SYNTHESIS AND BIOASSAYS OF *Chilo partellus* FEMALE SEX PHEROMONES AND THEIR ANALOGUES.

BY

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The work in this Thesis has not been presented for any Degree in any other University.

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DEDICATION

To Nambutie and Situma

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Abstract

Synthetic sex pheromones could provide valuable alternative means of pest control in Integrated Pest Management (IPM) strategies. Their uses could include population monitoring with pheromone traps to guide other control methods, by mass trapping and mating disruption.

(Z)-11-Hexadecenal (Z-11-$C_4H_9CH=CH(CH_2)_9CHO$) and (Z)-11-hexadecen-1-ol (Z-11-$C_4H_9CH=CH(CH_2)_9CH_2OH$), the major components of spotted stalk borer, *Chilo partellus* (swinhoe) female sex pheromone, were prepared in good yields from readily available 1,10-decanediol by an acetylenic route, and (Z)-9-tetradecenyl formate (Z-9-$C_4H_9CH=CH(CH_2)_7CH_2O-CHO$), an analogous structure was prepared from 1,8-octanediol by the analogous acetylenic route. Simple distillation of the 1-bromo-10-(2-tetrahydropyranyloxy) decane, coupling with 1-hexyne in liquid ammonia and lithamide followed by deprotection gave 11-hexadecyn-1-ol ($C_4H_9C\equiv C(CH_2)_9CH_2OH$) (b.p 96-98^0 C/0.04 mmHg; 75% yield). Partial hydrogenation of 11-hexadecyn-1-ol on a Lindlar catalyst gave (Z)-11-hexadecen-1-ol (b.p 75^0 C/0.01 mmHg, 95% yield) which on oxidation gave (Z)-11-hexadecenal (Z-11-$C_4H_9CH=CH(CH_2)_9CHO$) (b.p 71^0 C/0.01 mmHg; 80% yield) containing 1.3% of the (E) isomer by GC analysis on a methyl silicone column. (E)-11-Hexadecenal and (E)-11-hexadecen-1-ol were prepared *via* sodium/liquid ammonia reduction of the intermediate $C_4H_9C\equiv C(CH_2)_{10}OTHP$, giving the final product containing 1% of the (Z)-isomer. Pyridinium chlorochromate in dichloromethane was used for the oxidation of the olefinic alcohols to the corresponding

aldehydes (yield, 76-79%). The other analogues were obtained by derivatizing the aldehydes or alcohols of the intermediates or the final products in the synthesis.

The Electroantennographic (EAG) tests showed that the compounds differed in their ability to evoke EAG responses and they were lower than (Z)-11-hexadecenal. The EAG responses confirmed that (Z)-11-hexadecenal was a better stimulant than the corresponding alcohol (Z)-11-hexadecen-1-ol.

CHAPTER 1.

INTRODUCTION.

1.0 General Considerations.

The survival of the human species depends on the availability of food, which is either directly or indirectly supplied by plants. Man, therefore, has to control pests of all kinds that destroy the greater part of crops, both before and after harvest. Although great effort has been put in eradicating these pests, one third of the annual harvest in the world is destroyed by pests (Marini-Bettolo, 1976). Apart from other causes of food losses like diseases and weeds, pests consume upto forty percent of Africa's food reserves (Mohyuddin and Attique, 1978).

Tropical countries suffer the most severe crop loses due to pests because of conducive climatic conditions. The use of insecticides has been the most effective method of crop protection against insect pests (Mandava, 1985), although there has been a number of drawbacks. Firstly, most insecticides are synthetic, non-selective and toxic chemicals which have led to serious social and environmental repercussions. Human poisoning by insecticides is very common particularly in developing countries where safe handling and application of chemicals is not always feasible due to several socio-economic factors (Saxena, 1989).

Insect resistance by some pests has led to the use of higher doses or more powerful insecticides, which are not only uneconomical but also elevates this problem of resistance and

contamination of the environment (Saxena, 1989). Ecological imbalance may be brought about by the use of some insecticides like organo-chlorine derivatives which are degraded very slowly, or not at all, by biological factors such that they may last in the environment for many years (Marini-Bettolo, 1976). This calls for development of better, more specific and biodegradable pesticides (Mandava, 1985; Jacobson, 1975).

