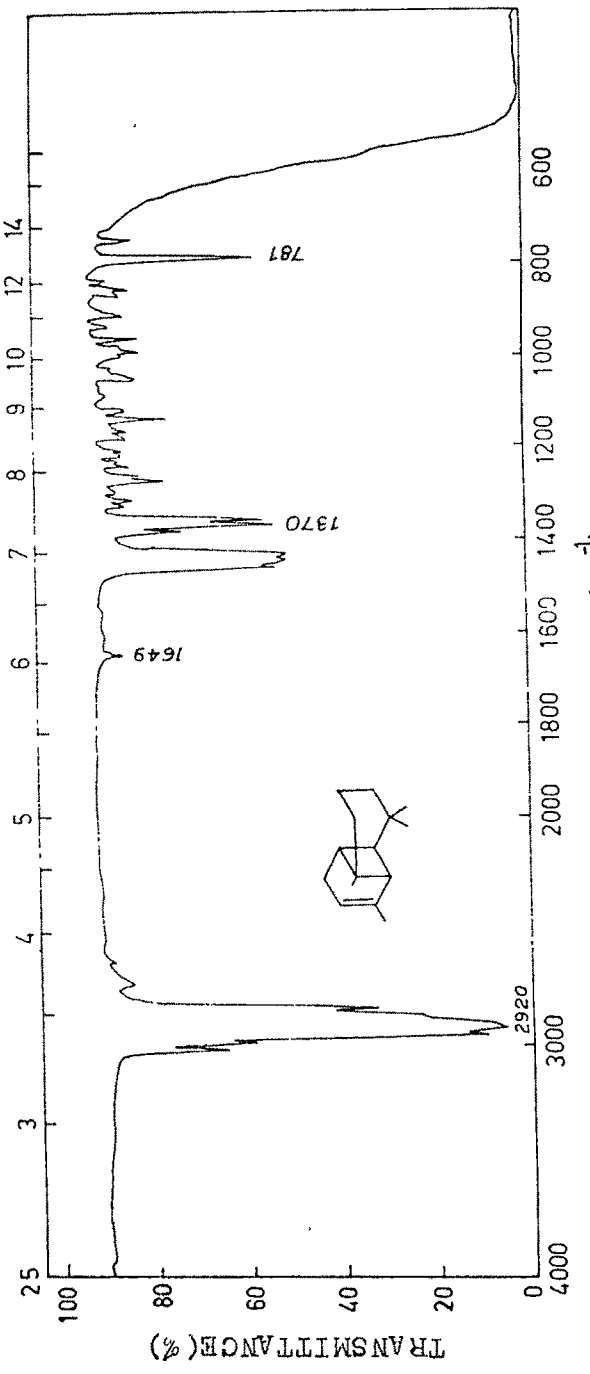


FIG.7 : IR SPECTRUM OF (+)-LONGIPINENE(1)

