C H A P T E R - II

MODELLING OF POTENTIAL INSECTICIDES ON SOME ACTIVE ESSENTIAL OIL COMPONENTS

ABSTRACT

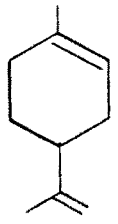
A maiden report is presented for the insecticidal substance - elemicin, obtained from the essential oil of Cinnamomum cecidodaphne.

Oil of Luvunga scandens showed insecticidal principles to be methyl cinnamate, 4-terpineol, α-terpineol and linalool. This is the first reported occurrence of 4-terpineol in this oil.

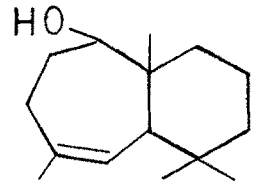
Systematic modelling on linalool for potential insecticides delivered an interesting series of ethynyl carbinols, not so far accounted in the literature, which are much potent than the base molecule towards more resistant pest - Tribolium castaneum.

INTRODUCTION

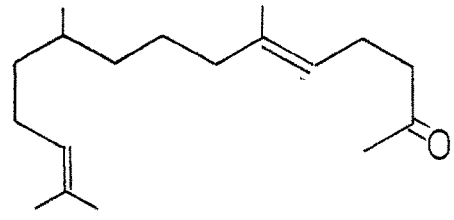
Up to the close of eighteenfifties, 'Essential Oils' were, in large part, unknown for their multitude of biological properties and, occupied a place of preeminence in cosmetic preparations[1] only though,insect repellent activity of the oil of citronella[2] has been known since then. Today, the concepts describing essential oils like, 'Quinta essentia' by Paracelsion,[3] have proved to be precursors to our present knowledge of their biological role, which has led to a steady accretion of information concerning their constitution[4] (Fig. 1).



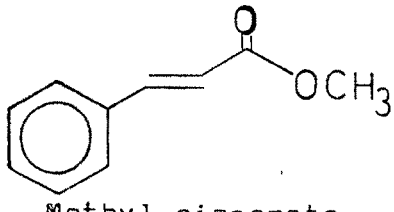
Limonene
(Monoterpenoid)



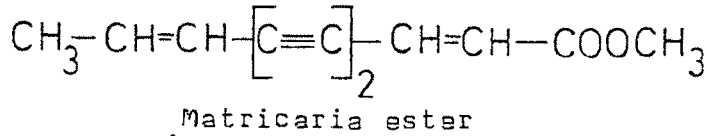
Allohimachalol
(Sesquiterpenoid)



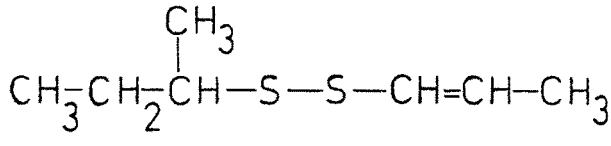
Farnesyl acetone
(Diterpenoid)



Methyl cinnamate
(Phenyl propanoids)



Matricaria ester
(Alkane derivatives)



Disulphide

Fig. 1: Some essential oil constituents

Now it has become increasingly clear that these 'so-called' secondary metabolites, many of them emerging out from mevalonic acid derived cascade, are metabolically active.[5] In spite of their defensive nature and instant synthesis against herbivory[6], there is a view of these being simply an overflow of primary metabolism.[7]

That the genesis of essential oils divulges the role in plant maintenance, it is prudent to consider a possible delivery of the insecticidal lead from such natural material. It is well-known fact that essential oils demonstrate effects against bacteria and fungi[8a,b] on one hand and on the other some insect ovicidal,[9] larvicidal[10a,b] and insecticidal properties are also well-known.

Recently a comprehensive account[11] regarding the role of essential oils in the insect control has been published. The oils of Lemon, Grapefruit, Lime, Kamquat, Tangerine, Orange and Tangelo;[12] Viburnum japonicum,[13] Juniperus recurva,[14] Acorus calamus,[15] Artemesia absinthium, Pogostemon heyneanus, P. parviflorus and Origanum majorana[16] have been reported to possess insecticidal property against Callasobruchus maculatus, C. chinensis, Sitophilus oryzae, Musca domestica larvae, Drosophila melanogaster larvae, Culex pipiens, Dysdercus koenigii, Stagobium penicum and Tribolium castaneum.

Though insect control with phytochemicals[17] has been known since time immemorial, an impetus in the systematic essential

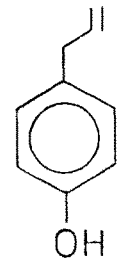
oil screening is a recent one. Some significant investigations are delineated below.

Foliage of Viburnum japonicum inhibited remarkably the growth of Drosophila melanogaster. H. Ohigashi et al.[13] attributed this activity to Chavicol (Fig. 2). Structure activity studies showed analogous compounds like methyl chavicol insecticidal to adults as well.

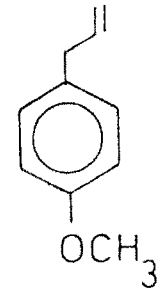
Cedrus deodara Linn has been shown to counter mites[18]. Systematic tracing revealed hydrocarbons α-, β - and Arhimachalene[19] as possible active components (Fig. 2).

Insecticidal activity of Juniperus recurva (heartwood) is due to thujopsene and 8-cedren-13-ol.[14] Laboratory studies on monoterpenes present in Pinus echinata and P. taeda have been found toxic to southern pine beetles (Dendroctonus frontalis Zimmerman) due to limonene.[11] A larvicidal (5E)-ocimenone isolated from Tegetes minuta was found active against Aedes aegypti.[20] Harayama et al. showed a norsesquiterpine acetylene dl-chamaecynone to be active isolate displaying a strong termiticidal action[21] (Fig. 2).

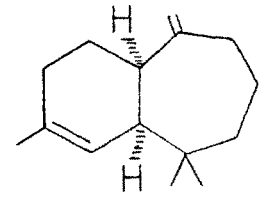
Many pure essential oil components such as camphene, carvacrol, carvone, citral, citronellol, eugenol, farnesol, β-phellandrene have lethal action against houseflies.[22] Activity of some components from essential oils on different insects is summarized[23] in the following table (Table 1).



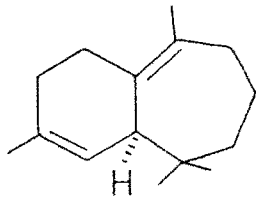
Chavicol



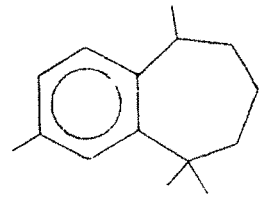
Methyl chavicol



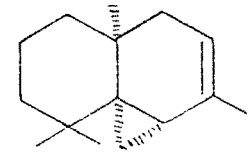
α-Himachalene



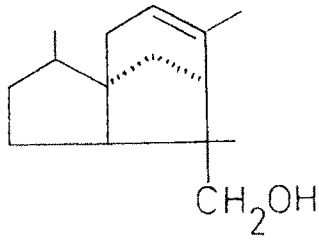
β-Himachalene



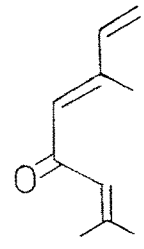
ar-Himachalene



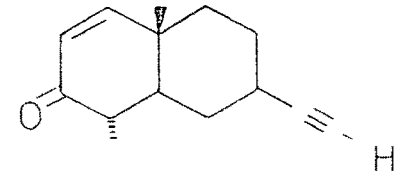
Thujoprene



8-Cedren-13-ol



(5E)-Ocimenone



(±)-Chamaecynone

Fig. 2 : Insecticidal principles from essential oils.

Table 1. Activity of some essential oil components on various insects.

Compound	Dose/insect OR %	Name of the insect tested	Insect stage	Percentage mortality
Cineol	40 µg	Musca domestica	Larva	12.5/62.5
Carvacrol	40 µg	Musca domestica	Larva	36.7/30.0
d-Limonene	40 µg	Musca domestica	Larva	20/33.3
Dihydro chavicol	20mg/3ml medium	Drosophila melanogaster	Larva	100
∠-Cedrene	33.5 µg	Culex pipiens	Adults	50
Cedrol	21.2 µg	Culex pipiens	Adults	50
Benzaldehyde	0.040%	Culex pipiens	Adults	50
Cinnamaldehyde*	0.00037%	Sitophilus oryzae	Adults	50
Eugenol*	0.0026%	Sitophilus oryzae	Adults	50

* Very low concentration of cinnamaldehyde and eugenol to kill S. oryzae adults is probably due to different bioassay procedure where direct glass surface contact method has been used. Such method usually blocks the respiratory system.

Not only of the academic interest but also the practical use of some essential oils has been proposed for controlling green house pests.[24] Myzus persicae and Trialeurodes vaporariorum, especially due to increasing resistance to insecticides. Amazingly enough insecticidal preparations using essential oils as second component with pyrethrum are found superior.[25] They are also blended in slow release insecticidal compositions.[26]

Retrospectively, although continued efforts to elicit new insecticidal molecules from nature and their modification programme is well-underway, Yet they have found limited applications in dairy, mosquito control and on stored grain pest.[27] Literature still awaits an efficient lead for the latter. It is well-known that the postharvest pest management is very crucial in view of the projected losses of 45 million tonnes[17] (valued at about 6 billion US dollars) only in cereals by 1985. The incidence is alarmingly devastating near tropical regions like India, where high temperature and humidity favours the growth of organisms.

Juxtaposing above facts our present endeavour has been confined to the control of stored grain pests by modelling of potential insecticides on natural leads obtained from the essential oils.

STRATEGY

In a bid to demonstrate the possibility of 'insecticide modelling on nature', a strategy was designed in two phases.

1. A preliminary screening of variety of essential oils against stored grain pest was undertaken followed by rapid unravelling of active oils for insecticidally active components by sequential bioassay, separation and identification programme.

2. Second phase dealt with the recognition of critical structural features in the active components for further modification and enhancement in the bioefficacy of these phytochemicals.

RESULTS AND DISCUSSION

1. Insecticidal components from essential oils

In all 33 essential oils belonging to 17 plant families were biologically evaluated for the insecticidal activity. Oils were obtained from the botanically identified plant material using modified clevenger apparatus.[28] Further processing furnished dry solvent free oils ready for entomological testing.

Bioassay was carried out employing Sitophilus oryzae (Rice weevil) and Tribolium castaneum (Red flour beetle)[29] as test insects, so as to get authentic and consistent results.[30] Likewise.recent interest in the pest control with preservative gas atmosphere[31] is in tune with the 'filter paper evaluation method' adopted here. All essential oils were tested at 0.39 mg/ cm^2 Concentration.Data is tabulated below (Table 2).

Table 2. Activity of some essential oils against S. oryzae and T. castaneum.

Entry No.	Name of the Plant	Family	Activity
1	Artemisia pallens	Compositae	-
2	Ageratum conyzoides	Compositae	-
3	Boswellia serrata	Burseraceae	-
4	Cinnamomum cecidodaphne	Lauraceae	+
5	Cinnamomum zeylanicum	Lauraceae	+

Table 2 continued

6	Callistemon lanceolatus	Myrtaceae	-
7	Cuminum cyminum	Umbelliferae	-
8	Cedrus deodara	Pinaceae	-
9	Coleus aromaticus	Labiatae	+
10	Dipterocarpus indicus	Dipterocarpaceae	-
11	Dipterocarpus pilosus	Dipterocarpaceae	-
12	Daucus carota	Umbelliferae	-
13	Eugenia caryophyllata	Myrtaceae	+
14	Eucalyptus citriodora	Myrtaceae	-
15	Foeniculum vulgare	Umbelliferae	-
16	Hyptis suaveolens	Labiatae	-
17	Juniperus species	Cupressaceae	-
18	Luvunga scandens	Rutaceae	+
19	Myristica fragrans	Myristicaceae	-
20	Moschosma polystachyum	Labiatae	-
21	Murraya koenigii	Rutaceae	-
22	Mangifera indica	Anacardiaceae	-
23	Murraya exotica	Rutaceae	-
24	Ocimum basilicum	Labiatae	+
25	Ocimum canum	Labiatae	+
26	Pogostemon patchouli	Labiatae	-
27	Santalum album	Santalaceae	-
28	Skimmia laureola	Rutaceae	-
29	Seseli indicum	Umbelliferae	-
30	Thuja orientalis	Pinaceae	-

Table 2 continued

31	Tagetus erecta	Compositae	-
32	Vaterao indica	Dipterocarpaceae	-
33	Zingiber zerumbet	Zingiberaceae	-

The oils of Cinnamomum cecidodaphne, Cinnamomum zeylanicum, Coleus aromaticus, Eugenia caryophyllata, Luvunga scandens, Ocimum basilicum and Ocimum canum exhibited insecticidal potential. Out of these C. Zeylanicum, E. caryophyllata, O. basilicum and O. canum were single component rich oils containing eugenol (84%), eugenol (90%), methyl chavicol (87%), and linalool (80%) respectively, which are known to be insecticidal against various insect species of stored grain pest.[16,32] While eugenol has a LD_{50} of 0.0026% against S. oryzae; methyl chavicol shows LD_{50} of 0.0084%. However, the latter has median lethal concentration of 0.0625% against Tribolium castaneum as well.[32] Although C. aromaticus oil was rich in carvacrol (60%), yet in the absence of its biological efficacy against stored grain pests,[33] this oil was subjected to detailed separation and evaluation.

In fact three essential oils were processed further i.e. C. aromaticus, C. cecidodaphne and L. scandens to isolate active components not known as insecticidal against stored grain pests hitherto.

Coleus aromaticus

Essential oil of Coleus aromaticus, on GLC probe (Fig. 3) showed 60% of carvacrol contents (coinjection with the authentic sample; 10% CW, 200°; R_t: 12.2 min.).

It was chromatographed on silica gel to furnish two fractions. The fraction devoid of carvacrol was inactive, however, carvacrol rich fraction (90% glc purity) exhibited insecticidal action. Hence this active compound was further evaluated for LC_{50} values against T. castaneum & S. oryzae (Table 3.).

Table 3. Percentage mortality of S. oryzae and T. Castaneum adults due to carvacrol after 24 hours.

Test insect	% mortality at mg/cm^2			LC_{50} mg/cm^2
	0.078	0.230	0.390	
S. oryzae	50.0	90.0	100.0	0.078
T. castaneum	40.0	85.0	100.0	0.117

Cinnamomum cecidodaphne

Oil of C. cecidodaphne was subjected to preliminary analytical analysis like chromatoplates and gas liquid chromatography. GLC (10% CW, 200°C) revealed 8 major components 1 to 8 (Fig. 4). Fractionation of the oil was carried out on an adiabatic annular teflon still (80 theoretical plates) which furnished fractions of

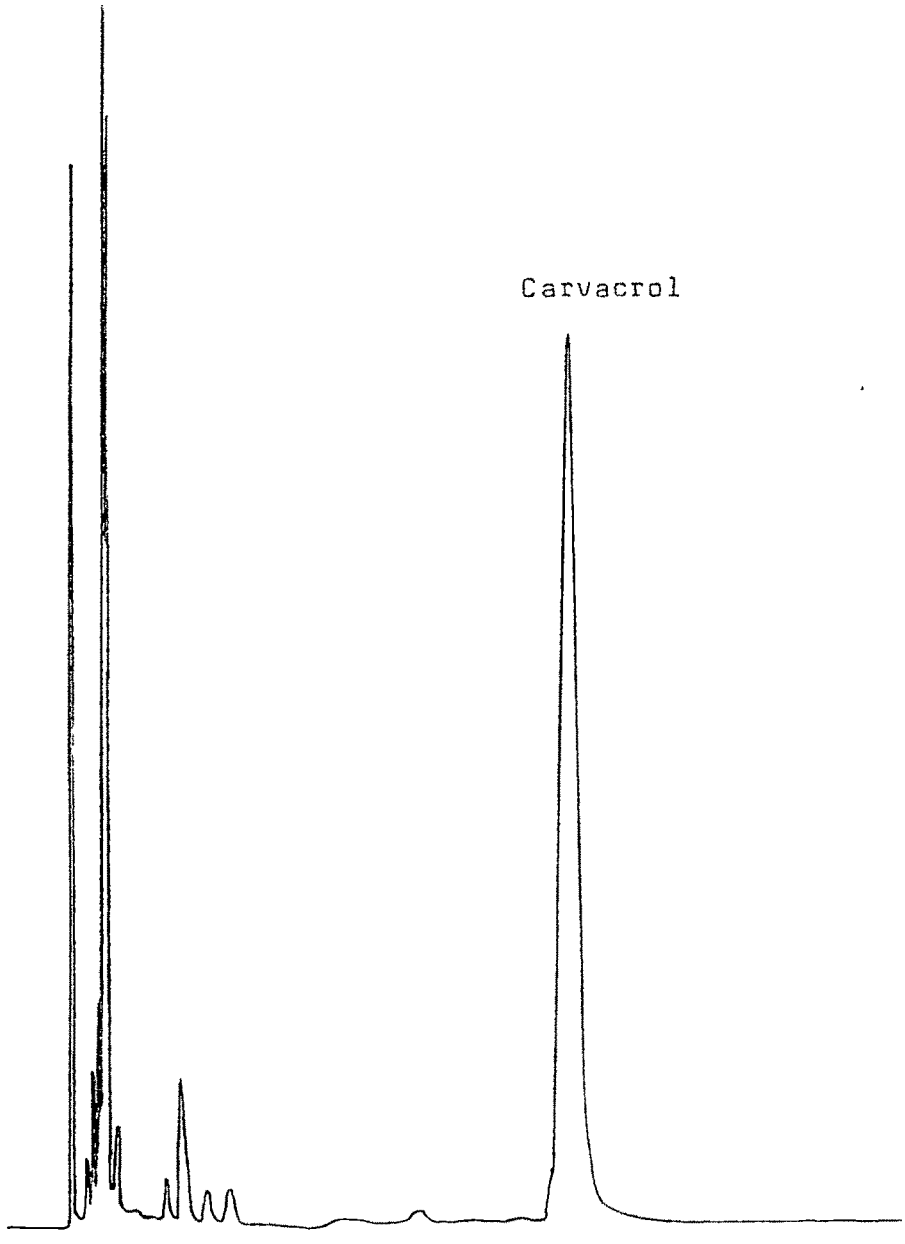


Fig. 3: GLC of the essential oil of Coleus aromaticus

components 1 to 6 (of GLC) in different purities. However, these compounds were inactive against test insects T. castaneum and S. oryzae. Components 7 & 8 which were obtained in 99.0% purity by column chromatographic separation, were found to be active (Table 4).

These two compounds corroborated well with the analytical data of elemicin[34a] and myrisiticin[35a] respectively.