Scientists therefore have a task ahead of them to improve the already existing methods of pest control, develop others and implement new methods. Use of natural products derived from metabolic activities of plants and animals has been one of the approaches. These approaches seem feasible because plants are the richest source of bioactive organic chemicals. Natural products from these plants have the advantages as insecticides because they are renewable, biodegradable, more selective and less resistant to biological factors (Alkofahi et al., 1989). Semiochemicals emitted by pests are also of great importance and field application of stem borer pheromones in monitoring, mass trapping, and mating disruption of these pests, as a control strategy is in progress (Unithan and Paye, 1991; Campion and Nesbitt, 1983). The advantages of the pheromone based control methods lie in the species specificity of the pheromones (Nesbitt et al., 1979) in their biological activity which means that only relatively small amounts are required, and in their negligible toxic effect on the environment (Campion and Nesbitt, 1983).

1.1 Scope of *Chilo partellus*.

Chilo partellus (swinhoe) is one of the most widespread pests in tropical Africa and the Indian sub-continent (Harris, 1985; Alighali, 1985). This pest is not endemic to Africa but was brought here by ships from the Indian sub-continent early this century (Hill, 1983). The pest found the local environment hospitable and successfully invaded and colonised most countries in eastern and southern Africa. Forty one species of the genus *Chilo* are known of which twenty five of these, including eighteen species which occur in Africa, infest cereals (Beevor et al., 1990). Essentially, it is a pest in hot lowland areas and is seldom found above an altitude of 1500M (Hill, 1983). The main hosts of *Chilo partellus* larvae are maize (Mohyuddin and Attique, 1978), sorghum (Seshu Reddy, 1988), sugarcane, rice, millet, and wheat (Harris, 1985). The larvae may attack various parts of the crop and at various stages of the plant growth. However, the larvae prefer feeding on the young plant rather than the older ones (Singh and Rana, 1989; Teetes et al., 1983). The severity of infestation largely depends on climatic conditions and the number of aestivating/diapausing larvae which perpetuate infestation from season to season. Control of these stem borers is difficult because the damage is inside the stalks where they are protected from insecticides.

The survival of *Chilo* spp. in the dry season depends upon alternate host and wild grasses, although the greatest proportion of carryover is in residues left in the field (Wheatly, 1961). The

maize stubbles, stalks, and cobs have been found to harbour borers. In Nigeria, Adeyemi (1969) found that the maize stubbles left after the early season harvest had an average of 27 borers per 100 stubbles with *Busseola fusca* and *Sesamia calamistis* as most abundant. Grain yield loss caused by *C. partellus* larvae in sorghum alone have been reported to be upto 80% in Kenya (Pathak, 1991). The residual infestation, together with confirmed possibility of *Chilo* spp. in Kenya (Scheltez, 1978) to diapause, makes the borer population sufficiently large to ensure infestation of the succeeding crop. Hargreaves (1939), working in Uganda, found that continuous sowing undertaken by local farmers provide continuous supply of young plants, which provide continuous food for the borer to complete its life cycle and infest other young plants. While studying the seasonal incidence of *Chilo* spp. it was found that the average number of larvae was greater in cooler than in hot season, but the average number of pupae, the percentage stem length tunnelled and percentage internode attacked were greater in the hot season, indicating that the attack was more severe in the hot season than in the cool season (Khan and Khan, 1969; Sarup et al., 1978). *Chilo* spp. are particularly more difficult to control largely because of the cryptic, nocturnal habits of the adult moths, and the protection afforded by the stem or cob of the host crop to the developing stages (Singh and Rana, 1989). Insecticidal control measures have proved difficult to apply effectively. In case of sugar cane, high crop density makes access for ground spraying almost impossible. In rice cultivation, insecticide

application is detrimental to beneficial insects and can cause serious pollution of waterways and to fish (Beevor et al., 1990). To control the borers it is important to destroy all stubbles during the dry season. This enables the initial infestation in the long rain crop to remain low and allow the crop to pass through the most susceptible stage before larvae borer population builds up. The disposal of such residues containing the resting phase larvae by burning, composting or as cattle beddings could reduce re-infestation. From early 1920's, legislation to secure a maize free period in Kenya has been used as a means of stalk borer control (Anderson, 1929). It was doubtful, however, how such a method would be administered efficiently because compelling farmers to burn or destroy all crop residues after harvest, instituting a close season with no volunteer maize and destruction of all graminaceous wild species cannot be practicable (Ingram, 1958). The commonest method of control of borers is the use of insecticides which have the advantage that their immediately obvious effectiveness to the farmer. This, however, is expensive, difficult to obtain, and occasionally may be phytotoxic and hazardous to other forms of life (Sukhani and Jotwani, 1977). In Kenya, the use of insecticides on cereal borer control is not widespread. Indeed, their use has been limited to the commercial large-scale farmers as the costs have been prohibitive to the many small-scale farmers. Ingram (1958) reported that chemical control needed many applications to control the continuous attack of *Chilo* spp. and *Sesamia* spp. However, these treatments do not show