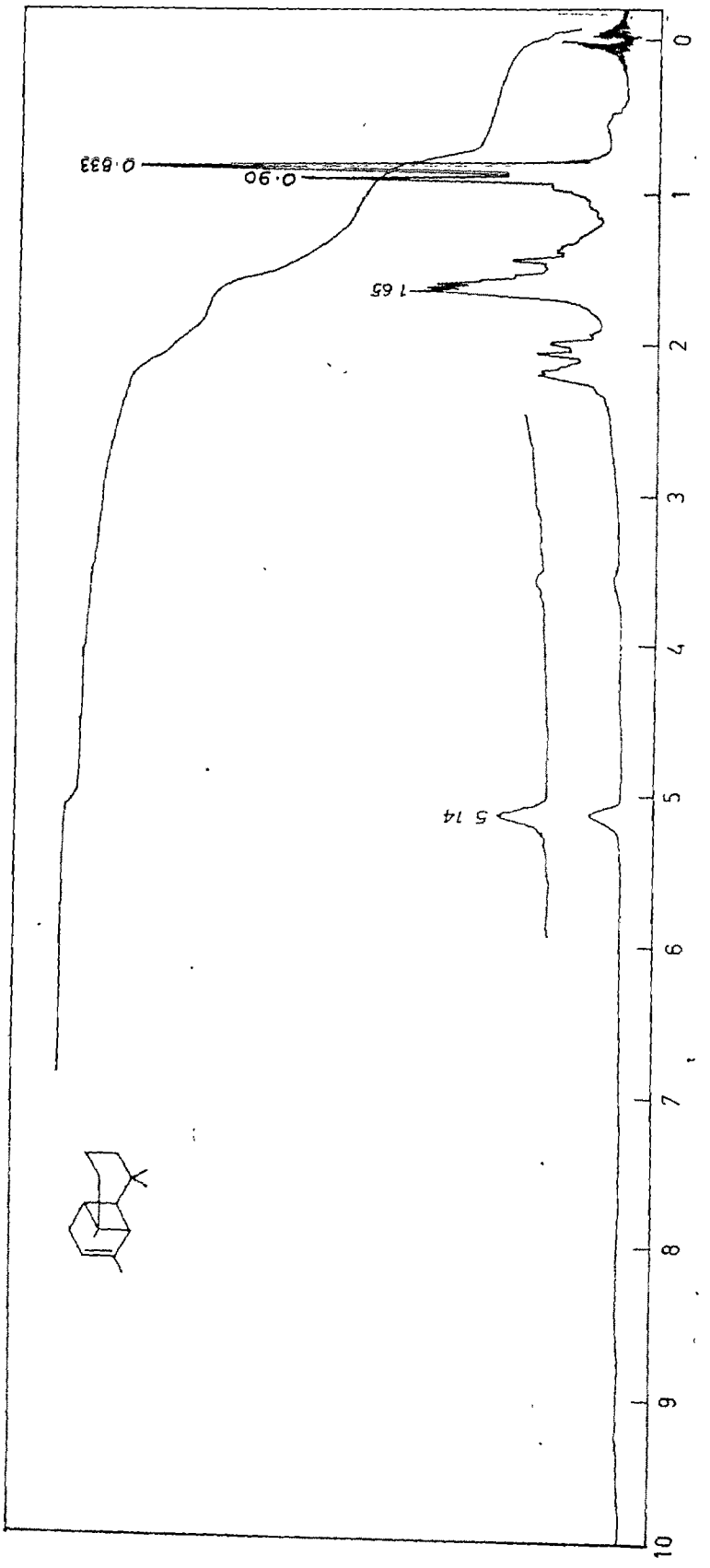
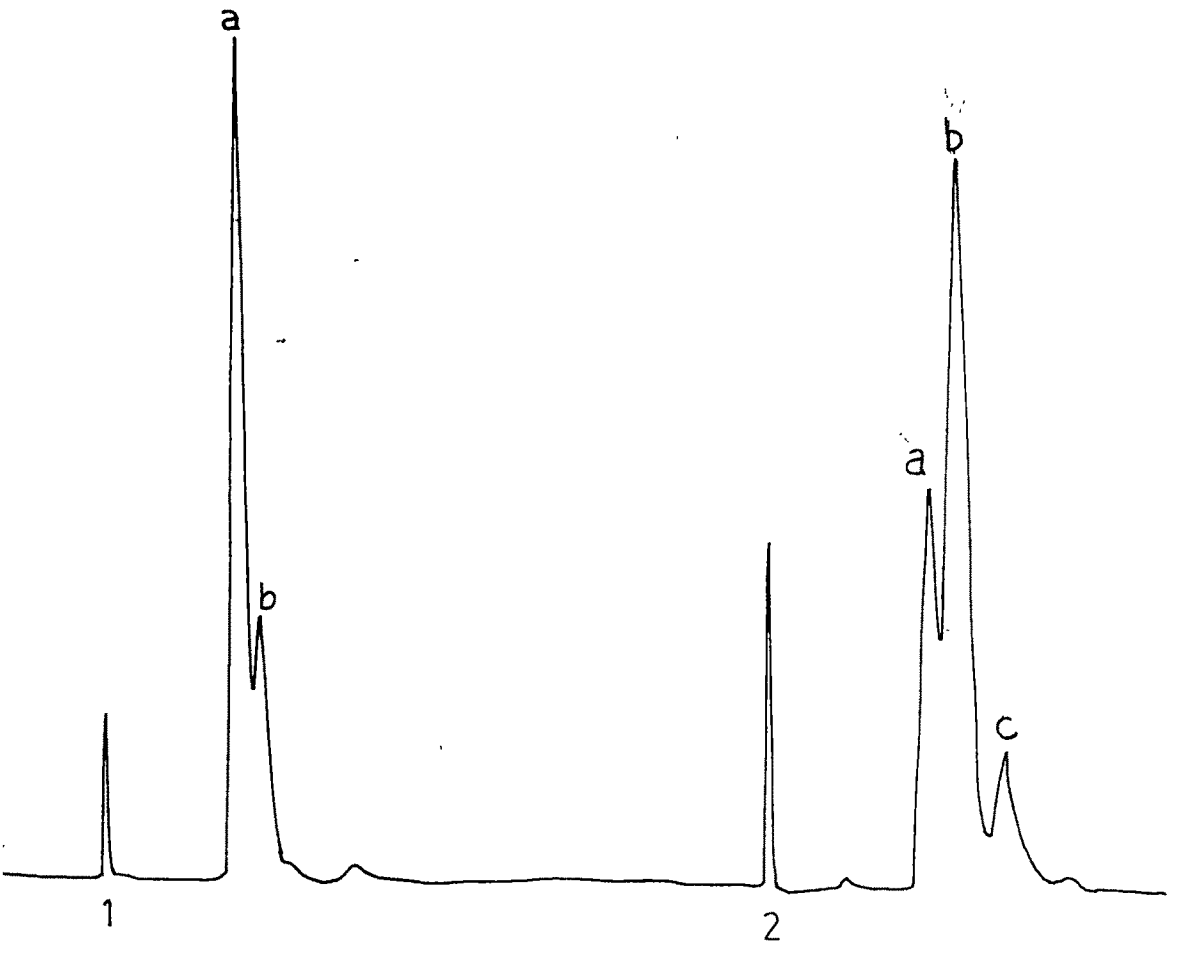