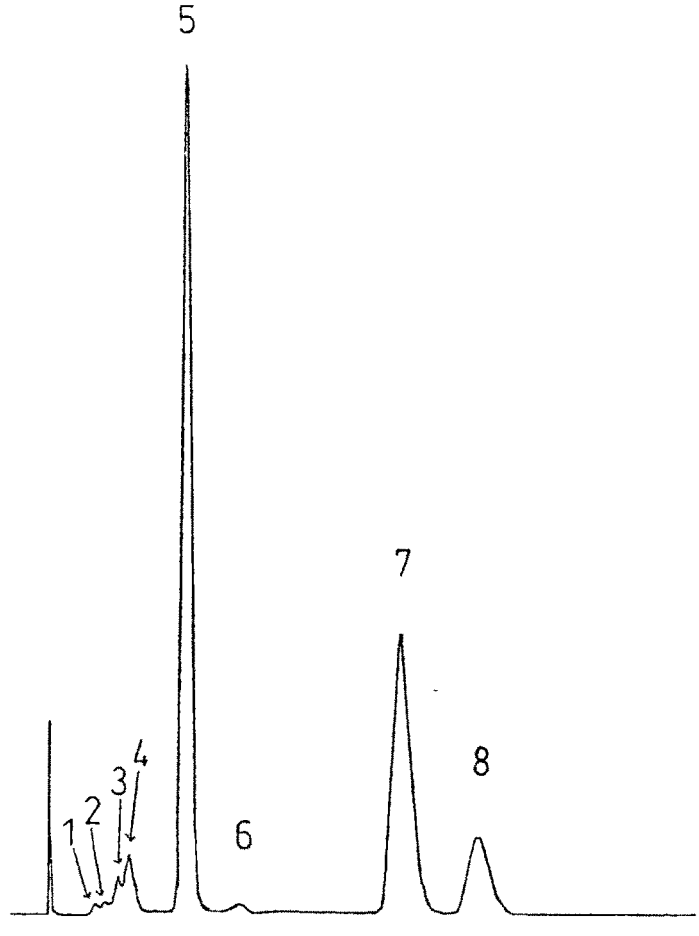


Fig. 4: GLC of the essential of the Cinnamomum cecidodaphne

Table 4. Percentage mortality of S. oryzae and T. castaneum adults due to elemicin & and myrisiticin treatment by contact method

Test insect	Elemicin					Myrisiticin			
	% kill at mg/cm^2				LC_{50} mg/cm^2	% kill at mg/cm^2			LC_{50} mg/cm^2
	0.39	0.31	0.195	0.078		0.078	0.039	0.0195	
T. castaneum	100.0	55.0	30.0	12.0	0.257	100.0	70.0	10.0	0.033
S. oryzae	100.0	60.0	25.0	17.0	0.273	100.0	85.0	15.0	0.028

While myrisiticin was found to be known insecticidal compound for Drosophila sps[36] after it was isolated from Pastinaca sativa L., there was no such record for elemicin.

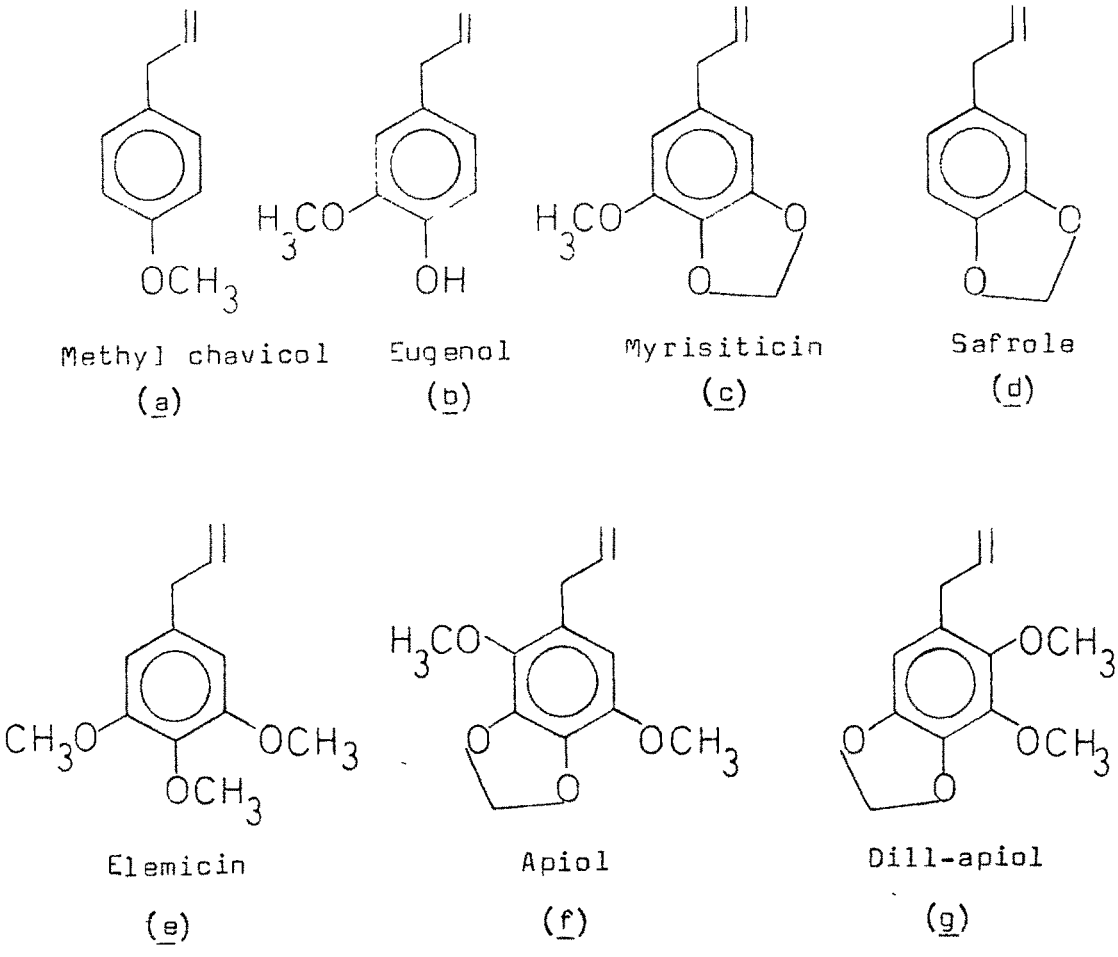
Thus elemicin being a new insecticidal molecule, it was evaluated together with myrisiticin, on other insect species (Table 5). Elemicin although effective on houseflies (Musca domestica), red cotton bugs (Dysdercus koenigii) and cockroaches (Periplaneta americana) was found to require high concentration to induce any kill among these insects species. The compound did not appear to be neurotoxic but cytotoxic, inhibiting some metabolic processes. This was because of the fact that a delayed induction of mortality (after 3 to 4 hr), even by topical application, was observed.

Table 5. Activity of Cinnamomum cecidodaphne oil and its active components on various insects

Insect	Mortality(%) due to essential oil at				Mortality(%) due to Myrisiticin at				Mortality(%) due to Elemicin at				
	1.0%	2.0%	3.0%	4.0%	0.5%	1.0%	2.0%	4.0%	0.5%	1.0%	2.5%	3.0%	4.0%
Musca domestica	-	-	56[a]	100[a]	-	3.3[a]	13.0[a]	21.0[a]	-	15.0[a]	100[a]	-	-
	40[b]	58[b]	80[b]	100[b]	-	-	20.0[b]	23.0[b]	-	10.0[b]	70[b]	100[b]	-
Periplanata americana	-	-	10[c]	75[c]	-	-	-	12.5[c]	-	-	10[c]	80[c]	100[c]
Dysdercus koenigii	20[d]	100[d]	-	-	-	-	-	-	13.3[d]	90[d]	-	-	-
Tribolium castaneum	100[d]	-	-	-	100[d]	-	-	-	-	12[d]	30[d]	-	-
Sitophilus oryzae	100[d]	-	-	-	100[d]	-	-	-	-	17[d]	25[d]	-	-
Bruchus chinensis	100[d]	-	-	-	100[d]	-	-	-	-	11[d]	20[d]	-	-
Trogoderma granarium	100[d]	-	-	-	47.5[d]	100[d]	-	-	0.3[d]	3.3[d]	12.5[d]	-	-

a = Topical application (1 µl/fly); b = direct spray (peet grady 2 ml/2.48 M^3); c = direct spray; d = contact toxicity (48 h)

The activity of elemicin appears to be interesting in view of so far incompletely accounted sequence of biogenetically close active allyl benzenes like methyl chavicol(a)[13], eugenol (b)[16], myrisiticin (c),[36] safrole (d)[13], apiol (f) and dill-apiol(g)[35a] (Fig. 5), effective in insect control. This would help in rationalizing 'structure activity relationship' of related bioanalogues.



Methyl chavicol (a) | Eugenol (b) | Myrisiticin (c) | Safrole (d)

Elemicin (e) | Apiol (f) | Dill-apiol (g)

Fig. 5: Biologically active allyl benzenes

Component 5 (Fig. 4), though not active, was identified as safrole, just for the curiosity. Though safrole is known to exhibit larvicidal activity,[13] it was inactive against the above stored grain pest.

Thus it could be concluded that the insecticidal activity of the oil of Cinnamomum cecidodaphne was due to active components, elemicin and myrisiticin.

Luvunga scandens

Essential oil of Luvunga scandens at 0.39 mg/cm^2 revealed its insecticidal action against Sitophilus oryzae and Tribolium castaneum with a kill of 80% and 100% respectively. At 0.23 mg/cm^2 only 10% of Sitophilus and 40% Tribolium were killed. This oil though used for curing various ailments[37] has not been known for the insecticidal activity so far.

It was evident at the out set, from the gas chromatogram that the oil is a blend of 29 components (GLC, Fig. 6). This deleted simple separation methodology. Therefore by column chromatography on neutral alumina (grade III),[38] broad separation into i) hydrocarbons, ii) esters, iii) alcohols and iv) acids was effected using step gradient elution. Separation profile was continuously monitored by IR spectrophotometer. These fractions A,B,C,D,E & F were evaluated further for the activity to give following data (Table 6).

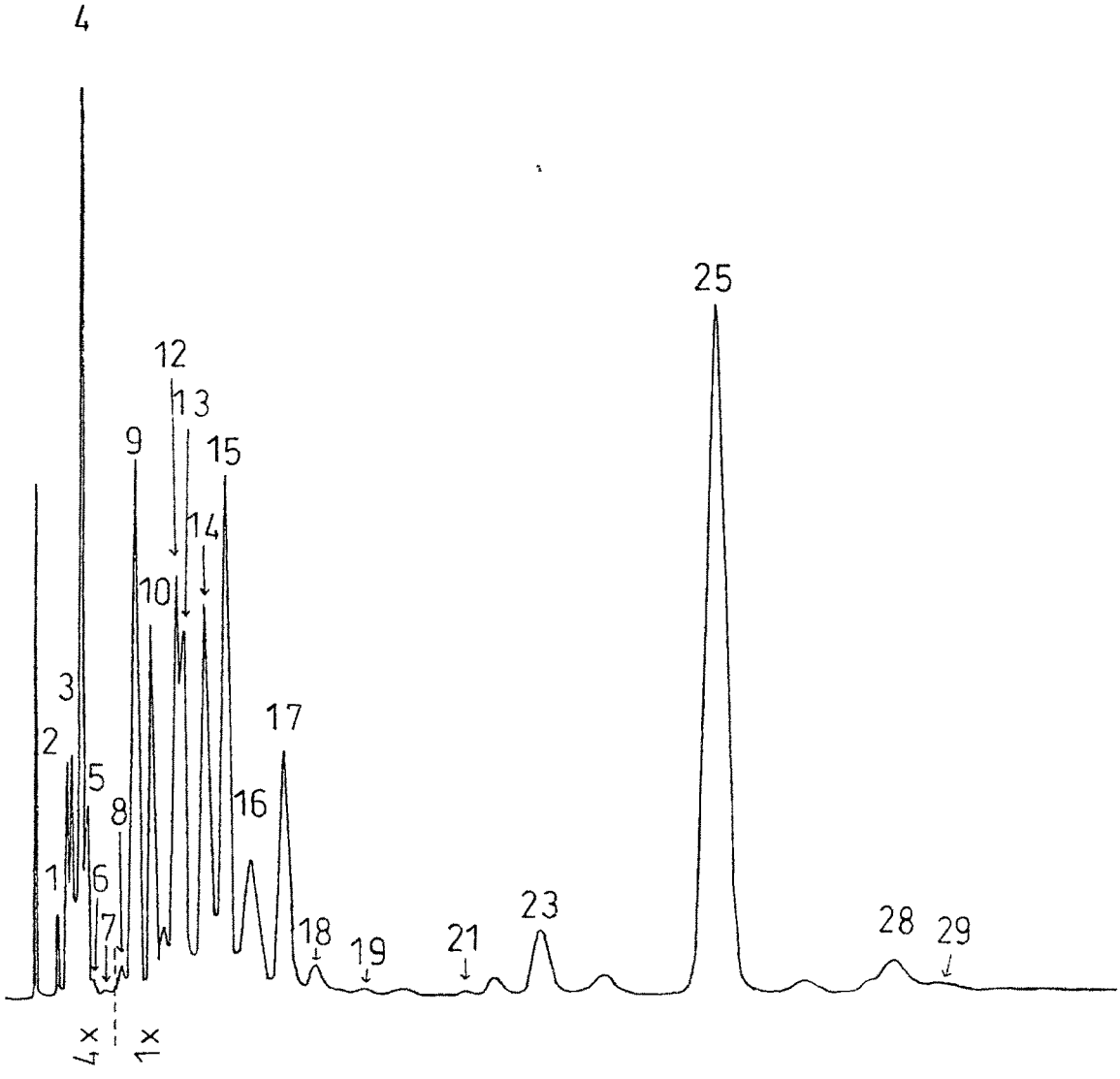


Fig. 6: GLC of the oil of Luvunga scandens

Table 6. Percentage mortality due to sub-fractions of *L. scandens* oil

Fraction	% mortality at 0.39 mg/cm^2		% mortality at 0.23 mg/cm^2	
	Sitophilus oryzae	Tribolium castaneum	Sitophilus oryzae	Tribolium castaneum
A	00	00	00	00
*B	100	100	90	90
*C	95	00	30	00
*D	45	00	5	00
E	00	00	00	00
F	20	00	00	00

Fractions B,C,D were the most active fractions.

The activity of fraction B was attributed to component 25 (~47% of the fraction) identified as methyl cinnamate[39] by the analytical data. Authentic sample preparation and coinjection extended confirmation. Methyl cinnamate is already known for its activity against *Sitophilus oryzae* and *Tribolium castaneum* at lower concentrations.[32]

Fraction C, which was an alcohol rich fraction (IR 3460 cm^{-1}), could induce mortality among *Sitophilus oryzae* alone. GLC

displayed two major components 9 (32%) and 13 (28%). Column chromatographic separation and analytical probe showed compound 9 to be linalool.[40] However, 13 could be obtained only by preparative GLC. Analytical data of this component was in perfect accordance with the literature data of 4-terpineol.[41] Confirmation of the above compounds linalool and 4-terpineol came by coinjection with the authentic samples. Residual fractions, after isolation of the above components, was not insecticidal in nature. These isolates were active against S. oryzae as was the parent fraction (Table 7).

Fraction D, a comparatively less effective fraction showed two isothermal peaks on glc, as major constituents, computing with 9 and 15. Component 9 was previously identified as linalool. Compound 15 obtained by column chromatographic purification matched with the spectroscopic data of α-terpineol.[42] Pooled fraction devoid of 9 and 15 failed to show insecticidal response. α-Terpineol was lethal to Sitophilus oryzae (Table 7).

Table 7. Percentage mortality due to linalool, 4-terpineol & α-terpineol

Insect	% Mortality due to linalool at		% Mortality due to 4-terpineol at		% Mortality due to α-terpineol at	
	0.39 mg/cm^2	0.23 mg/cm^2	0.39 mg/cm^2	0.23 mg/cm^2	0.39 mg/cm^2	0.23 mg/cm^2
Sitophilus oryzae	80	70	80	40	70	0
Tribolium castaneum	0	0	0	0	0	0

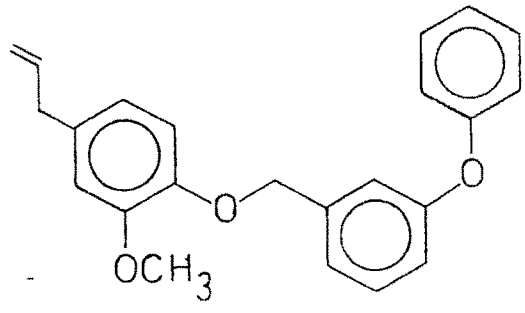
Thus gradual unfolding of essential oil of L. scandens revealed active principles to be methyl cinnamate, linalool, 4-terpineol and ∝-terpineol. These monoterpene alcohols were effective only on Sitophilus oryzae. Though linalool and ∝-terpineol are known contributors to the oil,[43] this is the first reported occurrence of 4-terpineol. Linalool was considered to be an effective material in controlling S. oryzae as the same component was found in O. canum as well, which was insecticidal to S. oryzae adults.

2. Modelling on Linalool

The key concept in our present approach was the recognition of structural preferences in the insecticidal leads, which could be suitably transcribed and manipulated further towards enhancement in the activity. The work in this direction forms the subject matter of this section.

From the active molecules obtained during activity screening elemicin- as a new insecticidal lead was processed further for 'modelling on nature' in our laboratory[44] and delivered compound H, showing ten-fold enhancement in the activity.

As the main concern right from the inception of this work resided in the discerning observation, for all terpenoid alcohol leads linalool, 4-terpineol and ∝-terpineol were singularly active against Sitophilus oryzae. Here it was considered challenging



H

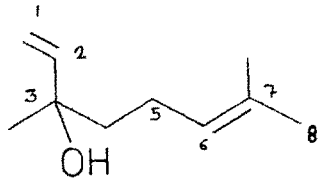
4-(m-Phenoxybenzyloxy),3-methoxy-allyl benzene

to understand the problem and modify one of these leads so as to render it effective on comparatively resistant Tribolium castaneum. For this purpose linalool was deemed to be a suitable base molecule, in view of

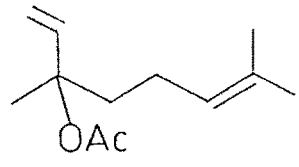
i) the ready accessibility as it is a main constituent in several essential oils,[45] and

ii) recent impetus in utilizing this perfumery molecule in the formulations used for insect repellency.[46a,b]

It is an established fact that the insecticidal response is an extremely delicate function of chemical and stereochemical changes, as only the specific conformational fit at the receptor is responsible to trigger the desired action.[47] Hence for identifying features relaying insecticidal response in linalool, sequential bioassay programme was sketched.