substantial increase in crop yield and, therefore, makes the control uneconomical for peasant farmers (Warui and Kuria, 1983). Thus alternative control measures that would overcome the drawbacks of insecticides are required. It was with the purpose of limiting the use of these pesticides that the search for suitable biological control methods was initiated. In 1991, with funding from the government of Netherlands, the International Centre of Insect Physiology and Ecology (I.C.I.P.E) and the Wageningen Agricultural University (in Netherlands) initiated a project on the biological control of the spotted stem-borer. Biological control using wasps (in Pakistan) on stem borers proved highly effective method of control. The wasps were released into the cereals infected by stem borers and was expected to control the population of the borer. Feeding deterrents are being tried as one of the measures (Lwande et al., 1986; Hassanali et al., 1986). This would be desirable when environmental factors are considered. Moreover antifeedants and feeding stimulants from plants provide knowledge about the inter-relationships between insects and plants. This in turn will contribute toward the development insect-resistant crop varieties.

Several cultural practices have a profound influence on the insect survival. The persistence of insects in a particular environment cause damage to the crop and each cultural practice needs to be evaluated as part of the total crop production system. Sometimes even a slight population reduction brought about by these practices delays the build up of insect numbers which consequently reduces the plant damage (Pathak and Dhaliwal, 1986). Although

these methods are simple and easy to follow, they are subject to the variability of agroecological zones, weather and are sometimes heavily dependant on the cooperation of neighbouring farmers. Therefore, cultural control practices as a component of IPM, to be successful and cost effective, will need group endeavour on the populations of cereals stem borers. Some cultural practices which are intended to disrupt or slow-down the population build-up of *Chilo* spp in cereals are tillage and mulching, sanitation, destruction of volunteer and alternate host plants, time of planting, plant density, exploitation of ovipositional behaviour, removal of infested plants, intercropping and irrigation.

1.1.1 Mating and Egg Fertility of *Chilo* spp.

An understanding of the mating process and the factors controlling it, and its effects on the reproductive potential is essential, if pheromonal control strategies aimed at interfering with the pests reproduction are to be developed. Kumar and Saxena (1985) reported that maximum mating activity in *Chilo partellus* takes place after midnight, on night following emergence, and egg laying occurs between 1600 and 1800 hours on the first day after mating.

In the Asiatic rice borer, *Chilo suppressalis* (Walker), mating activity of males remained at a high level for 8 days and that of the females for 4 days (Kanno and Satto, 1978). In *Perctinophora gossypiella* (saunders) also fewer females mated when pairing was delayed (Lingren et al., 1988). Decline in mating frequency with

age has also been noted in other species of moths (Ellis and Stele, 1982; Henneberry and Clayton, 1985). Female longevity in *Chilo partellus* is related to mating, and long lifespan in late mated females is not accompanied by an extended reproductive period (Unithan and Paye, 1991). Increased female longevity associated with delayed mating has also been observed in other lepidoptera (Ellis and Steele, 1982; Proshold et al., 1982; Henneberry and Clayton, 1985; Lingren et al., 1988).

Realization of the reproductive potential in *C. partellus* is dependent on the females age at mating, since late mated females lay fewer eggs than early mated females (Unithan and Paye, 1991). This was also observed in other species of moths e.g *Ephestia cautella* (walker) (Barrer, 1976), *Heliothis virescens* (F) (Proshold et al., 1982).

Chilo partellus males are able to mate once per night for several nights and the spermatophores transferred are effective in producing fertile eggs (Unithan and Paye, 1991), and in the laboratory it was shown that the males emerge 2-3 days earlier than the females (Ochieng et al., 1985). If this is also true in the field then males ability to mate repeatedly over 5-6 days ensures insemination of the late emerging females. A single spermatophore received at a night is enough to fertilize eggs throughout the females life. Females very seldom mate more than once, and the mated females are less attractive to males in the field (Unithan and Paye, 1991), and therefore males are likely to mate with only unmated females. The ability of the males to mate several times

has been reported also in other Lepidoptera e.g *Prodenia litura* (Jarczyk and Hertle, 1960), *Earias insulana* (Kehat and Gordon, 1977), and *S. littoralis* (Kehat and Gordon, 1975).