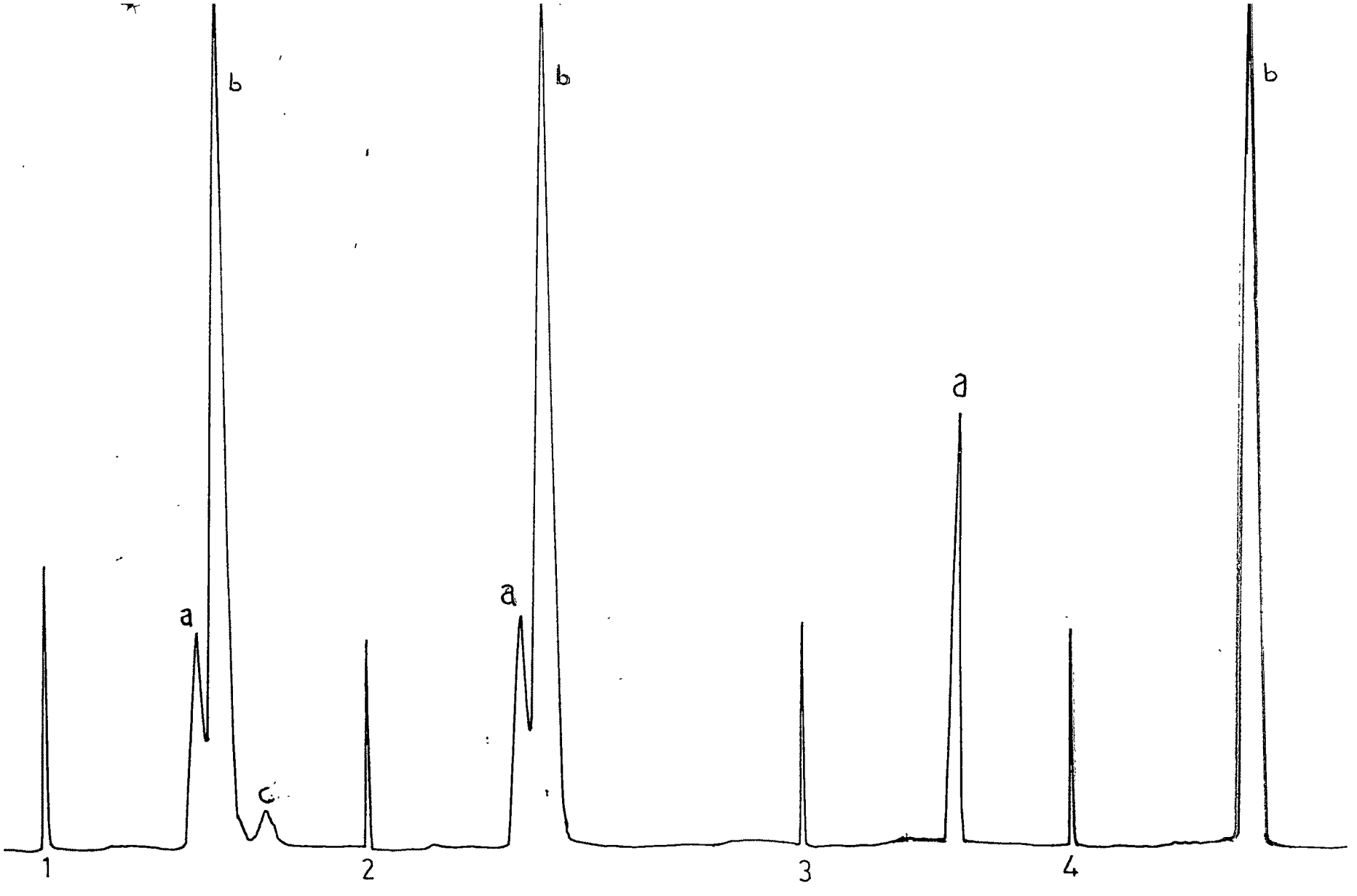