Linalool
(1)

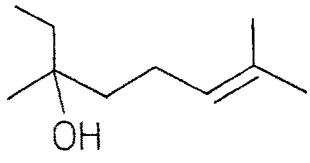


Linalyl acetate
(2)

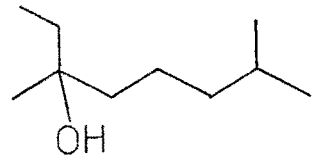
Linalool (1) has a typical terminal olefinic linkage in its acyclic monoterpenoid skeleton with a tertiary allylic hydroxyl function. It was presumed that the insecticidal action of linalool could be attributed either to 1) tertiary-OH function at C-3, ii) electron rich segments at C-1 and C-6 or iii) the basic carbon skeleton. Striking similarity in 4-terpineol, α-terpineol and linalool is a tertiary-OH group. Its role in the insecticidal action could be evaluated by testing authentic sample of linalyl acetate (2). Experiment showed a definite drop in the activity (Table 8) asserting strategic importance of a free hydroxyl function. This is understandable, particularly in terms of better molecular docking on the possible hydrophilic sites via H-bonding[48] as shown in some enzymatic inhibitory processes.

The effect of terminal olefinic segment at C-1 could be evaluated by making 1,2-dihydrolinalool (3) by selective hydrogenation of linalool over P-1 nickel boride catalyst. Compound (3) exhibited equivalent activity as that of linalool (Table 8). This led to destroy unsaturation of C-6 as well.

Hydrogenation of linalool using 10% Pd/C afforded tetrahydrolinalool (4).[49]

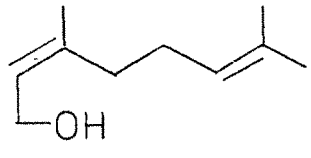


(3)

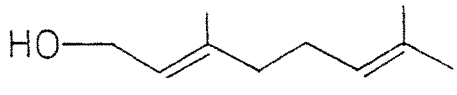


(4)

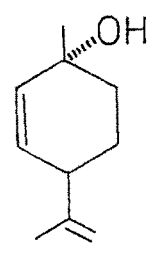
Compound (4) did not cause significant perturbation in the activity. Thus olefinic linkages at C-1 and C-6 did not exert any significant difference and pest showed similar preference towards linalool (1), 1,2-dihydrolinalool(3) and tetrahydrolinalool (4). This helped us to believe that the site of action is not specific enough. If it is so then some other complementary models of allylic alcohols also should work as linalool. The problem was urgent and called for an immediate solution. Hence some other monoterpenic allylic alcohols, two of them acyclic primary, nerol (5), geraniol (6) and a monocyclic tertiary, cis-p-mentha-2,8-diene-1-ol (7) were evaluated.



(5)



(6)



(7)

All of them failed to show activity even at 0.39 mg/cm^2 (Table 8). This disproved our earlier contention. Conceivably enough, site of action may not be sufficiently evolved to involve better understanding of stereoelectronic factors, but exhibits a definite preference for stereoconformational computation with linalool skeletal backbone, embodying toxic action.

Table 8. Percentage mortality due to compounds (1) to (7)

Compd. No.	Compound	% Mortality of *S. oryzae* at		% Mortality of *T. castaneum* at	
		0.39 mg/cm^2	0.23 mg/cm^2	0.39 mg/cm^2	0.23 mg/cm^2
1	Linalool	80	70	0	0
2	Linalyl acetate	60	30	0	0
3	1,2-dihydrolinalool	80	60	0	0
4	Tetrahydrolinalool	80	80	0	0
5	Nerol	0	0	0	0
6	Geraniol	0	0	0	0
7	*cis*-p-Mentha-2,8-diene-1-ol	0	0	0	0

Having reached at the basic skeleton our main concern was the ineffectiveness of these compounds on *Tribolium castaneum*.

For a long time we were intrigued with this problem. With this prime aim, extensive literature survey was carried out.

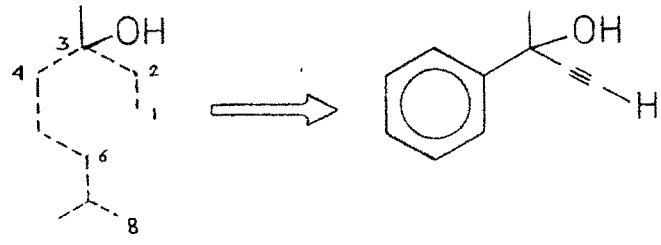
Keen observation of the previous efficacy data[50] on stored grain pest led to an empirical observation, that many aromatic compounds like methyl chavicol, methyl cinnamate, elemicin, myrisiticin, carvacrol were effective on Tribolium castaneum. In view of this it was thought that the receptor site in T. castaneum may demand, in particular, electron rich centres in the molecule for proper anchorage with the cationgenic sites, thereby offering a possible blockade in the electron transport.[51] As well, some enzyme inhibitory processes have been shown to incorporate 'charge transfer complex sites' (CTC binding sites), provided by the aromatic nucleus,[48] entering into pi-pi complexation. Ethynyl linkages also would have similar effect.

Literature on acetylenes revealed termiticidal compound chamaecynone.[21] Some diacetylenes[52] are also known to exhibit insecticidal properties. Likewise, in the segment studies of pyrethrin I, some ethynyl linkages have displayed enhancing effect in segments A & C.[53]

For examining these two concepts a model compound (8) was prepared in which

i) electron flow was injected by shunting of phenyl sextet at C-3 in tetrahydrolinalool (4), deleting C-4 to C-8 segment, and

ii) displacing C-2 linkage by ethynyl terminal.



(4) (8)

Here our intention of keeping intact methyl group on the carbinol carbon and tertiary -OH function is easily understandable. Compound (8) could be prepared from methylphenyl ketone and acetylide salt from dry purified acetylene.

Strategy for constructing this new C-C bond clearly involves abstraction of acidic acetylenic proton by a base to form corresponding acetylide. The carbanion thus formed attacks cationic carbonyl carbon to give ethynyl carbinols. For generating acetylides, bases like $NaNH_2$/liq. NH_3,[54] fused KOH,[55] dimisyl sodium[54] ($CH_3SO_2\bar{C}H_2Na^+$) were extensively used. When methyl phenyl ketone was exposed to sodium acetylide prepared in situ, using sodium metal in liq. NH_3, even after 24 hrs significant quantity of starting material could be detected (GLC monitor). Prolonged reaction time did not exert significant effect.

For this reaction to occur prime requisite is the facile attack of generated carbanion on the keto function. In fact

in the above case carbanion species formed in the medium gets highly solvated[56] due to the large excess of liquid ammonia- a protic solvent, and is expected to be encumbered to find a rapid access to the Sp^2 carbon. Low temperature (-33^oC) also would have a detrimental effect on the progressive reaction. Though fused KOH method appears to circumvent this problem, it is inconvenient for the small scale preparations.

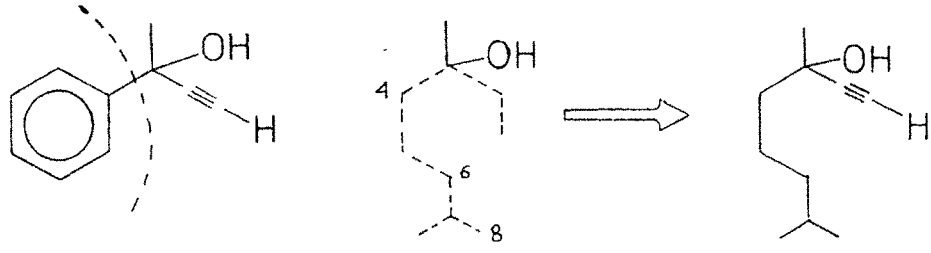
However, when the reaction was carried out using potassium t-amylate as base in dry ether at -15^oC, clean product was obtained in 8 hr (80% yield). In 1935 Gould et al.[57] used this base for condensation of acetylene with β-ionone and tetrahydroionone. Since then only few reports appeared in the literature[58] for the preparation of similar molecules. Hence a typical method was developed and all ethynyl carbinols described here after were prepared accordingly. This base is inherently stronger than other alkoxide bases.

^{1}H-NMR of (8) showed diagnostic singlet at 2.47δ (1H) for acetylenic proton. Strong absorption in IR spectra at 3280 cm^{-1} (C-H stretch) and 2110 cm^{-1} (C≡C stretch support the desired product. Other analytical details are concurrent with the literature data.[59]

Bioefficacy of (8) definitely showed improvement in the activity than the base molecule linalool, though still active only on S. oryzae. It was 100% lethal even at 0.23 mg/cm^2 and

exhibited 30% kill at 0.08 mg/cm^2 showing activity relative to linalool to be 201.0 (Table 9). However, Pyrethrum oleoresin was almost twice active than this compound.

At this point it was considered worthwhile to replicate C-4 to C-8 segment of tetrahydrolinalool (4) by deporting aryl group in (8) (Fig. 7).



(8) (4) (9)

Fig. 7

This led to 3,7-dimethyl-1-octyn-3-ol (9)-dehydrodihydrolinalool. Compound (9) was prepared by acetylene addition to 6-methylheptan -2-one.[58a] Ketone was obtained by retrograde aldol procedure[60] from citral followed by hydrogenation[61] on 10% Pd/C (Ref. 61: uses 10% Pd supported on $BaSO_4$. Other conditions were identical; Fig. 8).

A significant spurt in the activity was observed. Mortality for resistant Tribolium castaneum increased seventy-fold responding conceptually, to the electrophilic face at the site

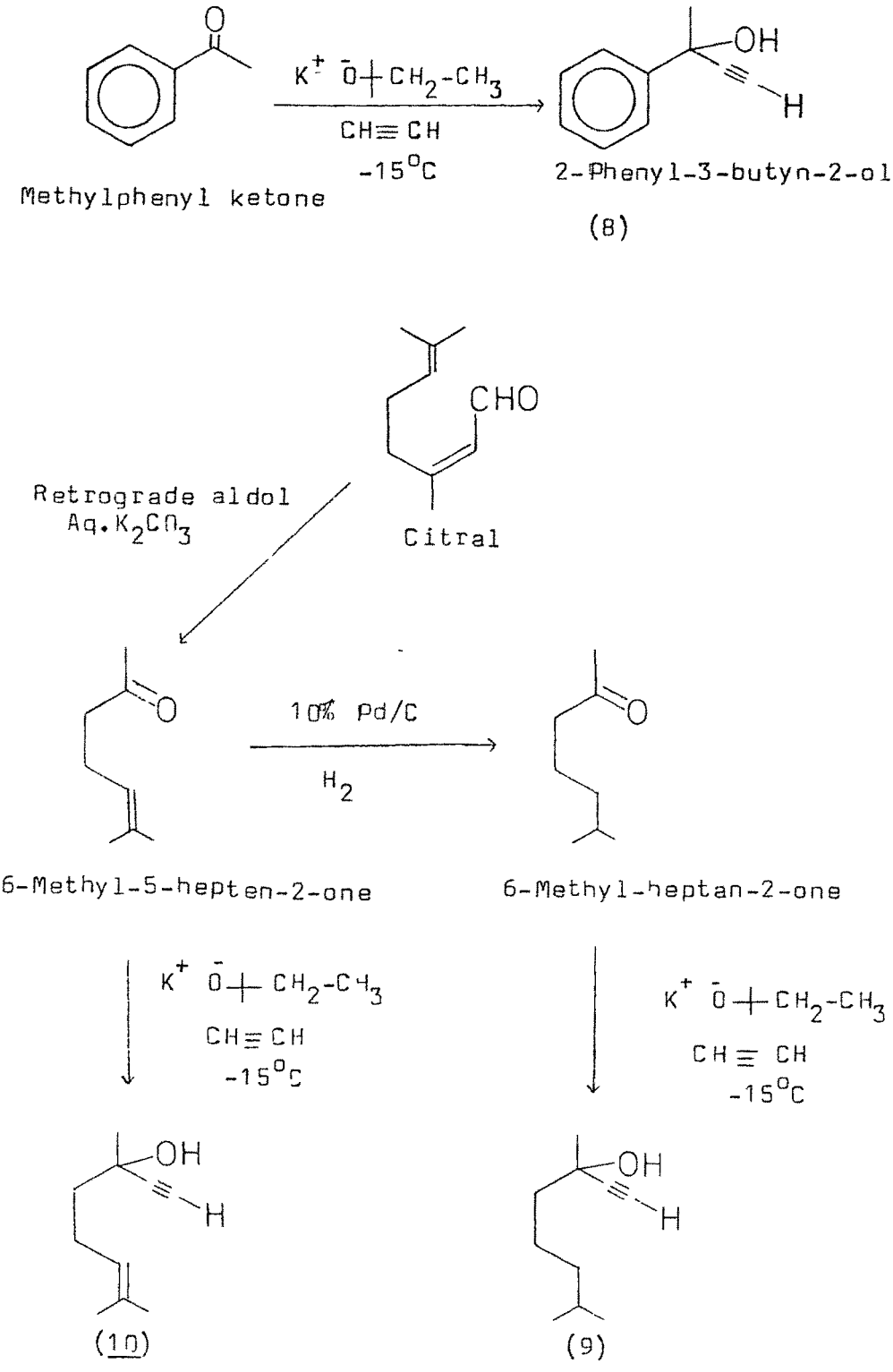


Fig. 8: Scheme for compounds (8), (9) and (10)

of action, retaining its effectiveness against Sitophilus oryzae (Table 9). The compound (9) emerged out to be strategic in the present sequence. Hence on similar lines compound (10)-dehydrolinalool (3,7-dimethyl-6-octen-1-yn-3-ol) was prepared (Fig. 8), in which C-4 to C-8 segment comes from linalool. Bioassay of (10) showed slight degradation in the activity (Table 9).

Thus, systematic study could render the molecule active on both the pest insects. We were obviously influenced by our approach in having rationalized terminal ethynyl carbinol skeleton. Entomological logic would now dictate optimization in terms of lipophilicity. During this time a literature note on the lipids from Tribolium castaneum[62] described large contribution by C_{16} to C_{18} acids. Total hydrocarbons as well show n-alkanes (C_{25} to C_{31}), 3-methyl alkanes (C_{26} to C_{32}) and internally branched mono- and dimethyl alkanes C_{27} to C_{32} and C_{29}-C_{31} respectively. Similarly, lipid examination of Sitophilus oryzae has shown[63] predominance of long chain fatty acids. Palmitic and oleic acids comprising 70%. Polyunsaturated C_{18} acids were also selectively deposited in the phospholipid fraction.

This instigated an idea of increasing carbon chain for better lipohilicity to ensure smooth penetration through cuticle. This would further give a better idea regarding probable mode of action(systemic or respiratory blockers etc.). These

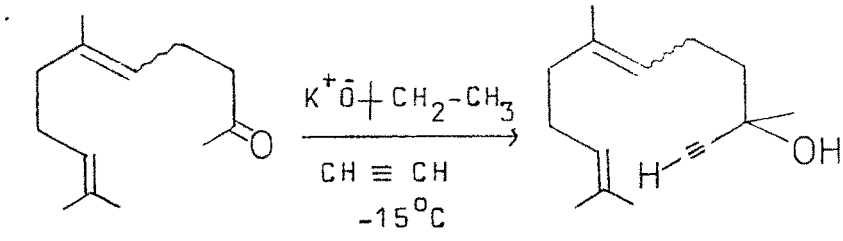
studies of C_{10} molecules were further extended to sesqui- and diterpenic ethynylcarbinols by keeping intact HO-C(CH_3)-C≡C-H segment. Acetylenes - dehydronerolidol (12) and dehydroisophytol(13) were prepared by a typical acetylene addition procedure (Fig. 9). These were evaluated together with the authentic samples of sesqui-and diterpenic alcohols, nerolidol (14) and isophytol (15) as equivalents of linalool for a comparative profile. This modification had an alleviating effect on the activity. All these compounds (11) to (15) were totally inactive.

Hence, in order to optimize lipophilicity, ethynyl carbinols of lower carbon number were synthesized. 1-Ethynyl cycloheptanol (16) (C_9) and 1-ethynyl cyclohexanol[58b] (17) (C_8) (Fig. 9) were effective against both the insects (Table 9).

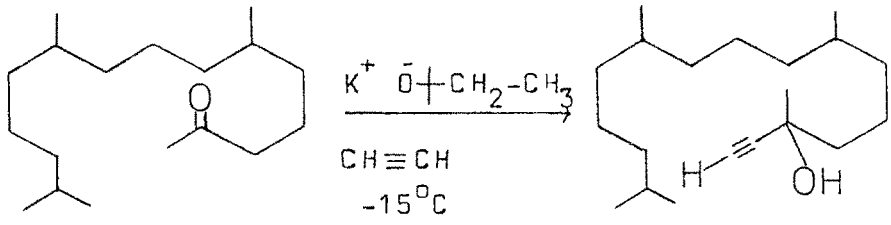
1-Ethynyl cyclohexanol, was the most active compound in the series and seems to have optimum lipophilicity. The rigid ring also may have some role to play. Further manipulations regarding the carbon number on lower side were not tried, as would have the adverse effect on volatility.

To the best of our knowledge all these ethynyl carbinols (8), (9), (10), (16) and (17) have not been known as insecticidal compounds.

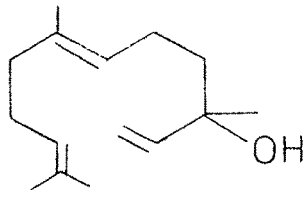
Though present sequence revolves round comparatively less intricate molecular assemblage, the novelty lies in the delivery



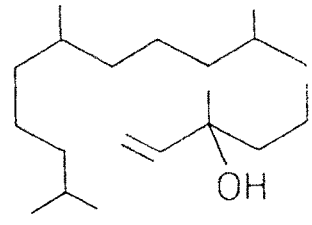
Geranyl acetone (12)



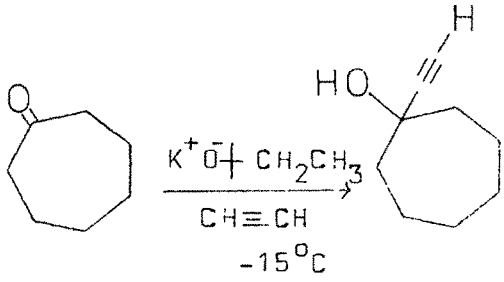
Phytone (13)



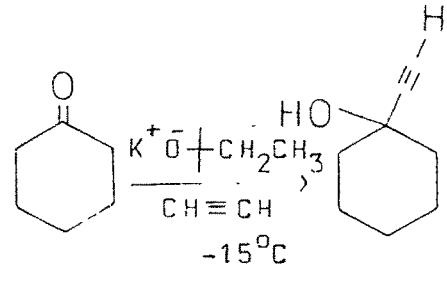
Nerolidol (14)



Isophytol (15)



(16)



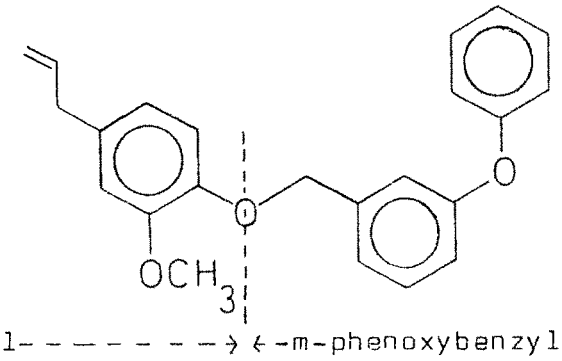
(17)

Fig. 9: Preparation of compounds (12), (13), (16) and (17)

of structurally closer active molecules (9) and (10), with respect to the base molecule-linalool.