1.2 Pheromones.

A pheromone is a chemical messenger which is secreted by a member of an animal species and which elicits a definite behavioural response in another member of the same species (Campion and Nesbitt, 1983). In many lepidopterous pests, sex pheromones are secreted by one sex, usually the female, to attract members of the opposite sex for mating. Pheromones have now been isolated and identified for many species. Typically but with some exceptions, moth sex pheromones are produced in the glands located in the terminal abdominal segments and are long chain, unsaturated esters, alcohols, or aldehydes (Ando et al., 1977). Pheromones generally consists of more than one component and these may be isomers with respect to geometry or the position of the unsaturation. Or, they may be structurally related compounds differing in chain length or the nature of the functional group (Beevor et al., 1981; Campion and Nesbitt, 1983). In most cases the components are secreted in very precise ratio (Campion and Nesbitt, 1983).

1.2.1 Utilization of Sex Pheromones for the Control of Stem Borers.

Pheromone baited traps are now widely used in monitoring to assess the population levels of insect pests in both Agriculture and Forestry (Unithan and Paye, 1991). The simplicity of

construction and maintenance of the traps and their species-specificity gives them many advantages over light traps and other methods of population sampling (Campion and Nesbitt, 1983; Klassen et al., 1982). Two principle methods of using sex pheromones for population suppression of insect pest exist. In mass trapping, large numbers of traps are used to reduce populations to an acceptable levels. In mating disruption, the area under treatment is permeated with synthetic pheromones so that the male moths cannot detect the relatively small amount of pheromone produced by a female moth and mating and subsequent larval infestations are prevented. The advantage of pheromone-based control methods over the conventional methods lie in species specificity of pheromones (Nesbitt et al., 1979). The relevance of pheromones in general to the needs of developing countries was discussed by Campion and Nesbitt, (1981). The major problem in the study of lepidopterous stem borers is in determining the origin of the parent moths which initiate the infestation, since an attack is normally noticed when the larvae appears shortly after beginning of the growing season. The use of pheromone traps could serve at least initially to define more clearly the periods of the moths flight and to indicate when the crop is at high risk so that the individual farmers can take appropriate action.

Mating disruption does seem promising for control of *Chilo* spp and mass trapping of males using pheromone traps will be effective in reducing the production of viable eggs if a high proportion of the males can be removed from the population. Similar reservations

have been expressed in the case of other species of moths (Kehat and Gordon, 1975; 1977; Campion and Nesbitt, 1983). On the other hand, in *C. partellus* delayed mating can result in a marked reduction in the number of viable eggs due to combined effects of reduced fecundity and reduced fertility (Unithan and Paye, 1991). Thus delaying or disrupting mating by permeating the field with pheromone components, if achieved, could be an effective strategy for suppressing *C. partellus* population.

1.2.2 Identification of Stem-borer Pheromones.

The sex pheromones and attractants for lepidopterous stem-borers which have been identified are listed in Table 1 below. The species include the African stalk-borer, *Busseola fusca* (Fuller) (Nesbitt et al., 1980a; Hall et al., 1981) and three *Chilo* species- (Nesbitt et al., 1975b; 1979; 1980b Ohta et al., 1976) the sugar cane borer, *Chilo sachariphagus* (Bojer), an important pest in Java and Mauritius, *Chilo partellus* (Swinhoe) a pest of cereals, rice, and sugar-cane in Eastern and Southern Africa and Indian subcontinent, and *Chilo suppressalis* (Walker), a major pest of rice in S.E.Asia. The European corn-borer, *Ostrinia nubilalis* (Hubner) (Klun and Junk, 1977) is the most important pest of maize in Europe, and N.America. *Ostrinia furnicalis* (Guenee) (Chieng et al., 1981) occupies a similar position in Asia. The pheromones of two *Sesamia* species (Zagatti et al., 1981) have been investigated. An attractant has been found for *Sesamia cretica* (Led) (Arsura et al., 1977) which attacks sorghum in Southern

Europe and North Africa and the major component of the pheromone of *Sesamia inferens* (Walker) has been identified. The latter is a pest attacking most graminaceous crops throughout Asia. The African sugar-cane borer, *Eldana saccharina* (Walker) (Kunesch et al., 1981) is unusual in that like the other species of the Galleriinae the male moth produces a sex pheromone from appropriate sex glands. The sex pheromones of the two New World stem borers, *Diatraea saccharalis* and *D. rufescens*, are currently under investigations and the structure of one component of the pheromone of the former species has been identified.