FIG. 8 : ^{1}H-NMR SPECTRUM OF (+)-LONGIPINENE(1)



Column: 10% CW 20M (360 cm x 0.6 cm, Al- column)
Hydrogen flow: 60 ml/min, Temp.: 150°
a) Longipinene, b) Longicyclene
d) Longifolene

Fig. 9. GLC OF 1) POOL No. 3 and 2) POOL No. 5



Column: 10% CW 20M (360 cm x 0.6 cm, Al.column)
Hydrogen flow: 60 ml/min, Temp. 150°
1) Pool No. 4, 2) Pool No. 4 after PAA treatment
a&3) Cyclosativene, b&4) Longicyclene, c) Longifolene

Fig. 10. GLC OF POOL No. 4 BEFORE AND AFTER PAA TREATMENT

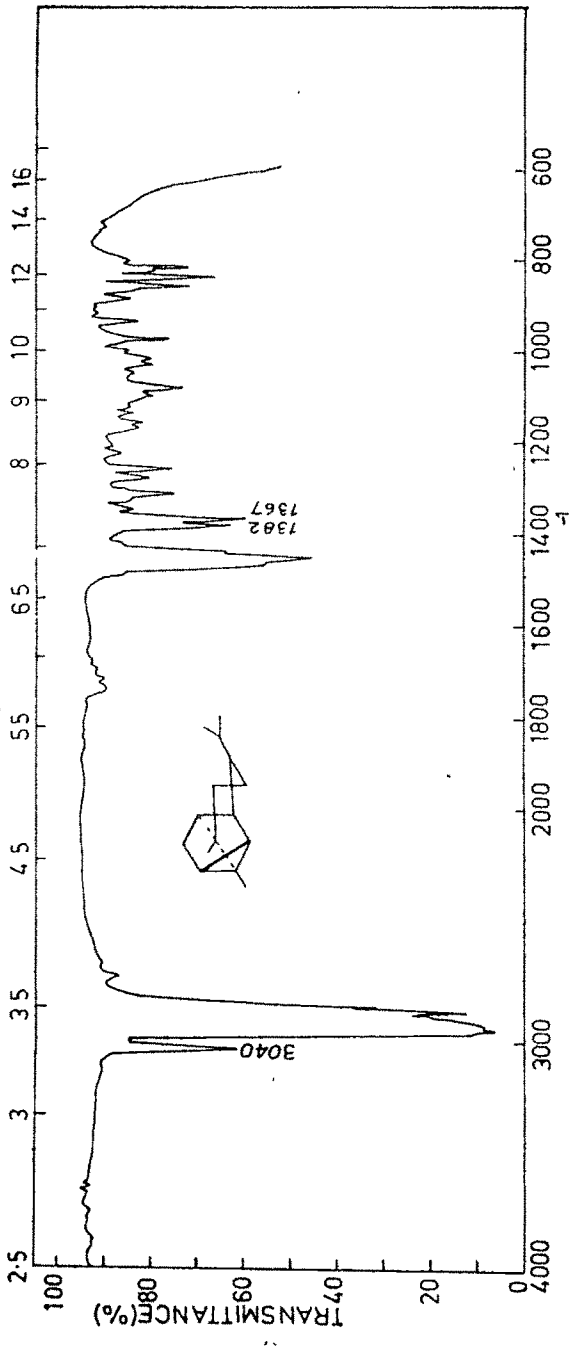


FIG.11 : IR SPECTRUM OF (+)-CYCLOSATIVENE(3)