3. Analogues based on methyl cinnamate

In connection with our ongoing research on 'elemicin' as a model,[44] led to 4-(m-phenoxybenzyloxy), 3-methoxy-allyl benzene (H), which showed improvement in the activity.



←Eugenol- - - - - - - →←-m-phenoxybenzyl alcohol→

H

Fundamentally this molecule is a combination of insecticidally active units eugenol and m-phenoxybenzyl alcohol. In the present study it was thought to create analogous situation by combining active residues trans-cinnamic acid with eugenol and m-phenoxybenzyl alcohol, individually, via an ester function as in methyl cinnamate.

m-Phenoxybenzyl cinnamate (18) was prepared by p-TSA catalyzed esterification of trans-cinnamic acid in large excess of m-phenoxybenzyl alcohol in refluxing benzene (Fig. 10).

Chromatographic separation on silica-gel furnished highly viscous ester in 99.0% GLC purity. Structure was fully borne out on the basis of spectral features.

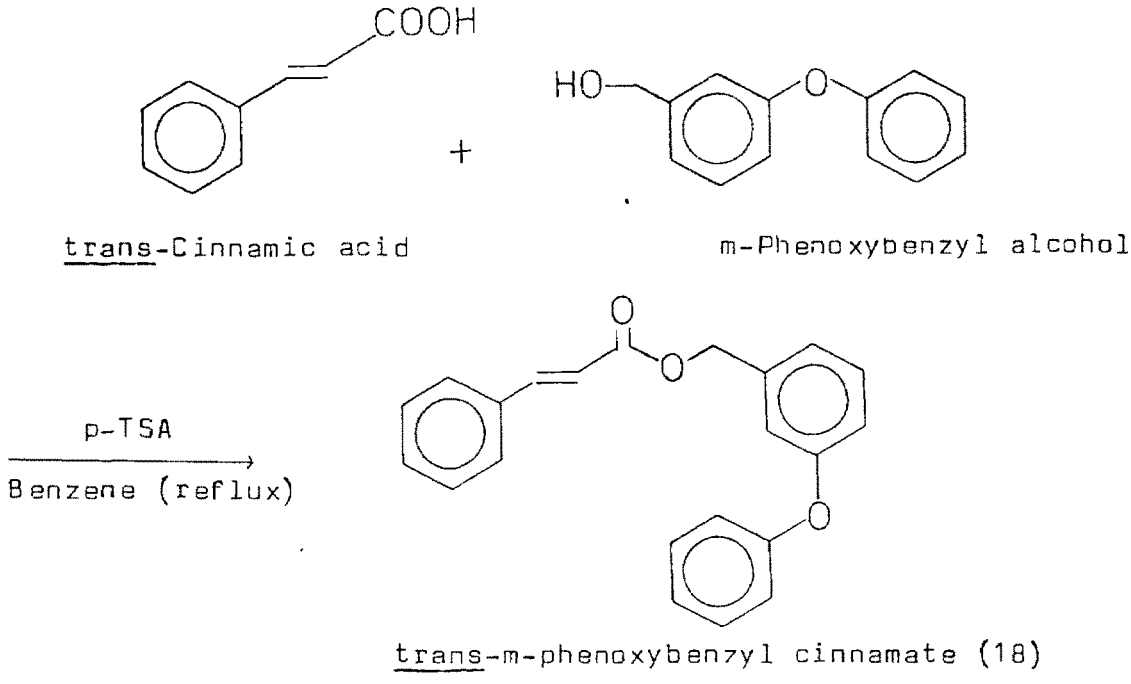
^{1}H-NMR (CCl_4): 5.12 (s, 2H, -COO-C$\underline{H}_2$-Ph-O-Ph), 6.37 (d, 1H, J = 16Hz trans, Ph-CH=C$\underline{H}$-COO-), 6.7-7.5 (m, 14H, aromatic), 7.60 (d, 1H, J = 16Hz trans, Ph-C$\underline{H}$=CH-COO-);

IR (neat): 1710 cm^{-1} (C=O stretch of Ar-C=C-COO-), 1250 cm^{-1} (C-O stretch, ester):

Mass - M^+ 330 and microanalysis.

Formation of trans-eugenyl cinnamate (19) was mediated by, in situ formation of reactive acid chloride using freshly distilled thionyl chloride under inert condition (Fig. 10). ^{1}H-NMR spectra of (19) was a simple addition of peaks contributed by acid and alcohol residues. IR was reminiscent with the structure (ester 1735 cm^{-1}).

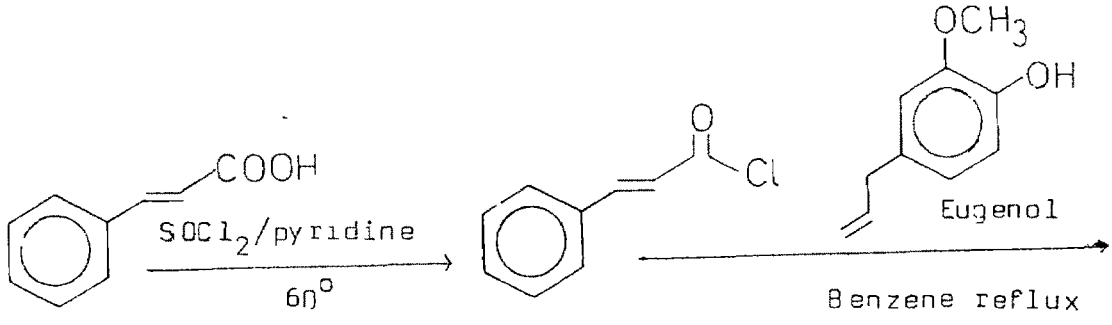
These molecules (18) and (19) were inactive hence further conclusions could not be drawn. Indeed the alcohol residue in methyl cinnamate seems to have specific function in imparting insecticidal activity. Methyl cinnamate analogues with different alkyl chain lengths at α & β carbons have been tested previously for synergistic activity[64] with pyrethrum.

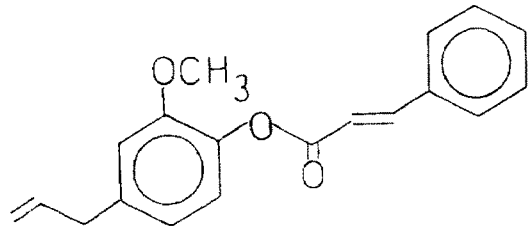


trans-Cinnamic acid

m-Phenoxybenzyl alcohol

trans-m-phenoxybenzyl cinnamate (18)





trans-Eugenyl cinnamate (19)

Fig. 10: Scheme for compounds (18) and (19)

Table 9. Relative insecticidal activity of Linalool[a] and various compounds against S. oryza and T. castaneum*

Entry No.	Compd. No.	Compound	Sitophilus oryzae	Tribolium castaneum
1	1	Linalool	100.0	NT[b]
2	2	Linalyl acetate	58.6	NT
3	3	3,7-Dimethyl-6-octen-3-ol	95.8	NT
4	4	Tetrahydrolinalool	114.7	NT
5	8	2-Phenyl-3-butyn-2-ol	201.0	NT
6	9	3,7-Dimethyl-1-octyn-3-ol	114.7	70.5
7	10	3,7-Dimethyl-6-octen-1-yn-3-ol	104.4	68.4
8	12	Dehydronerolidol	NT	NT
9	13	Dehydroisophytol	NT	NT
10	16	1-Ethynyl-cycloheptanol	75.1	46.1
11	17	1-Ethynyl-cyclohexanol	133.5	109.3

[a] Activity relative to linalool (=100) by contact method. (LC_{50} of linalool = 0.187 mg/cm^2).

[b] NT = nontoxic upto 0.39 mg/cm^2 level

* In comparison pyrethrum oleoresin showed better LC_{50} values against both insects (S. oryzae = 0.051 mg/cm^2 and T. castaneum = 0.048 mg/cm^2).

EXPERIMENTAL

For General Methodology, see Experimental of Chapter I.

1. Insecticidal components from essential oils

Hydrodistillation of essential oils

Fresh herbage of botanically identified plant material was hydrodistilled using modified cleavenger apparatus.[28] Duration of distillation varied from 5 to 7 hrs. Oils thus obtained were processed as usual and dried on anhyd. Na_2SO_4. Solvent stripping furnished oils for entomological evaluation.

Coleus aromaticus. Oil of Coleus aromaticus on GLC examination (10% CW, 200°C) showed 60% of carvacrol contents (R_t: 11.2 min). It was chromatographed on silica gel to separate carvacrol and other components (TLC monitoring: 10% EtOAc in pet. ether, I_2 vapours).

Chromatogram I

Wt. of oil: 1.0 g ; Wt. of silica gel: 40.0 g

Grade: IIA-IIB , Column dimensions: 1.2 cm x 78 cm.

Frac.No.	Eluent	Vol. of eluent(ml)	Wt. of eluate (g)	Remarks
1	Pet. ether	300	0.305	Mixture, R_f:0.86
2	4% EtOAc in pet ether	700	0.579	Carvacrol, R_f: 0.50
	Total		0.884	GLC purity: 90%

Carvacrol showed insecticidal activity(Frac.No.2), while fraction devoid of carvacrol (Frac. No. 1) was inactive.

Cinnamomum cecidodaphne

GLC of this oil (10% CW, 200°C) showed eight peaks (Fig. 4). Retention times and GLC percentages were as follows:

Table 10. Retentions times and GLC percentages of the components of oil of C. cecidodaphne

Component No. on GLC	R_t min	GLC percentage
1-4	0.8-2.0	9
5	2.88	39
6	4.20	2
7	7.6	37
8	9.28	13

Low boilers were separated employing high efficiency fractionating column- Adiabatic Annular Teflon Spinning Band Still with 80 theoretical plates: NFT 51 (see General Methodology, experimental, Chapter I). This simplified separation at the later stage. Following fractions were collected while monitoring separation profile by GLC (10% CW, 200°C) (Table 11).

Table 11. Fractionation of C. cecidedodaphne oil

Fraction No.	Pressure mm	Pot temp ^{o}C	B.p. ^{o}C	Reflux ratio	% composition on glc components					Wt. (g)
					1-4	5	6	7	8	
1	12	126	100	25:1	0.9	99.1	minor <1%	-	-	0.499
2-6	11.5	127	102	35:1	1.0	99.0	"	-	-	5.020
7-8	11.5	127	102	35:1	-	99.9	-	-	-	4.685
9	11.5	127	102	35:1	37.57	49.64	minor <1%	-	-	0.900
10	11.5	127	104	35:1	44.96	6.8	-	14.74	25.17	2.360
11	11.5	127	104	30:1	2.85	-	-	42.85	54.28	1.510
Pot										10.150
										25.124

Total charge of the oil : 25.4 g

Fractions (1 to 9) constituting components 1 to 6 on GLC, failed to show insecticidal activity. Active fraction (11) was rich in components 7 (42.9%) and 8 (54.3%).

Separation & identification of myrisiticin and elemicin: Fraction (11); 0.5454g was charged for column chromatographic separation on silica gel (grade, IIIA, 35.0g; 1.6 x 43 cm) and following fractions were collected using benzene: petroleum ether system. Monitor: TLC; solvent system: benzene.

Chromatogram-II

Frac. No.	Eluent	Vol. of eluent(ml)	Weight of eluate(g)	Component No. on GLC	%GLC purity
1	60% Benzene in pet.ether	20			
2	-do-	20	0.0600	1 to 5	Mixture
3	-do-	20			
4	-do-	20			
5	-do-	20	0.1753	8	99.9% R_f:0.57
6	80% -do-	20			
7	-do-	20	0.0800	8 + 7	Mixture
8	-do-	20			
9	-do-	20			
10	-do-	20	0.1039	7	99.9%
11	90% -do-	20			R_f: 0.2
12	-do-	20			
13	-do-	20			
14	50% benzene in acetone	60	0.0880	-	Column washing
	Total		0.5072		

Compound 8 was identified as myrisiticin by physical and analytical data[35a] (^{1}H-NMR and IR).

Physical data: b.p. 136-137°/8 torr (lit.[35b] b.p. 149.5°/15 torr

R_f: 0.57 (solvent system- benzene)

R_t: 9.28 min (10% CW, 200°).

^{1}H-NMR (CCl_4) (Fig. 11): 6.24 (d, 2H, J = 2Hz, meta, Ar-H) 6.0-5.61 (m, 1H, -CH=), 5.85 (s, 2H, -O-CH_2-O-), 5.17-4.87 (m, 2H, CH_2 =), 3.84 (s, 3H, -OCH_3), 3.24 (d, 2H, J = 7Hz, CH_2-)

IR (neat) (Fig. 12): 2900, 1630, 1508, 1430, 1315, 1192, 1130, 1090, 1045, 995, 915, 805, 690 cm^{-1}.

Spectral data of 7 was concurrent with that of elemicin[34a]

Physical data: b.p. 143-145°/8 torr (Lit.[34b], b.p. 144-7°/10 torr).

R_f: 0.2 (solvent system: benzene)

R_t: 7.6 min (10% CW, 200°).

^{1}H-NMR (CCl_4) (Fig.13): 6.32(s, 2H, Ar-H), 6.25-5.6 (m, 1H, $CH=CH_2$), 5.25-4.9 (m, 2H, $CH=CH_2$), 3.82 (s, 6H, 2-OCH_3), 3.75 (s, 3H, -OCH_3), 3.3 (d, 2H, J = 7Hz-CH_2-C=).

IR (neat) (Fig. 14): 1640, 1590, 1460, 1420, 1330, 1240, 1180, 1010, 980, 915, 825 cm^{-1}.

Both the above compounds were active against the test pest.

Lavunga scandens

GLC of the total oil (10% CW, 200°, Fig. 6) displayed 28 components. Hence it was taken up for broad cut separation.

Broad separation: Total oil (25.0g) was chromatographed on freshly prepared neutral alumina, grade III[38] (100-200 mesh, 750g, 4 x 83cm). For separation into i) hydrocarbons, ii) esters, iii) alcohols and iv) acids by polarity upgradation as follows. Separation profile was monitored by IR spectrophotometer.

Chromatogram III

Frac. No.	Eluent	Vol. of eluent(L)	Wt. of eluate (g)	IR analysis remarks
A	Petroleum ether	1.25	9.1	Hydrocarbons
*B	Petroleum ether	7.50	6.3	Esters
*C	3% EtOAc/pet. ether	2.5	1.5	Alcohols
*D	5% EtOAc/pet. ether	2.5	2.0	Alcohols
E	10% EtOAc/pet. ether	1.25	1.2	Alcohols + Acids
	15% EtOAc/pet. ether	2.5		
	25% EtOAc/pet. ether	2.5		
F	50% EtOAc/pet. etner	2.5	1.1	Acids
	Ethyl acetate	2.5		
	8% Methanol/ethyl acetate	1.25		
	Methanol	1.25		
		Total	21.2	

* Active fractions B, C and D were further processed individually for insecticidal substances.

Fraction B. This fraction of R_f 0.7 (20% EtOAc/pet.ether) on GLC (10% CW, 200°) displayed four major peaks at R_ts: 0.92 min, 2.48 min, 10.08 min and 15.84 min and computed with the peaks 4, 10, 25 and 28 on GLC of the total oil (Fig. 15). Component 25 was 47%.

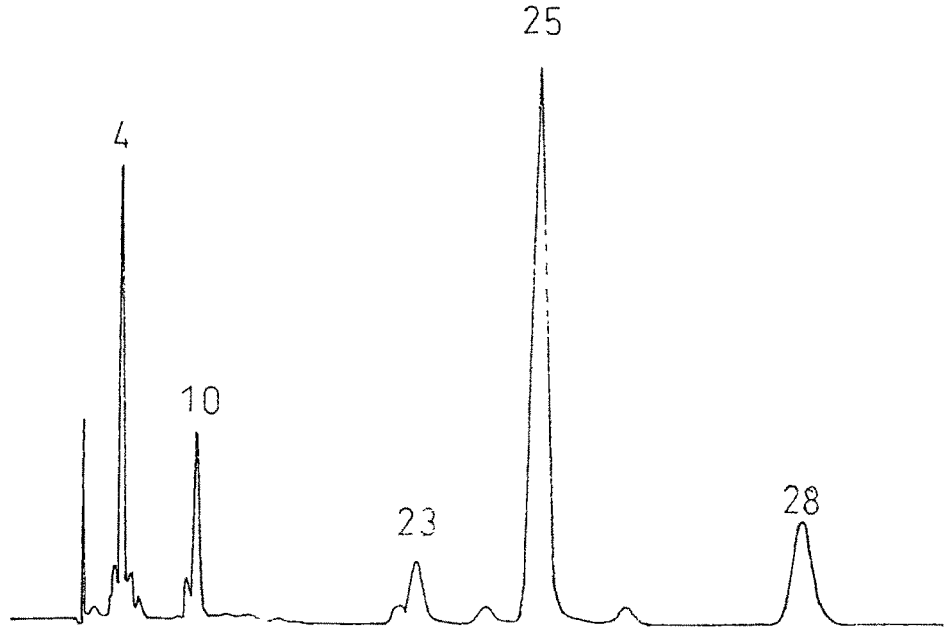


Fig. 15: GLC of Frac. B of L. scandens oil

^{1}H-NMR of this fraction showed prominence of all the peaks characteristic of methyl cinnamate.[39] IR absorptions for the same, as well, could be correlated with the available information.[39] Authentic sample preparation and coinjection extended confirmation. Methyl cinnamate is already known to be active against S. oryzae and T. castaneum[32] in low concentrations.

Fraction C. This homogenous alcohol rich fraction (R_f: 0.55, 20% EtOAc/pet.ether; IR: 3460 cm^{-1}, OH) showed on glc (10% CW, 200°) two major peaks 9 (32.4%, R_t: 2.6 min) and 13 (28.2%, R_t: 3.8 min) (Fig. 16).