Table 1. *Identification of lepidopterous stem-borer pheromones and attractants.*

Species	Host crop	Pheromone component	Ref.
Busseola fusca	Maize, Sorghum	(Z)-11-tetradecenyl acetate (E)-11-tetradecenyl acetate (Z)-9-tetradecenyl acetate	Nesbitt et al., 1980a ,, Hall et al., 1981
Chilo sacchariphagus	Sugar-cane	(Z)-13-octadecenyl acetate	Nesbitt et al., 1980b
Chilo partellus	Sugar-cane, Sorghum, Rice	(Z)-11-hexadecenal (Z)-11-hexadecen-1-ol	Nesbitt et al., 1979 ,,
Chilo suppressalis	Rice	(Z)-11-hexadecenal (Z)-13-octadecenal	Nesbitt et al., 1975b Ohta et al., 1976
Ostrinia nubilalis	Maize	(Z)-11-tetradecenyl acetate (E)-11-tetradecenyl acetate	Klun et al., 1973 Klun and Junk, 1977 Mustea, 1973 Carde et al., 1975 Kochansky et al., 1975
Ostrinia furnacalis	Maize	(Z)-12-tetradecenyl acetate (E)-12-tetradecenyl acetate	Ando et al., 1980 Klun et al., 1980 Chieng et al., 1981
Eldana saccharina	Sugar-cane	(E)-4-methyl-5-(3-methyl-2-butenyl)tetrahydrofuran-2-one	Kunesch et al., 1981
Sesmia inferens	Maize, Rice Sugar-cane	(Z)-11-hexadecenyl acetate	Nesbitt et al., 1976 Zagatti et al., 1981
Diatraea saccharalis	Sugar-cane	(Z,E)-9,11-hexadecadienal	Hammond et al., 1980
Sesmia cretica	Sorghum, Maize Sugar-cane	(Z)-9-tetradecen-1-ol (Z)-9-tetradecenyl acetate	Arsura et al., 1977 ,,

1.2.3 Field Application of Stem-borer Pheromones.

Control of insect population can be attempted by treating the target crop with the appropriate pheromone to prevent male moths from locating "calling" females and thus suppress mating. This has led to the development of slow release formulations which will maintain relatively high concentrations within the crop for up to several weeks after their application.

Initially this was achieved by using the same dispensing systems that were employed in traps and attaching large numbers of these by hand (Nesbitt et al., 1975b). More recently, commercial formulations have been developed consisting of hollow fibres, plastic laminates and microcapsules (Campion et al., 1978; Brookes et al., 1979). Several attempts have been made to control *O. nubilalis* by mating disruption, in some cases before the biology of the insect had been fully investigated. For example, it was found in both Canada and Switzerland that a substantial amount of mating occurred outside the maize crop (Showers et al., 1976; Buchi et al., 1981). This in itself would make the technique ineffective unless an area wide treatment was undertaken to include both the maize crop and all the adjacent cultivations. Even if control was achieved, it is probable that the cost of treatment would be economically unacceptable. Thus control is possible, but only if the treated area is well isolated and the movement of insects in and out greatly limited. Beevor et al., (1977) showed that air permeated with synthetic pheromone of *Ostrinia* spp caused communication disruption in small scale trials carried out in rice

fields in the Philipines. It was shown that the more stable pheromone analogues (Z)-9-tetradecenyl formate, and (Z)-11-hexadecenyl formate were effective disruptants. When these were dispensed from polyethylene vials surrounding a trap baited with synthetic pheromone, catches of male moths were virtually eliminated (Beevor et al., 1977). Mating suppression tests in field cages using (Z)-9-tetradecenyl formate, and (Z)-11-hexadecenyl formate dispensed from polyethylene vials indicated high levels of disruption activity (Beevor and Campion, 1979; Beevor et al., 1981). Similar tests on *O. nubilalis* were conducted in Japan with a number of other pheromone analogues, the most effective of which was the hydrocarbon, (Z)-5-hexadecene (Kanno et al., 1978). Open field trials in 0.2 ha rice plots using (Z)-5-hexadecene in rubber dispensers were also successful in that significant reduction in plant damage was achieved, in treated areas (Kanno et al., 1980; Tatsuki and Kanno, 1981). In Korea, a different type of microcapsulated formulation of the aldehyde pheromone component was shown to reduce catches of male moths of *Chilo* spp in traps baited with virgin females in the field (Lee et al., 1981). It was suggested by Rothschild (1981) that mating disruption is unlikely to be successful in situations where a crop is attacked by a complex of pests. Under such circumstances it would be advantageous to disrupt mating of both species simultaneously by using an appropriate combination of pheromones.