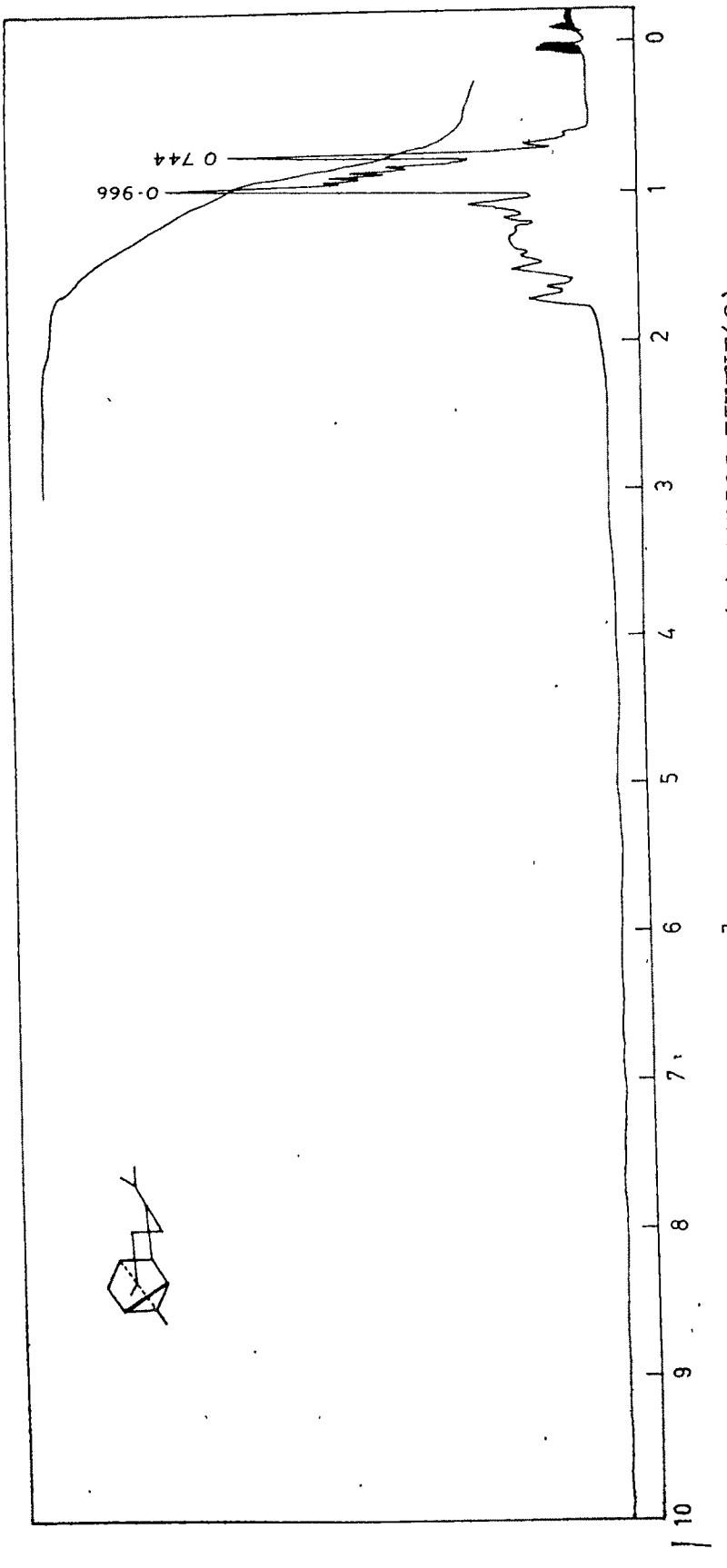


FIG.12 : ^{1}H-NMR SPECTRUM OF (+)-CYCLOSATIVENE(3)

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