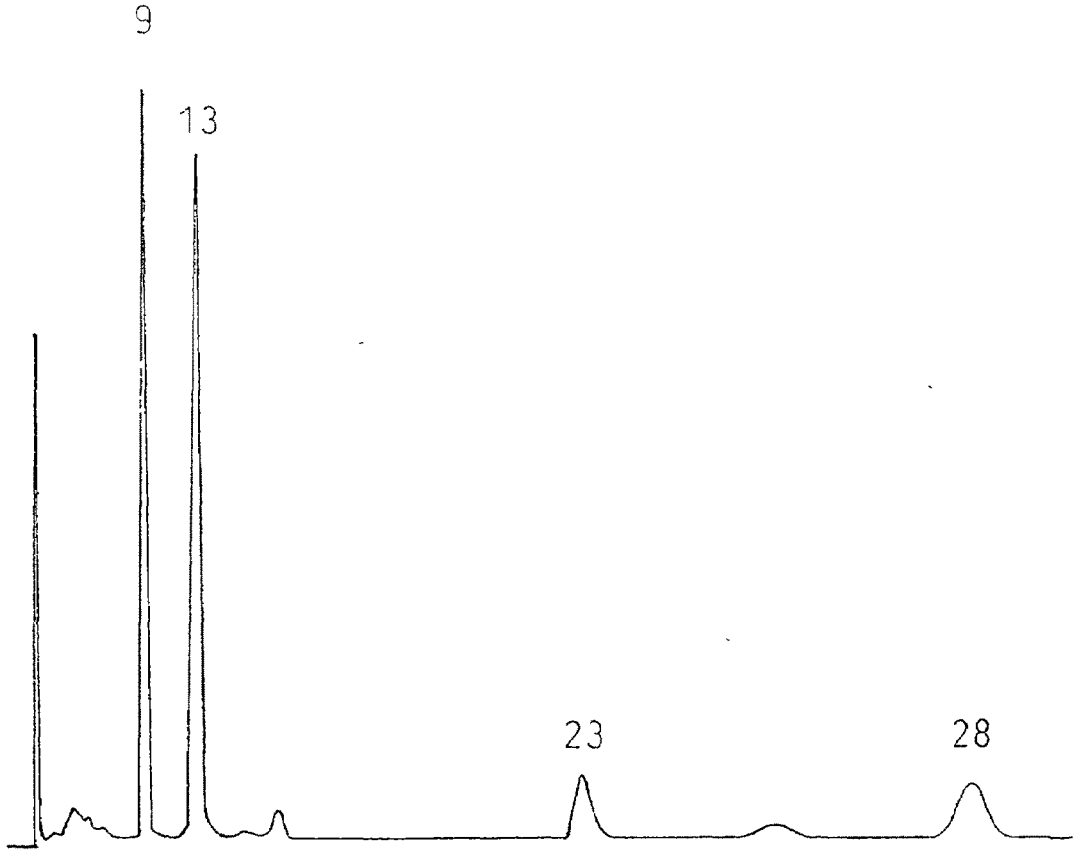


Fig. 16: GLC of Frac. C of L. scandens oil.

1.5 g of this fraction was column chromatographed on silica gel (IIA, 45 g, 1.8 x 51 cm) and fractions were collected.

Chromatogram IV

Fr. No.	Eluent	Vol. of eluent(ml)	Eluate wt(g)	Remarks
1-4	Petroleum ether	400	-	
5	0.5% EtOAc/pet. ether	450		
6-7	1.0% EtOAc/pet. ether	400		
8-9	1.5% -do-	400		
10-15	2.0% -do-	150	0.1190	
16-17	-do-	50	0.1032	
18-20	-do-	75	0.2503	
21-26	-do-	200	0.4754	
27-28	-do-	50	0.0241	
*29-33	-do-	125	0.0694	*92.0% GLC pure R_f: 0.28
34-38	-do-	125	0.0950	(10% EtOAc/ pet.ether)
39-42	-do-	100	0.0080	
43-44	10% EtOAc/pet.ether	500	0.1300	

Fraction (29-33) was 92.0% pure and showed R_t as that of peak 9 (2.6 min.). By ^{1}H-NMR and IR itwas identified as

linalool[40] and confirmed by coinjection with the authentic sample. No pure fraction of component 13 could be obtained. Preparative GLC (see general methodology for conditions) furnished 99.0% pure fraction of 13 which in turn on analytical probe revealed it to be 4-terpineol.[41] Coinjection with the authentic sample was uniformly isothermal.

R_t: 3.8 min(10% CW 200°); R_f: 0.32 (10% EtOAc/pet.ether). Linalool and 4-terpineol were active against Sitophilus oryzae as was the parent fraction.

Fraction D: This fraction was mainly composed of (GLC) 9 (18.4%) and 15 (64.4%) (Fig. 17). 9 has been already identified as linalool.

Above fraction (1.5 g) was chromatographed on silica gel. (IIA; 60.0g, 1.7 x 65 cm) and eluted with petroleum ether in increasing percentage of ethyl acetate as follows.

Chromatogram V

Frc. No.	Eluent	Vol. of eluent(ml)	Eluate Wt(g)	Remarks
1-3	Petroleum ether	450	-	
4-7	1% EtOAc/petroleum ether	500	-	
8-14	2% EtOAc/pet ether	600	0.0637	
15-18	-do-	200	0.0640	
19-24	-do-	300	0:2407	
*25.27	-do-	150	0.1374	*R_f: 0.15
28-41	-do-	800	0.1221	2% EtOAc/pet.
42-52	3% -do-	1000	0.0117	ether
53	Ethyl acetate	1000	0.7162	
		Total	1.3558	

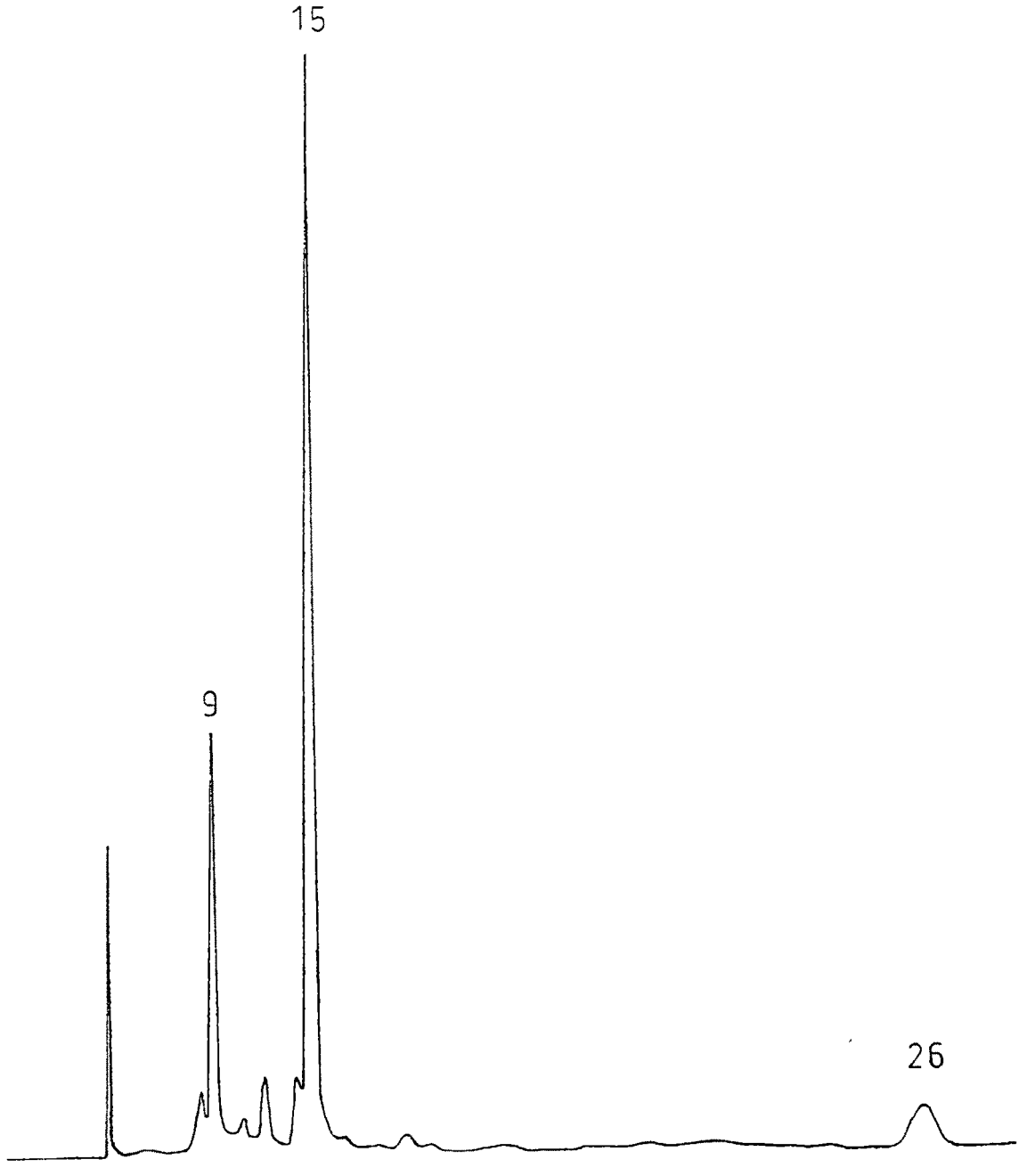


Fig. 17: GLC of Frc. D of L. scandance oil

Frc. (25-27) was homogeneous, showed GLC purity 95.0% (R_t: 5.0 min, 10% CW, 200°) and corresponded with(15). Analytical data (^{1}H-NMR, IR) matched with that of ∝-terpineol,[42] reported in the literature. This monoterpene alcohol showed kill for Sitophilus oryzae.

2. Modelling on linalool

3,7-Dimethyl-6-octen-3-ol (3). Linalool (2.0 g, 12.6 mmole) in EtOH (15 ml) was hydrogenated over (P-1)-nickel boride catalyst (prepared according to ref. 66), at 27°/755 mm till 318 ml (1 mole eq.) hydrogen was absorbed. The product was isolated as usual by filtration and distilled to furnish 1.87 g of 1,2-dihydrolinalool (3), as a colourless liquid (yield, 92.1%).

Physical data: b.p. 90° oil bath/15 torr (lit.[67] b.p. 89-90°/14 torr).

R_t: 4.72 min (10% CW, 150°); GLC purity 93.0%.

^{1}H-NMR ($CDCl_3$): 5.3-5.0 (m, 1H, =C<u>H</u>-), 2.28-1.9 (m, 2H, C<u>H</u>$_2$-CH=), 1.9-1.3 (m, 4H, CH_2S), 1.72 (s, 3H, =C-C<u>H</u>$_3$), 1.68 (s, 3H, =C-C<u>H</u>$_3$), 1.2 (s, 3H, -C(OH)-C<u>H</u>$_3$, 0.92 (t, 3H, J = 6Hz, -CH_3).

IR (neat): 3400, 2980, 2940, 1465, 1380, 1150, 1060, 1000, 940, 900, 840 cm^{-1}.

3,7-Dimethyl-octan-3-ol-(4): Linalool was fully hydrogenated on 10% Pd/C as described in the literature.[49]

Physical data: b.p. 80° oil bath/15 torr (lit.[49] b.p. 185-186°/715 torr).

R_t: 3.2 min (10% CW, 150°), GLC purity: 99.0%.

2-Phenyl-3-butyn-2-ol (8). (General procedure to obtain ethynyl carbinols). Dry ether (1.9 ml) was cooled to-18°C and saturated with dry purified acetylene gas by bubbling (15 lit/h), while magnetically stirred for 1 h. A solution of potassium tertiary amylate, prepared by dissolving freshly cut potassium (0.14g, 0.0036 g-atom) in dry t-amyl alcohol (2.3 ml) was injected to the above ether solution under vigorous stirring. Acetylene flow was raised to 32-35 lit/h. A solution of methyl phenyl ketone (0.23 g, 1.9 mmole) in dry ether (1.9 ml) was injected separately in portions so as to keep the reaction temperature at -10° to -15°C. After complete addition, mixture was stirred for ~4 h at 0°C till TLC (10% EtOAc/pet.ether, spray: 2,4DNP) showed no starting ketone.

The reaction mixture was then decomposed by cold water (10°C) and acidified with dil. H_2SO_4 (pH ~ 6). The product was taken up in ether (3 x 5 ml) washed with 5% aq. NH_4Cl (2 x 5 ml) followed by water till aqueous layer showed neutral pH. Final wash with brine (2 x 5 ml), drying over anhyd. Na_2SO_4 and solvent stripping furnished crude product which on bulb distillation yielded 0.23 g of required product (8) (yield 82%), which solidified on keeping.

Physical data: m.p. 50-51°C, b.p. 105-107° oil bath/7 torr (lit.[59] b.p. 90-95°/11 torr).

R_f: 0.23(10% EtOAc/pet.ether).

R_t: 12.48 min (10% SE 30, 100°) GLC purity + 99.0%.

^{1}H-NMR (CCl_4) (Fig. 18): 7.7-7.04 (m, 5H, Ar-H), 2.82(bs, 1H, -O-H exchangeable with D_2O), 2.47 (s, 1H, -C≡C-H), 1.66 (s, 3H, $-CH_3$).

IR[59](KBr) (Fig. 19): 2980, 2110; 3280, 1220, 1085, 1050, 930, 760, 695, 655 cm^{-1}.

6-Methyl-5-hepten-2-one. The above ketone was prepared from citral by retrograde aldol condensation as given in the literature.[60]

Analytical data: b.p. 100°/90 torr (lit.[60] b.p. 108-110°/100 torr).

R_t: 4.4 min (10% SE 30, 100°); GLC purity + 99.0%.

^{1}H-NMR and IR data as reported earlier.[60]

6-Methyl-heptan-2-one. Hydrogenation of 6-methyl-5-hepten-2-one (1.0 g, 7.9 mmole) on 10% Pd/C (50 mg) furnished required saturated ketone (0.92 g) yield: 90.2%.

Physical data: b.p. 90° oil bath/20 torr (lit.[68] b.p. 70°/20 torr).

R_t: 3.8 min (10% SE 30, 100°): GLC purity + 99.0%

^{1}H-NMR and IR data as in the literature.[61]

3,7-Dimethyl-1-octyn-3-ol (9). General procedure described for ethynyl carbinols under (8) was followed.

6-Methyl-heptan -2-one (2.0 g, 15.6 mmole) and potassium acetylide were allowed to react in cold to furnish required product (2.01 g) in 85.9% yield. Similar procedure described in the literature reports 41.0% yield.[57]

Physical data: b.p. 80°/10 torr (lit.[57] b.p. 65-67°/6 torr)

R_t : 1.76 min (10% SE 30, 150°); GLC purity 95.0%.

^{1}H-NMR ($CDCl_3$) (Fig. 20): 2.43 (s, 1H, -C≡C-H), 2.15 (bs, 1H, -O-H exchangeable with D_2O), 1.9-1.1 (bm, 7H, CH & CH_2S), 1.5 (s, 3H, -C(OH)-CH_3), 0.92 (d, 6H, J = 6Hz, C-$(CH_3)_2$).

IR (neat) (Fig. 21): 3310, 1250 (overtone); 3400, 2960, 1470, 1365, 1140, 910, 625 cm^{-1}.

3,7-Dimethyl-6-octen-1-yn-3-ol (10). 6-Methyl-5-hepten-2-one (2.68 g, 21.3 mmole) on acetylene addition employing general procedure described earlier (for Compd. 8) gave the title compound (10) (2.0 g) in 81% yield.

Physical data: b.p. 90-92°/8-9 torr (lit.[69] b.p. 65°/2 torr).

R_t: 8.8 min (10% SE 30, 100°). GLC purity: + 99.0%

^{1}H-NMR ($CDCl_3$) (Fig. 22): 5.14 (t, 1H, J = 8Hz, -C=CH-), 2.43 (s, 1H, ≡C-H), 2.4-1.57 (m, 5H, CH_2S and O-H), 1.65 (s submerged 3H, -C=C-CH_3), 1.62 (s submerged 3H, C=C-CH_3), 1.44 (s, 3H, -C(OH)-CH_3).

IR (neat) (Fig. 23): 3310, 2120; 3400, 2970, 2930, 1710, 1450, 1370, 1115, 900, 835, 620 cm^{-1}.

Dehydronerolidol (12). Dehydronerolidol (12) (mixture of E- and Z-isomers was prepared using 1.84 g(9.5 mmole) of geranyl acetone by general procedure described under (8) in 81.3% yield (1.69 g).

Physical data: b.p. 130° oil bath/1 torr (lit.[69] b.p. 110-116°/ 1 torr).

R_t : Z-isomer = 12.2 min, E-isomer: 14.0 min (10% CW, 200°) GLC purity 93.0%.

Confirmed by co-injection with the authentic sample.

Dehydroisophytol (13). This ethynyl carbinol was prepared from the corresponding ketone (4.1 g, 15.3 mmole) using appropriate quantity of base in 74.4% yield (3.35 g).

Physical data: Viscous liquid b.p. 130°/0.5 torr (lit.[69] b.p. 132-140/0.7 torr);

R_t: 5.28 min (10% OV_4, N_2-40 ml/min, Col. 180°, FID); GLC purity 98%.

Compared with the authentic sample by GLC.

1-Ethynyl-cycloheptanol (16). 0.28 g (2.5 mmole) of cycloheptanone was ethynylated (according to general procedure under 8) in 92.7% yield to give 0.32 g of required fractionated material.

Physical data: b.p. 95-98° bath/10 torr (lit.[70] b.p. 56-60°/0.65 torr)

R_t: 10.2 min(10% SE 30, 100°) GLC purity: + 99.9%

^{1}H-NMR(CCl_4) and IR (neat) as reported in the literature.[70]

1-Ethynyl-cyclohexanole (17). Title compound could be obtained by the same procedure as above (also described in the literature) using 1.0 g (10.0 mmole) of cyclohexanone in 88.0% yield (1.12 g).

Physical data: b.p. 74°/14 torr (lit.[69]: b.p. 80-82°/18 torr).

R_t: 10.0 min (10% CW, 150°) GLC purity 99.0%.

Confirmed by coinjection with the authentic sample.

3. Analogues based on methyl cinnamate.

3-Phenoxy-benzyl cinnamate (18). To a solution of trans-cinnamic acid (2.0 g, 13.5 mmole) in 20 ml benzene was added catalytic quantity of p-TSA (100 mg) and m-phenoxybenzyl alcohol (15.0 cc, excess). The mixture was refluxed for 14 hr , when tlc showed disappearance of trans-cinnamic acid. Reaction mixture was cooled. After addition of 30ml benzene, organic portion was washed with water (2 x 10 ml), followed by saturated solution of $NaHCO_3$ (2 x 20 ml). Final washing with water (till pH = 7) and brine furnished crude ester after solvent removal.

Crude product was chromatographed on silica gel column (100 g, 2.1 x 60 cm) and followed by tlc (10% EtOAc/pet.ether).

Frac. 1.	Pet. ether	600 ml	0.0080	impurity
Frac. 2	1% EtOAc/pet.ether	500 ml	2.1000	unwanted mixture
Frac. 3	-do-	250 ml	3.0500	Required ester
Frac. 4	-do-	200 ml	0.1000	R_f: 0.36
Frac. 5	-do-	200 ml	0.015	(10% EtOAc/ pet.ether)
		Total	5.2595	

3.165 g of straw coloured liquid showed following analysis (yield: 71.93%).

Physical data: Viscous straw coloured oil.