1.2.4 Method of Extraction and Identification of the Female Sex Pheromones of *Chilo partellus.*

The composition of the pheromone emitted by a single female moth was examined using charcoal air filters (Nesbitt et al., 1979; Grob and Zurich, 1976) to trap the volatiles. Gas chromatography (GC) analysis combined with simultaneous recording of male moth Electroantennograph (EAG) responses to the column effluent were carried out as described by Mourhouse et al., (1969) and Nesbitt et al., (1977). Purification of tip extracts after GC-EAG analysis established that the female tip extracts contained two olfactory stimulants for the male moths, an aldehyde, (Z)-11-hexadecenal ($Z\text{-}C_4H_9CH{=}CH(CH_2)_9CHO$) and an alcohol (Z)-11-hexadecen-1-ol ($Z\text{-}C_4H_9CH{=}CH(CH_2)_9CH_2OH$) were identified. These were purified by a method involving conversion of the aldehyde into a bisulfite adduct prior to various microchemical reactions (Nesbitt et al., 1979). Pure alcohol was obtained by collection of the appropriate peaks from Carbowax 20 M and Apiezon L. GC columns as described by Nesbitt et al., (1975a). The aldehyde was regenerated by addition of sodium carbonate to the aqueous phase after extraction of the alcohol with dichloromethane (Nesbitt et al., 1979). The major component (aldehyde) had a lower retention temperature on all columns and was approximately 20 ng/tip equivalent and the amount of minor component (alcohol) was approximately 3 ng/tip equivalent (Nesbitt et al., 1979). Field trials of the two compounds showed that the major aldehydic component alone , when dispensed from polyethylene vials, was comparable in attractiveness with the

female moth. However the exact function of the alcohol was uncertain, since during the initial field trials it was reported that addition of (Z)-11-hexadecen-1-ol to (Z)-11-hexadecenal in the 1:7 ratio found in female extracts (Campion et al., 1978) reduced the trap catches to level of unbaited traps (Nesbitt et al., 1979). The detection of the alcohol in the airborne volatiles from a female "calling" *Chilo partellus* indicates that the alcohol is actually emitted by the moth and would suggest that it has a definite function in premating behaviour, despite the observed effect on trap catches. The situation in *C. partellus* thus seems similar to that reported for the tortricid moth *Choristoneura fumiferana*. In the latter, the main attractant component of the female sex pheromone was identified as (E)-11-tetradecenal (Weatherston et al., 1971) and the attractiveness of the synthetic aldehyde to male moth was shown to be reduced by addition of (E)-11-tetradecen-1-ol (Sanders et al., 1972). Both the aldehyde and the alcohol were found in tip extract, but only the aldehyde was detected in washings from jars that had held female moths (Weatherston and Maclean, 1974) and trapped on porapak Q (Weatherston et al., 1975). Weatherston and Maclean, (1974) concluded that the alcohol was merely a biosynthetic precursor to the aldehyde. More recently re-examination of the pheromone obtained by rinsing out female moths containers showed it to be a mixture of the (E) and (Z) isomers of 11-tetradecenal in a ratio 94:4 and addition of small percentage of the (Z) isomer to the pure synthetic (E)-11-tetradecenal was found to be necessary to optimize

attraction (Saunders and Weatherston, 1976). No comment was made on the isomeric composition of the 11-tetradecen-1-ol in tip extract. (Z)-11-Hexadecenal has also been identified as the major component of the female sex pheromone of *Chilo suppressalis* where its attractiveness to the male moth is synergized by a second component (the homologous aldehyde, (Z)-13-octadecenal) (Nesbitt et al., 1975b; Beevor et al., 1977).

Although chemical analysis of *C. partellus* tip extracts showed two main components, (Z)-11-hexadecenal and (Z)-11-hexadecen-1-ol, thirteen additional minor components have been identified (Hassanali et al,.1992) all of them saturated or monosaturated aldehydes or alcohols (Table 2).

In both *C. partellus* and *C. suppressalis*, (Z)-9-tetradecenyl formate, a compound structurally related to (Z)-11-hexadecenal has been found to be a potent olfactory stimulant for the male moth (Nesbitt et al., 1975b; Nesbitt et al., 1977). With *C. suppressalis* this same formate has been shown to disrupt communication between male and female moths in the field (Beevor et al., 1977). (Z)-11-Hexadecenal has also been reported as a female sex pheromone component in the noctuid species virescens, *H. zea* (Roelofs et al., 1974; Tumlinson et al., 1975) and *H. armigera* (Piccardi et al., 1977) and in the plutellid, *Plutellid xylostella* (Tamaki et al., 1977; Chow et al., 1977). (Z)-11-Hexadecen-1-ol has been found as a pheromone component in the clover cutworm *Scotogrmma trifolii* (Underhill et al., 1976).

Table 2. Components identified from female *C. partellus* gland extracts.

Name	Structure
Tetradecanal	$CH_3(CH_2)_{12}CHO$
(Z)-9-Tetradecenal	$Z\text{-}9\text{-}C_4H_9CH{=}CH(CH_2)_7CHO$
(Z)-10-Pentadecenal	$Z\text{-}10\text{-}C_4H_9CH{=}CH(CH_2)_8CHO$
(Z)-11-Tetradecenal	$Z\text{-}11\text{-}C_2H_5CH{=}CH(CH_2)_9CHO$
Hexadecanal	$CH_3(CH_2)_{14}CHO$
(Z)-9-Hexadecenal	$Z\text{-}9\text{-}C_6H_{13}CH{=}CH(CH_2)_7CHO$
Tetradecan-1-ol	$CH_3(CH_2)_{12}CH_2OH$
(Z)-11-Hexadecenal	$Z\text{-}11\text{-}C_4H_9CH{=}CH(CH_2)_9CHO$
(Z)-10-Pentadecen-1-ol	$Z\text{-}10\text{-}C_4H_9CH{=}CH(CH_2)_8CH_2OH$
Octadecanal	$CH_3(CH_2)_{16}CHO$
Hexadecan-1-ol	$CH_3(CH_2)_{14}CH_2OH$
(Z)-13-Octadecenal	$C_4H_9CH{=}CH(CH_2)_{11}CHO$
(Z)-7-Hexadecen-1-ol	$C_8H_{17}CH{=}CH(CH_2)_5CH_2OH$
(Z)-11-Hexadecen-1-ol	$C_4H_9CH{=}CH(CH_2)_9CH_2OH$
Octadecan-1-ol	$CH_3(CH_2)_{16}CH_2OH$

1.3 Aim of the Project.

To synthesize the female *Chilo partellus* sex pheromones and their analogues and to measure EAG evoked by these compounds as a prelude to behavioural tests. The long-term aim of the project is to determine if: (a) the presence of small amounts of certain analogues would affect the attractancy performance of synthetic pheromones.

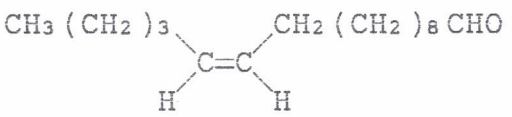
(b) effective disruptants of the action of pheromones can be identified.

The following compounds were synthesized

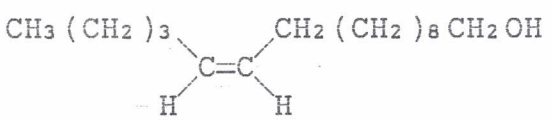
1.3.1 The Female Sex Pheromones.

Female *Chilo partellus* (swinhoe) abdominal tip extracts were found to produce two olfactory stimulants, which were identified as,

(Z)-11-hexadecenal.



(Z)-11-hexadecen-1-ol.

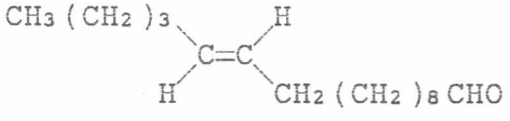


Both compounds were detected in the volatiles of "calling" female moth (Nesbitt et al., 1979). The female tip extracts from a female *Chilo partellus* revealed that the olfactory stimulants found in a "calling" female volatiles were produced in the ratio of 7:1, the aldehydic component being the major compound (Nesbitt et al., 1979; Campion et al., 1978). These compounds would be used as reference samples and their electroantennographic activities as a basis for comparing with those of the analogues.

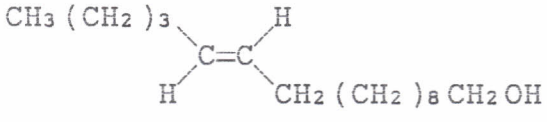
1.3.2 Analogues

1.3.2.1 **Geometric Isomers of the Pheromones**

(E)-11-hexadecenal.



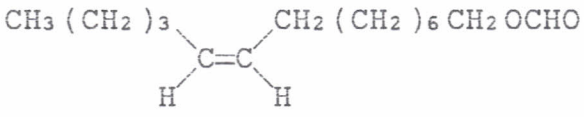
(E)-11-hexadecen-1-ol.