R_t: 5.6 min (10% DCQF, 230°) GLC purity + 99.0%

^{1}H-NMR (CCl_4) (Fig. 24): 7.60 (d, 1H, J = 16Hz, trans; Ph-C<u>H</u>=CH-), 7.5-6.7 (m, 14H, Ar-<u>H</u>), 6.37 (d, 1H, J = 16Hz trans; Ph-CH=C<u>H</u>-COO-), 5.12 (s, 2H, -O-C<u>H</u>$_2$-Ph);

IR (neat) (Fig. 25): 1710, 1250; 3075, 1640, 1585, 1490, 1450, 1310, 1160, 980, 760, 685 cm^{-1}.

Mass: M^+ 330

Elemental analysis: Found C, 79.51; H, 5.29; $C_{22}H_{18}O_3$ requires, C, 79.98; H, 5.49.

<u>Eugenyl cinnamate (19)</u>. To <u>trans</u>-cinnamic acid (1.33 g, 9.0 mmole),

in dry pyridine (1.0 g) was added freshly distilled thionyl chloride (7.0 ml) under an inert atmosphere of N_2 at 0^oC. The contents were then heated to 60^o for 1 hr, cautiously to control evolution of hydrogen chloride. Distilled off excess $SOCl_2$ and introduced eugenol (2.0g, 12.0mmol) into the reaction pot for an *in situ* esterification by refluxing in dry benzene for 4 hr. The solid contents were then poured into iced alkaline phase (pH~8.5) and extracted with petroleum ether (4 x 25 ml). Combined organic phase was washed with water till neutral and concentrated to get the crude sticky solid ester (2.1 g), which on crystallization (3% EtOAc in pet.ether, 30 ml) furnished 1.8 g of pure crystalline product **19** (yield: 68.0%).

Physical data: m.p. 93^o sharp.

R_f: 0.5 (10% EtOAc in petroleum ether).

^{1}H-NMR ($CDCl_3$) (Fig. 26): 7.85 (d, 1H, J = 16Hz *trans*, Ph-C<u>H</u>=CH-COO-), 7.74-6.77 (m,8H, Ar-<u>H</u>), 6.64 (d, 1H, J = 16Hz *trans*, Ph-CH=C<u>H</u>-COO-), 6.2-5.75 (m, 1H, CH_2=C<u>H</u>-CH_2), 5.28-4.94 (m, 2H, $C\underline{H}_2$=CH-CH_2-), 3.91 (s, 3H, -O$C\underline{H}_3$), 3.38 (d, 2H, J = 6Hz, = CH-$C\underline{H}_2$-Ph).

IR (KBr) (Fig. 27): 1735; 1638, 1510, 1305, 1268, 1135, 1038, 980, 860, 760, 680 cm^{-1}.

Mass: M^+ 294.

Elemental analysis: C, 77.11; H, 6.08 ; $C_{19}H_{18}O_3$ requires C, 77.53; H, 6.16.

BIOASSAY METHODS

In general evaluation of the presented essential oils or their isolates and other synthetic compounds was done against two common stored grain pests Tribolium castaneum and Sitophilus oryzae. However, for some specific evaluation other insects like Trogoderma granarium, Callosobruchus chinensis, Musca domestica, Periplanata americana and Dysdercus koenigii were also used. Three types of bioassays were used against these insects.

1. Contact method

This method was used mostly against stored grain pests like T. castaneum, S. oryzae, T. granarium and C. chinensis adults. All these insects were taken from our routine cultures maintained at 27 ± 1°C, 70 ± 5% RH and 16 hour photophase. Initially a standard concentration of 0.39 mg/cm^2 surface was used followed by the reduction in concentration level as required (see results and discussion).

In each treatment the oil or a chemical compound was dissolved in acetone to make a required concentration. This material was then impregnated into a filter paper (0.5 ml/g cm diameter size) placed in a glass petridish of the same size. 2-6 hour old adults (10 adults/petridish in 4 replicates) were confined in these petridishes and mortality was recorded after 24 hrs.

Percent kill was calculated and subjected to log analysis using gauss integral plotting on log graphs to calculate LC_{50} values, wherever required.

To check the efficacy as compared to natural Pyrethrum Oleoresin; the oleoresin was received through courtesy of Central Institute of Medicinal and Aromatic Plants, Srinagar Centre, J & K State. The efficacy was evaluated as mentioned above.

The contact toxicity against Dysdercus koenigii (red cotton bugs) was also tried using elemicin from C. cecidodaphne. In this case 5th instar larvae were released on to the treated surface and kill observed after 24 hrs (Table 5). In these treatments solvent based compound was spread in a glass jar and solvent dried completely, so that active compound remained coated as a thin film on the surface of the jar.

2. Topical application

This bioassay was used against houseflies (Musca domestica) only. The flies maintained at 27 ± 1°C and 16 hr photophase were used when 2-3-day old. The female flies were separated and applied with each test material at the rate of 1 µl/fly in acetone. In control only 1 µl acetone was used. The flies were then allowed to stay for 24 hrs with normal food. Percentage mortality was recorded after 24 hrs and plotted on log graph.

3. Direct spray test

This test was used against houseflies and cockroaches.

For houseflies,50 flies were released into a peet grady chamber ($2.48M^3$ space). 2 ml of the test material in each concentration was sprayed through an automizer at a pressure of 40 psig. The number of knocked down insects were counted after 10 minutes. This was followed by the collection of flies from the chamber and their transfer into recovery container to observe mortality after 24 hrs.

In case of cockroaches P. americana adults, which were bred on dog biscuits, were used in 1:1 sex ratio for testing when 2-3 weeks old. The breeding conditions were same as for other insects.

A direct spray of 0.5 ml kerosene-based concentrations were used when 10 cockroaches/test (3 replicates) were placed in a glass cylinder (15 x 20 cm) coated internally upto half with a thin film of liquid paraffin to prevent an escape. Mortality was recorded after 48 hrs. The various concentrations used are given in Table (Table 5).

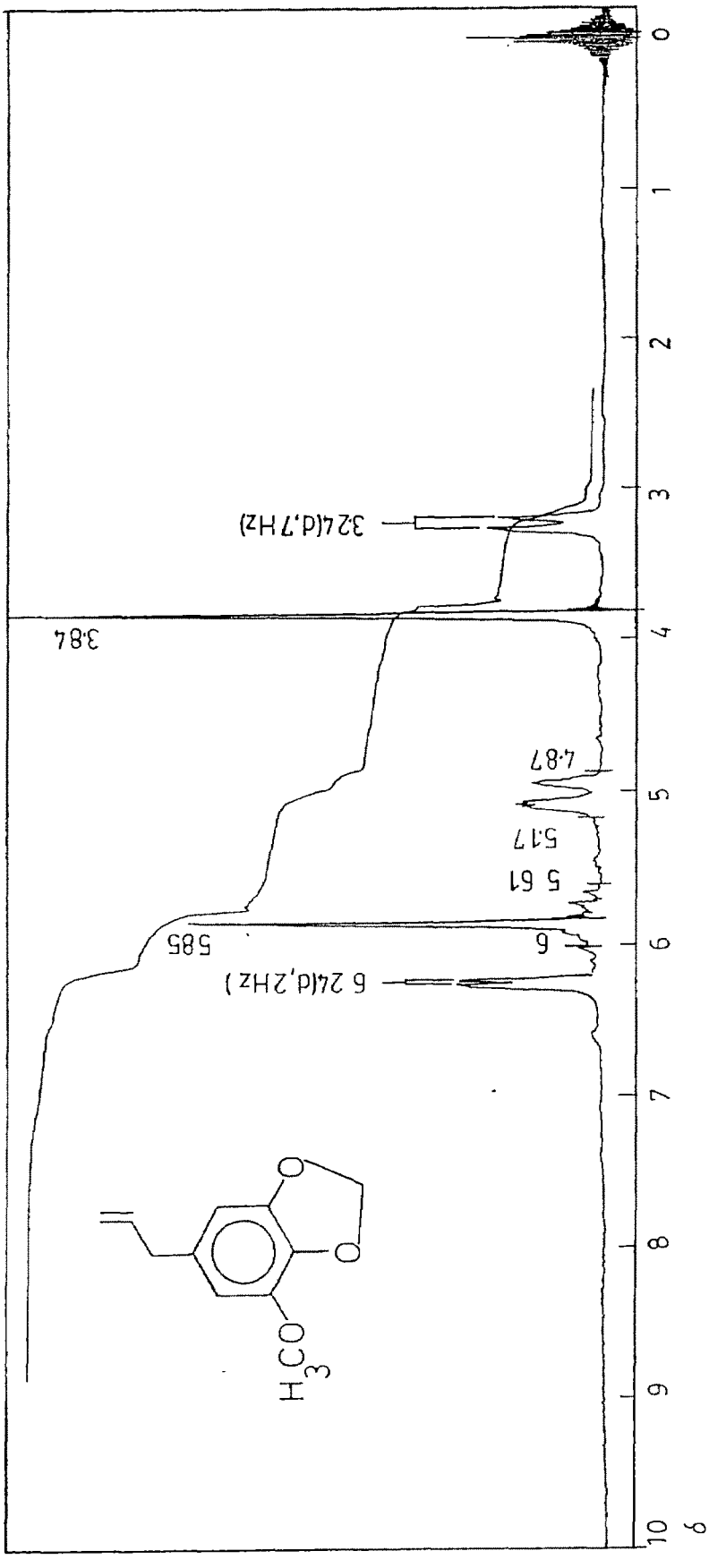


Fig. 11 : ^{1}H-NMR spectrum of Myristicin

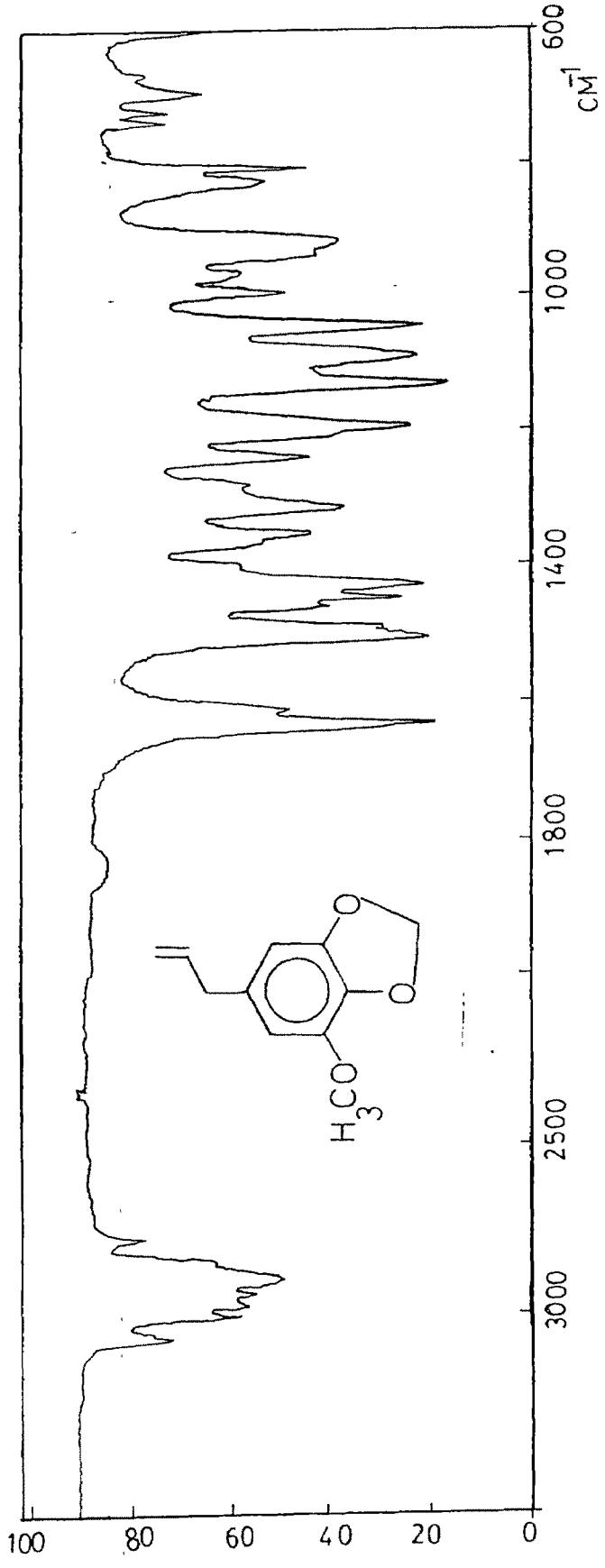


Fig. 12: IR spectrum of Myristicin

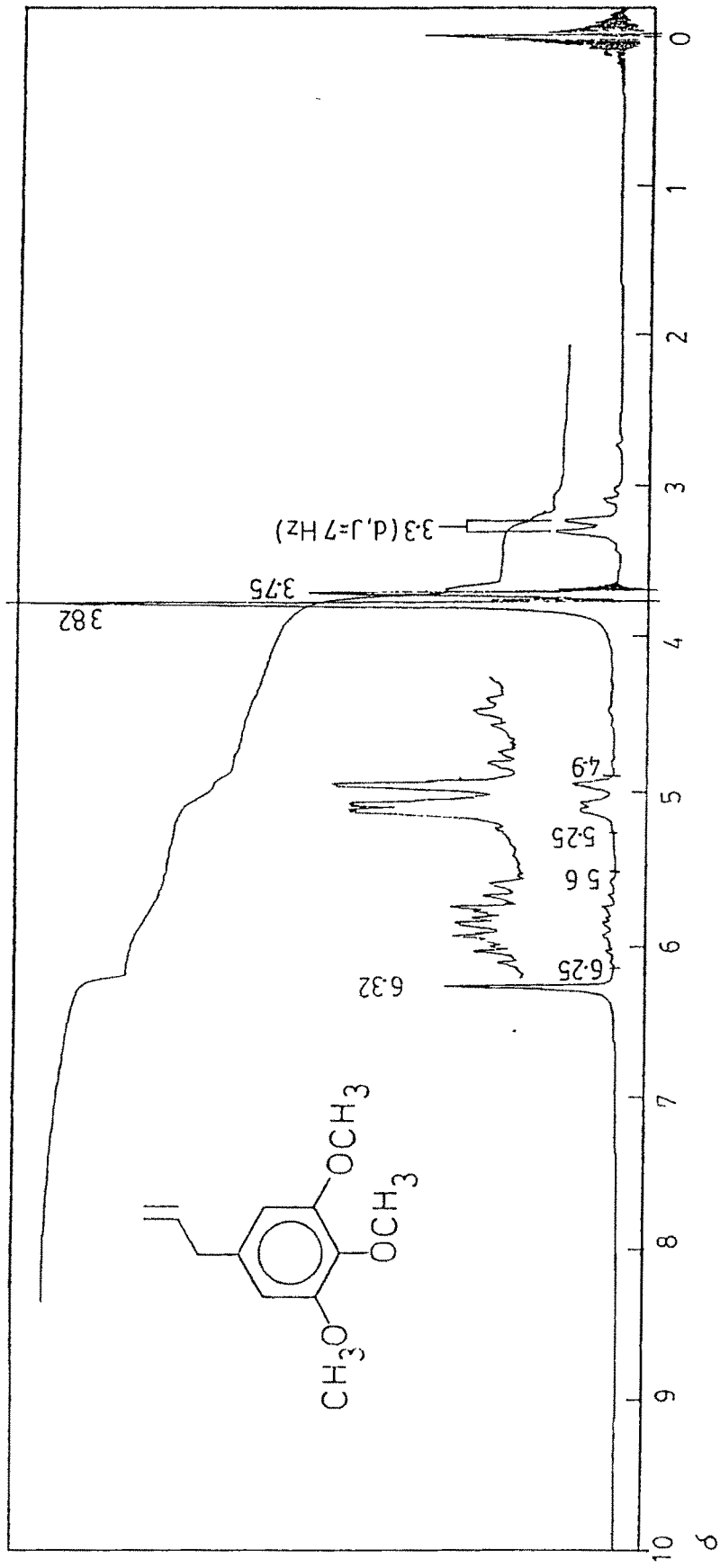


Fig. 13: ^{1}H-NMR spectrum of Elemicin

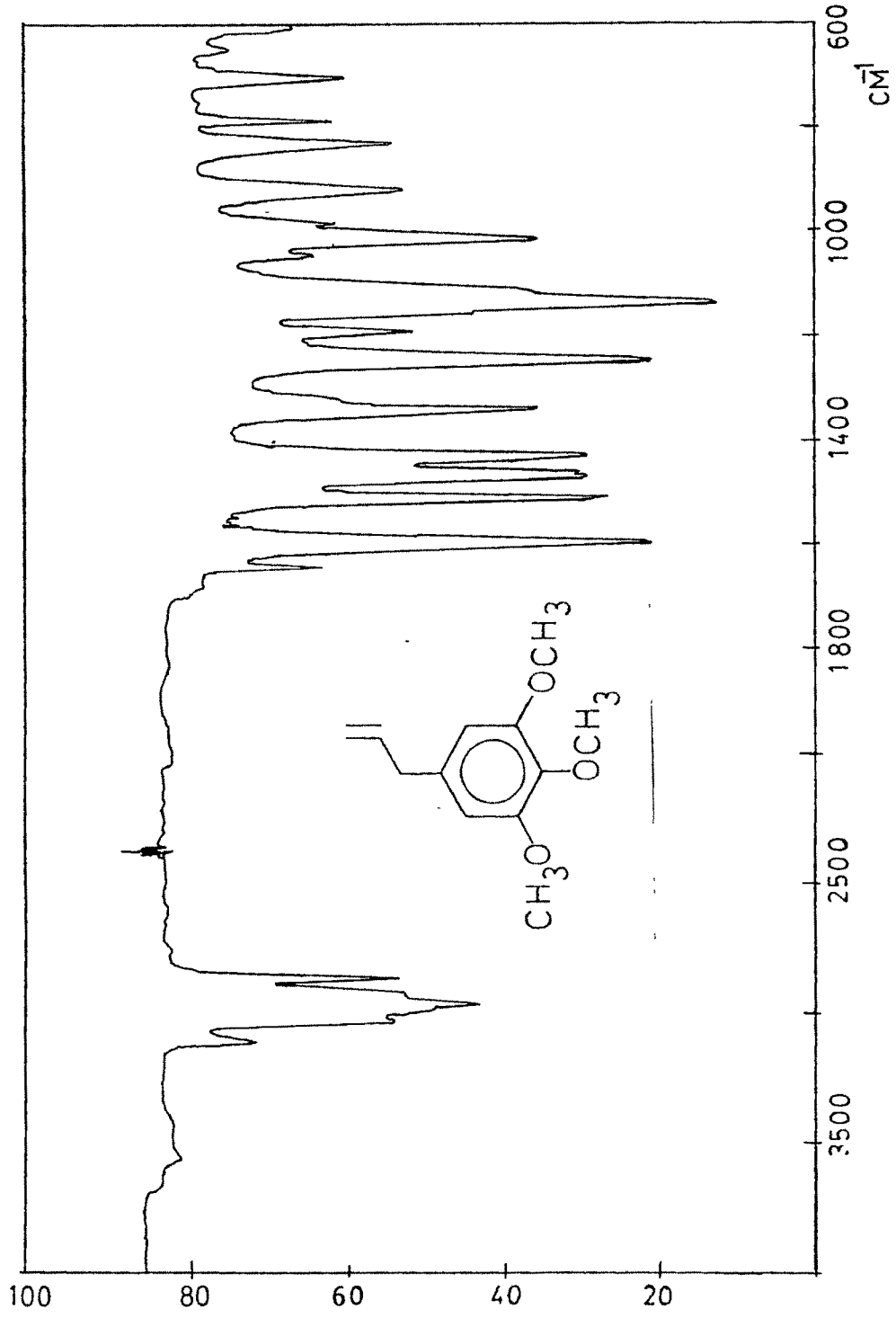


Fig. 14: IR spectrum of Elemicin

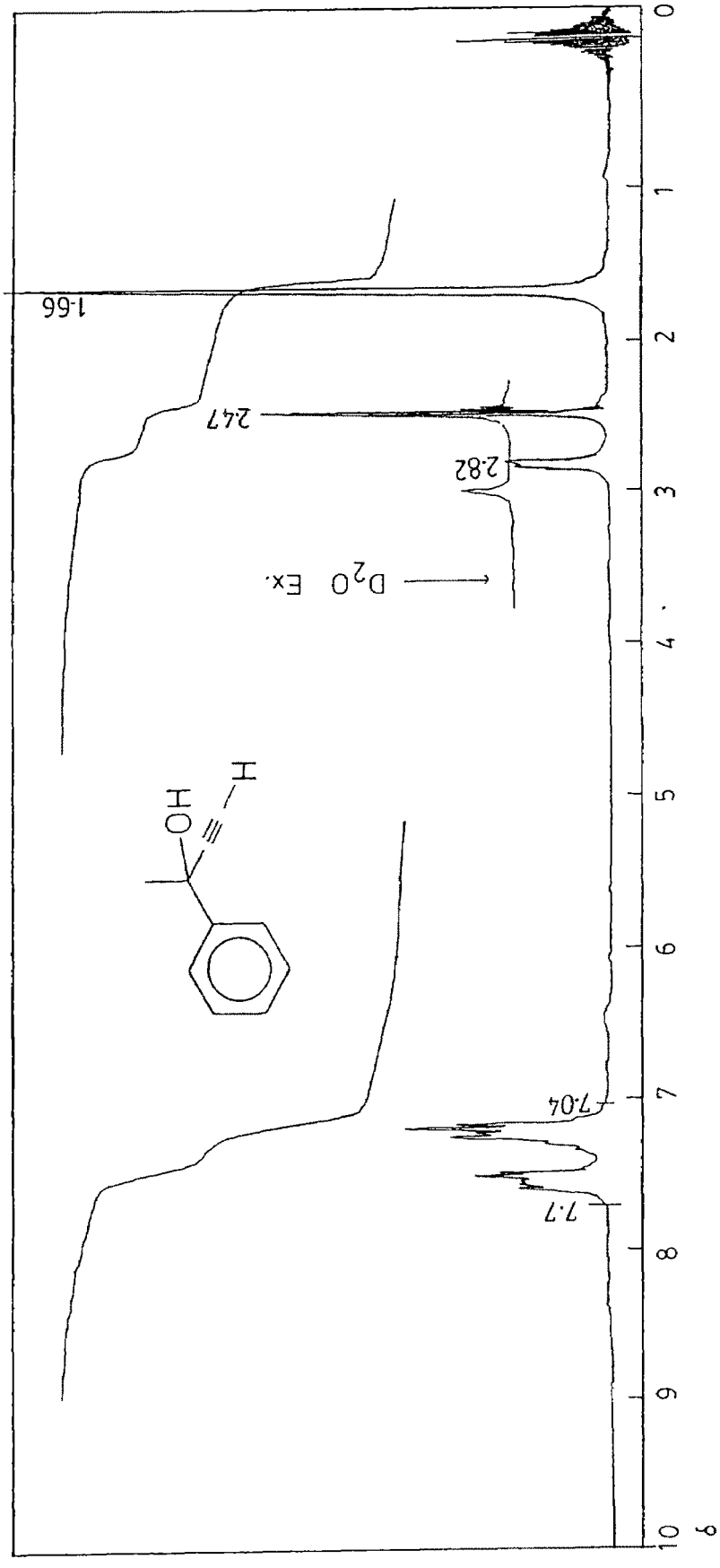


Fig. 18: ^{1}H-NMR spectrum of 2-Phenyl-3-butyn-2-ol (8)

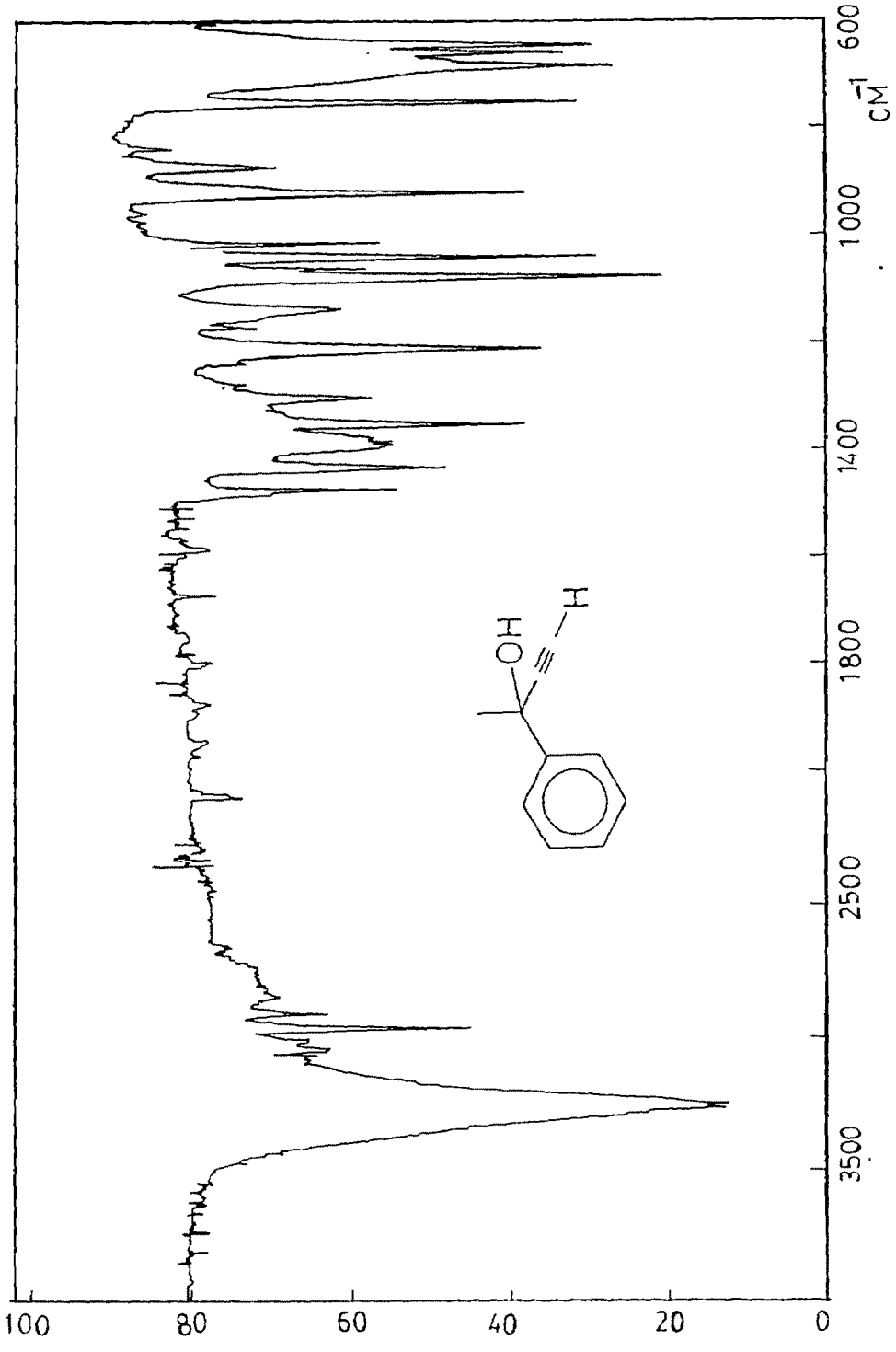


Fig. 19: IR spectrum of 2-Phenyl-3-butyn-2-ol (8)

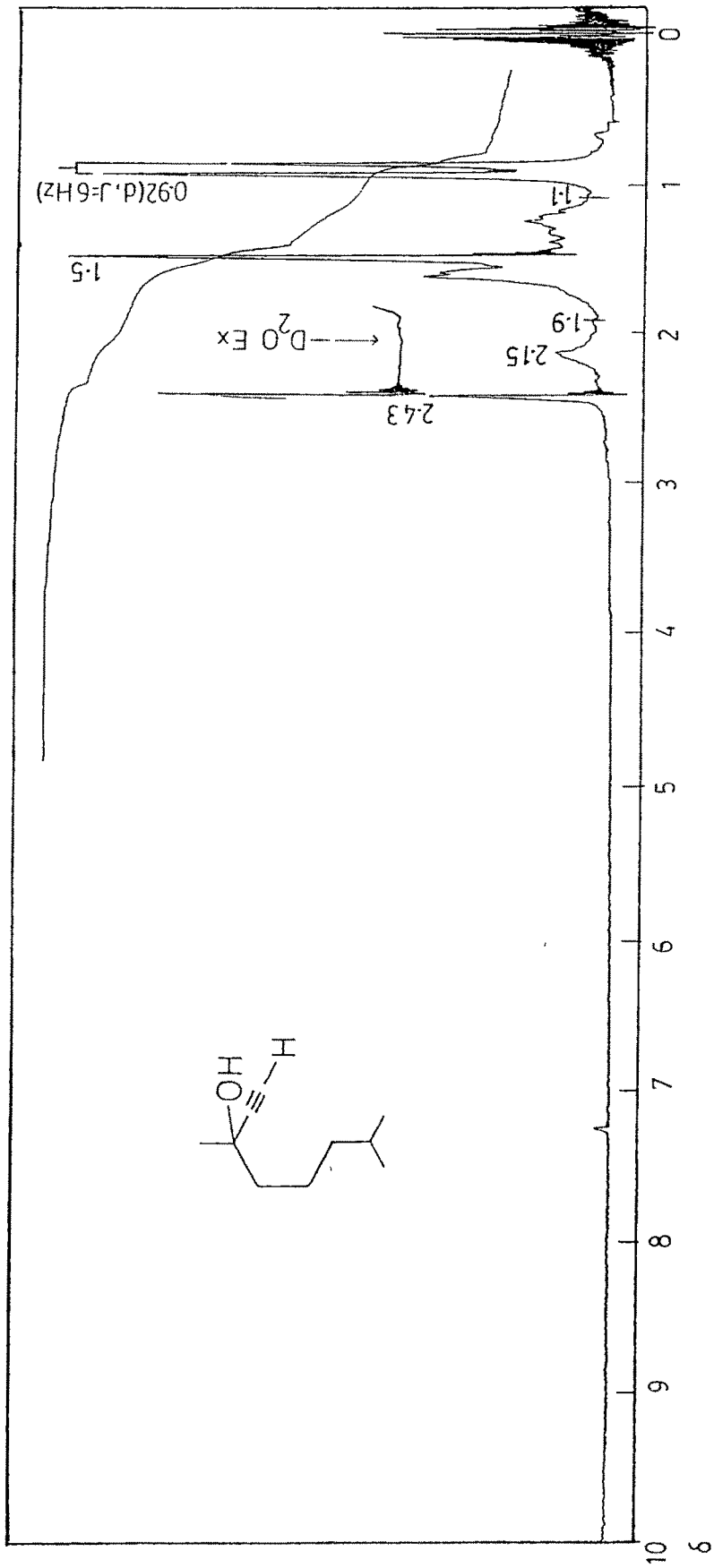


Fig. 20: ^{1}H-NMR spectrum of 3,7-Dimethyl-1-octyn-3-ol (9)

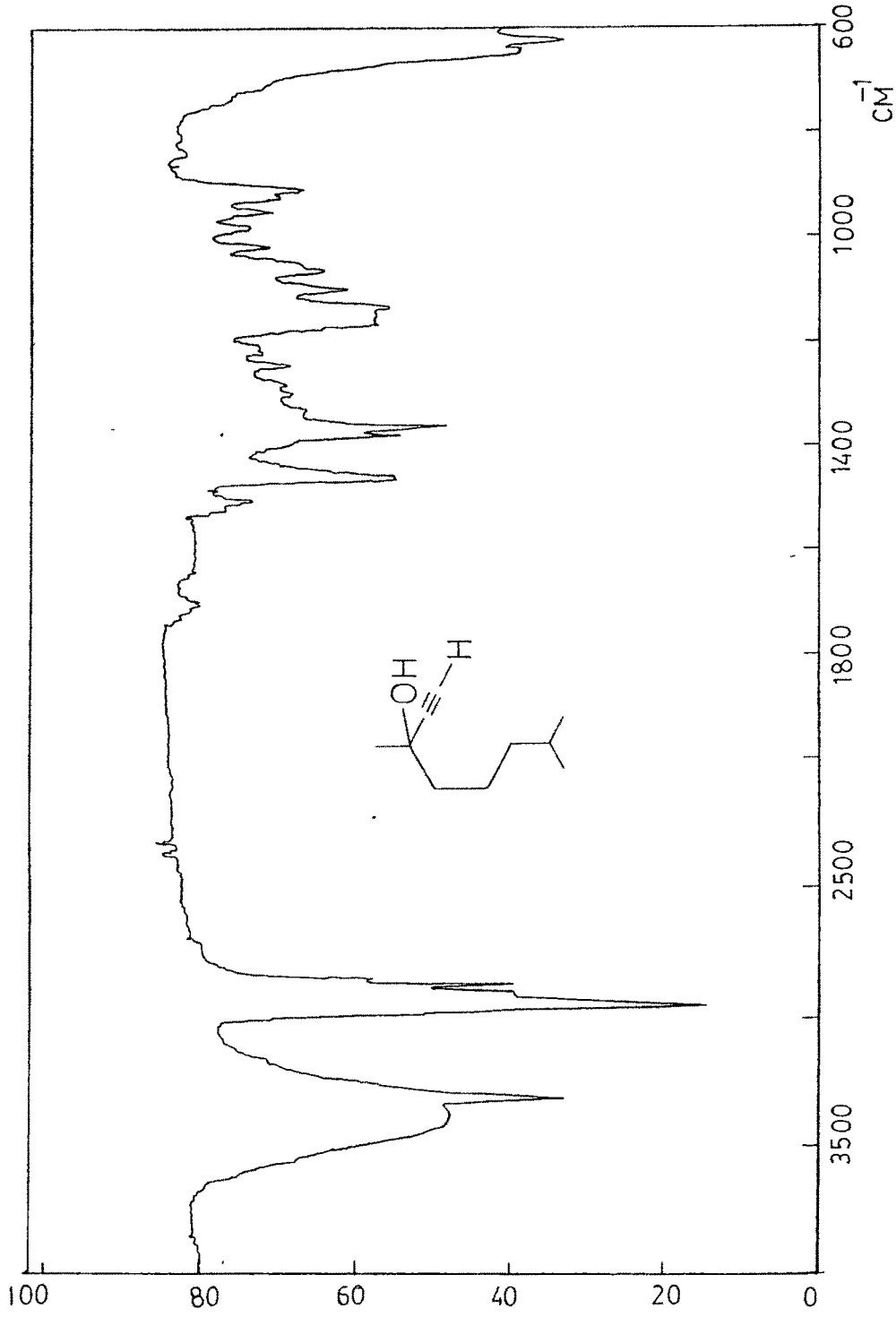


Fig. 21: IR spectrum of 3,7-Dimethyl-1-octyn-3-ol (9)

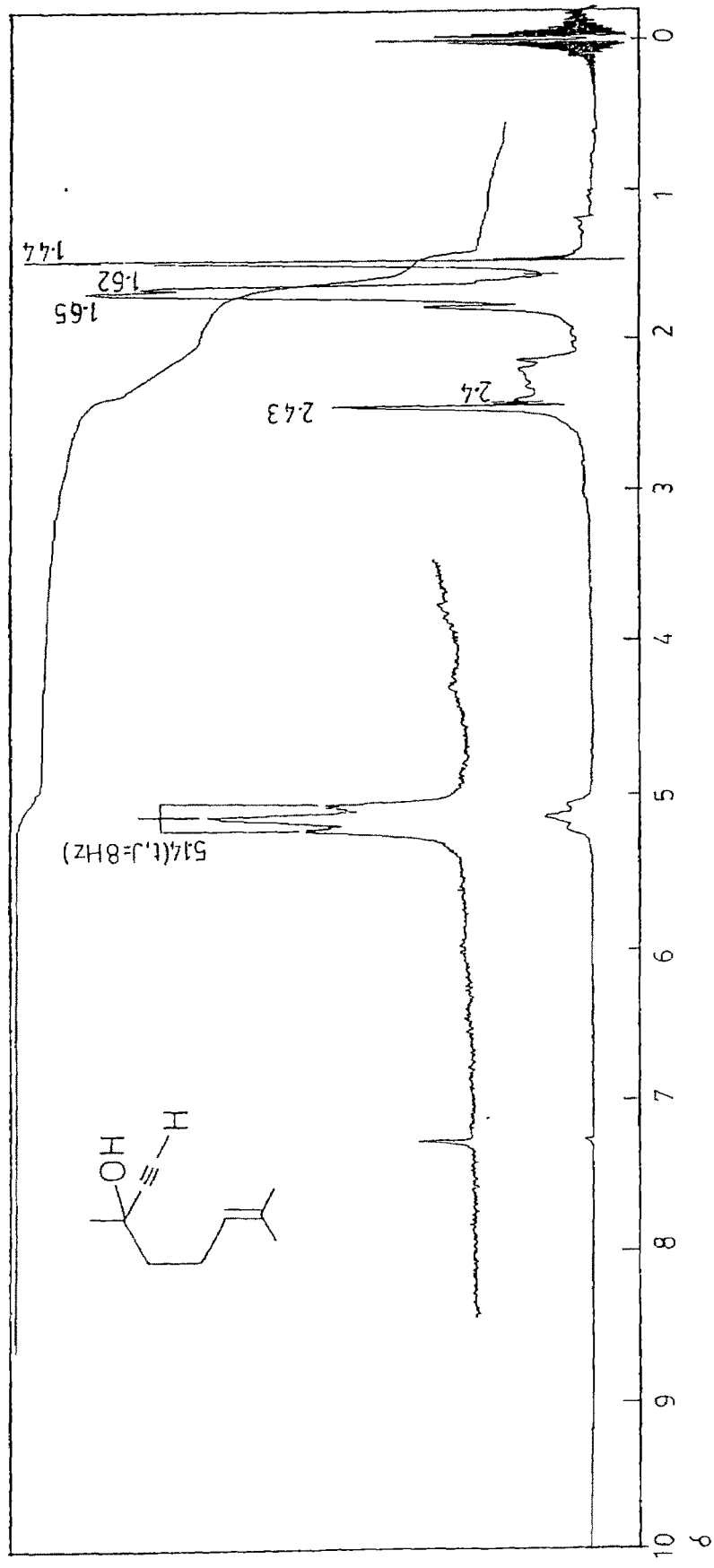


Fig. 22: ^{1}H-NMR spectrum of 3,7-Dimethyl-6-octen-1-yn-3-ol (10)

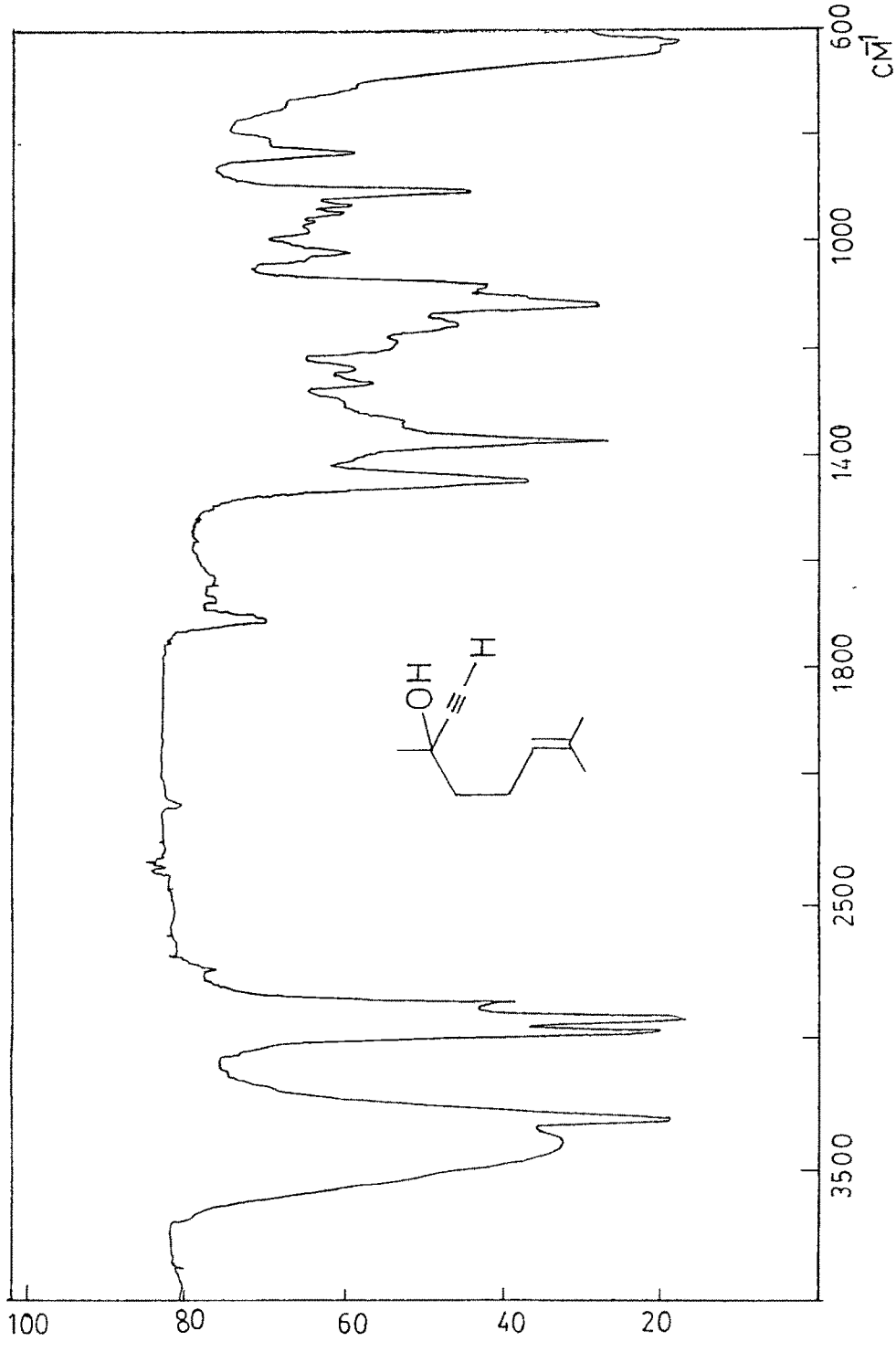


Fig. 23: IR spectrum of 3,7-Dimethyl-6-octen-1-yn-3-ol (10)

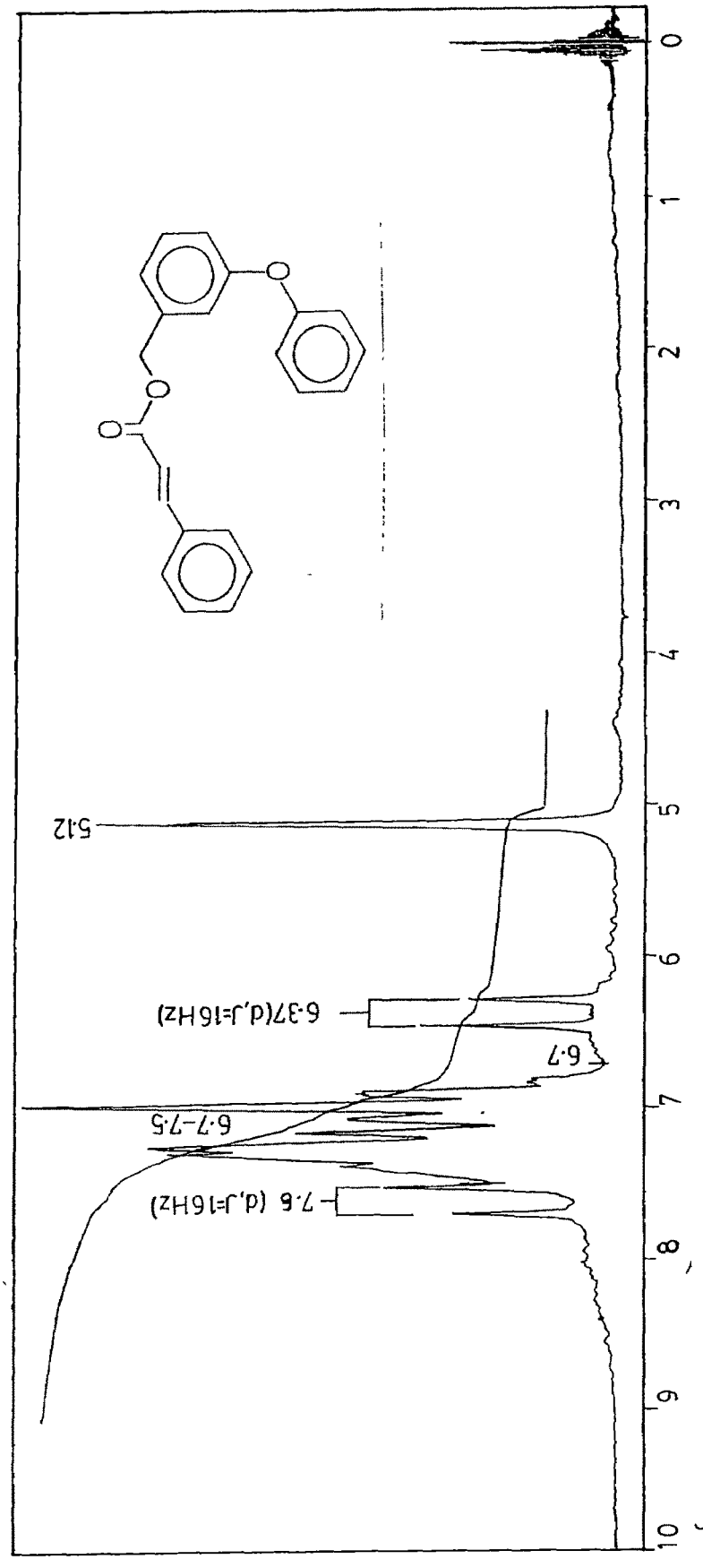


Fig. 24: ^{1}H-NMR spectrum of 3-Phenoxybenzyl cinnamate (18)

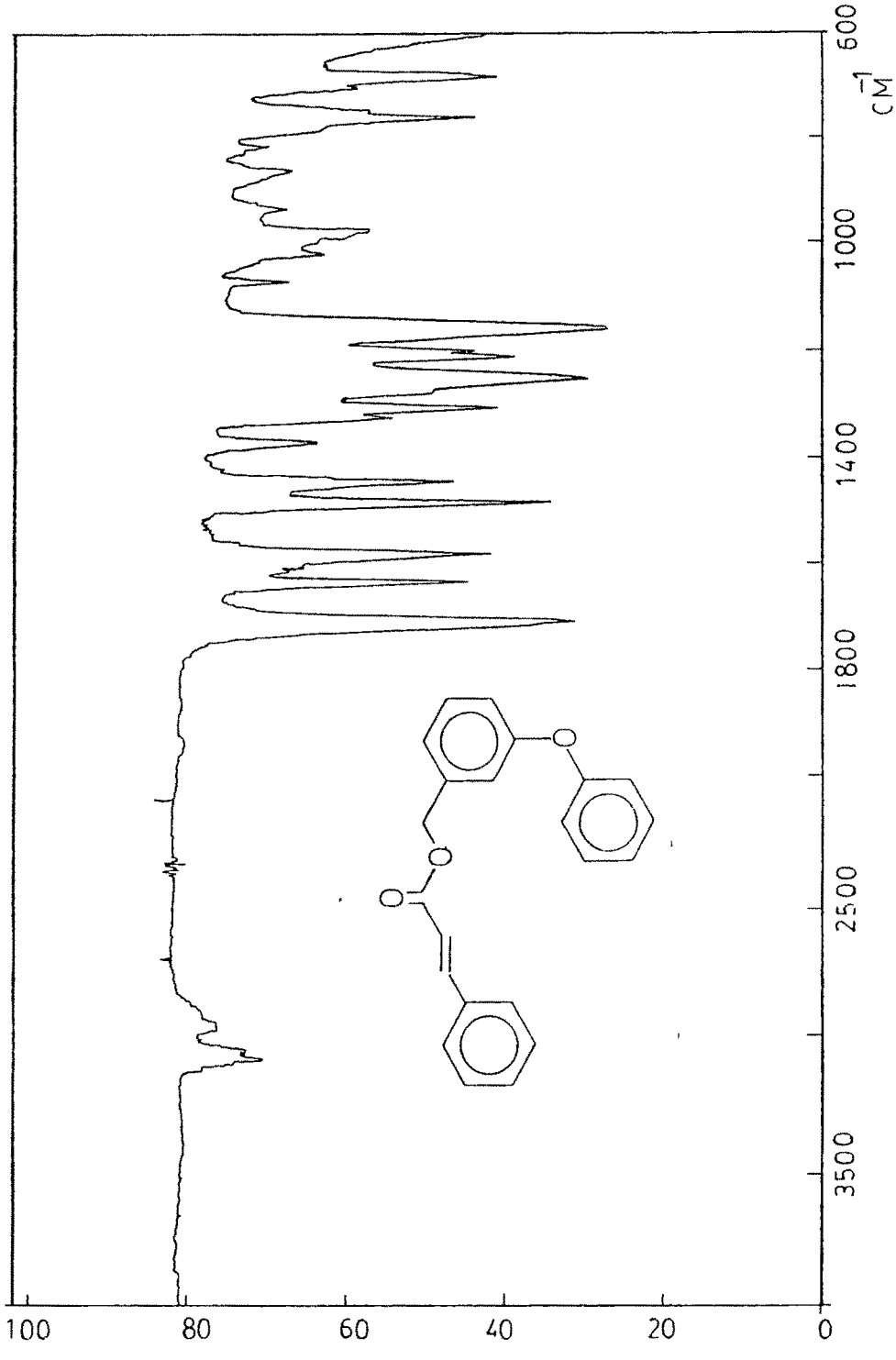


Fig. 25: IR spectrum of 3-Phenoxybenzyl cinnamate (18)

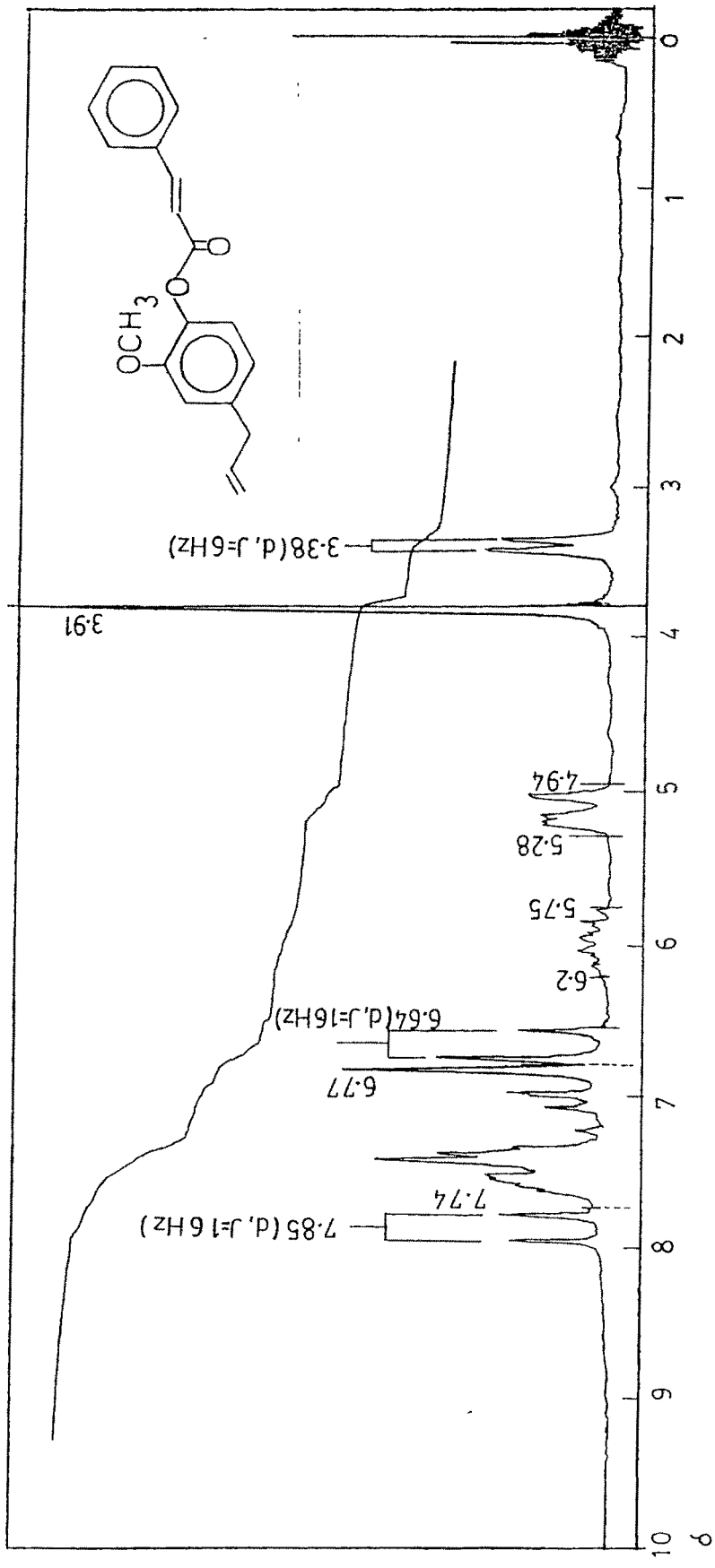


Fig. 26: ^{1}H-NMR Spectrum of Eugenyl cinnamate (19)

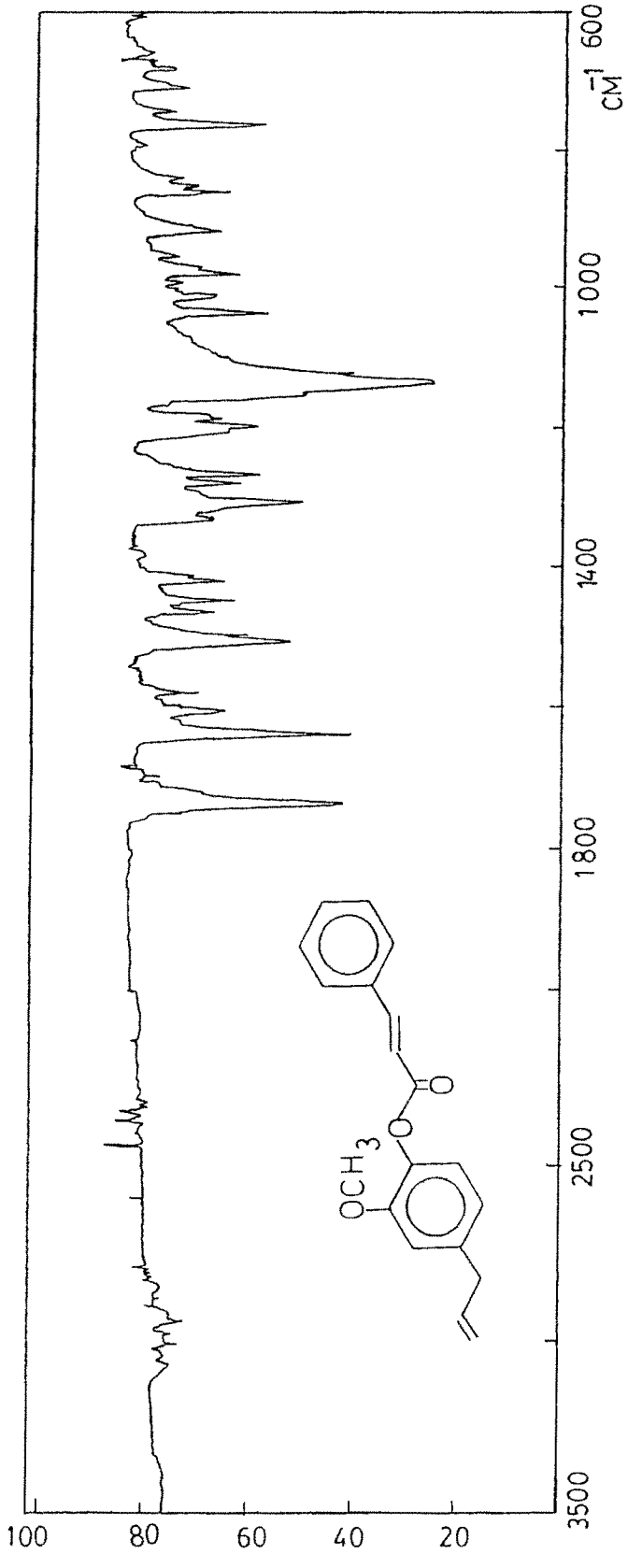


Fig. 27: IR spectrum of Eugenyl cinnamate (19)

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