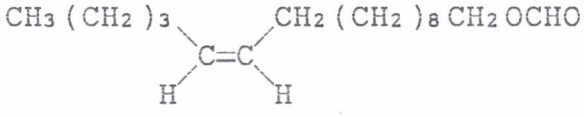
Since, however stereospecific the synthetic method used small amounts of these isomers would be expected to be present their biological activities if any would be important with respect to the performance of the synthetic pheromones.

1.3.2.2 **Formates**

(Z)-9-tetradecenyl formate

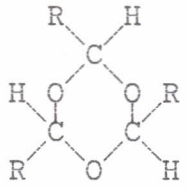


(Z)-11-hexadecenyl formate



It has been reported for some lepidoptera which have monosaturated aldehydes as their natural female sex pheromones that the formates derived by replacing the β-methylene group of the aldehyde by oxygen are potent olfactory stimulants for the male moth e.g in *Heliothis zea*, (Z)-9-tetradecenyl formate elicits a stronger EAG response than natural pheromone (Z)-11-hexadecenal (Priesner et al., 1975). Similarly (Z)-9-tetradecenyl formate and (Z)-11-hexadecenyl formate are strong olfactory stimulants for *Chilo suppressalis*, although, in this case less potent than the aldehydes of natural pheromones, (Z)-11-hexadecenal and (Z)-13-octadecenal) (Nesbitt et al., 1975b; Beevor et al., 1977). These findings prompts the synthesis of (Z)-9-tetradecenyl formate and (Z)-11-hexadecenyl formate and testing them on *Chilo partellus* males and their EAG response comparable to that of the synthetic aldehyde (Z)-11-hexadecenal.

1.3.2.3 **Trimer of the Aldehyde.**



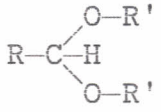
Where R is (Z)-$C_4H_9CH=CH(CH_2)_9$-

It has been reported that materials which contain large quantities of the trimer is unsuitable for use, even when applied at high dosages. This is probably due to the effect of the trimer on the dispensers (Dunkelblum et al., 1984). For example the sex pheromone of the spiny bollworm *Earias insulana* (Boisduval) has been identified as (E,E)-10,12-hexadecadienal (Hall et al., 1980; Klug et al., 1982). Field tests showed that funnel traps baited with 2 ng of the synthetic pheromone absorbed into the polyethylene vials captured *E. insulana* effectively (Kehat et al., 1981a). The sex pheromone traps were proposed as a potential means of improving control programs for *E. insulana* (Kehat et al., 1981b). Surprisingly, the pheromone prepared for the 1982 season gave erratic and significantly lower male catches than in previous years. Ultimately it was found that these samples trimerized extensively (Dunkelblum et al., 1984). Thus it would be interesting to note the response of male *Chilo partellus* towards the trimer of the aldehyde.

The characteristic response of the ethers from the trimer are associated with the stretching vibration of the C-O-C system. The vibrational characteristic of this system would be in the same

region as the C-C-C system, however since vibrations involving oxygen atoms result in greater dipole moment, changes other than those involving carbon atoms, more intence bands are observed for trimer ethers. In the IR spectra the most characteristic absorption is the band 1215 cm^{-1} (Fig 50) which diminishes on storage. A frehly prepared trimer was also be devoid of aldehydic properties. However, the trimer is a difficult compound to handle since it cannot be detected on a capillary GC as this would cause thermal decomposition of the trimer to a monomer (Dunkelblum et al., 1984).

1.3.2.4 **Acetal**



Where R is $(Z)\text{-}C_4H_9CH{=}CH(CH_2)_9\text{-}$

and R' is $(Z)\text{-}C_4H_9CH{=}CH(CH_2)_9CH_2\text{-}$

This compound is related to the trimer structurally, in that, both have the ether linkages. Since the compound is likely to be formed in a synthetic mixture of the aldehyde and the alcohol its effect on *C. partellus* would be interesting to study. The trimer and the acetal have comparable molecular weights, but the latter cannot undergo thermal decomposition on a capillary GC.

1.3.2.5 Effect of Acetylenic Bond.

$C_4H_9C{\equiv}C(CH_2)_9CHO$

The EAG response of *C. partellus* to this compound comparable to the aldehydal pheromone would be desirable. And also the bromo derivatives of the aldehyde and their analogous formates of related molecular weights will be studied.

1.3.2.6 Structural Determination .

The structures of these synthetic sex pheromones would be determined with the help of a combination of the following spectroscopic analytical methods:

1/.Proton nuclear magnetic resonance (^{1}H-NMR) spectra.

2/.Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra.

3/.Mass spectra.

4/.Gas chromatographic (GC) profiles.

5/.Infra-red (IR) spectra.

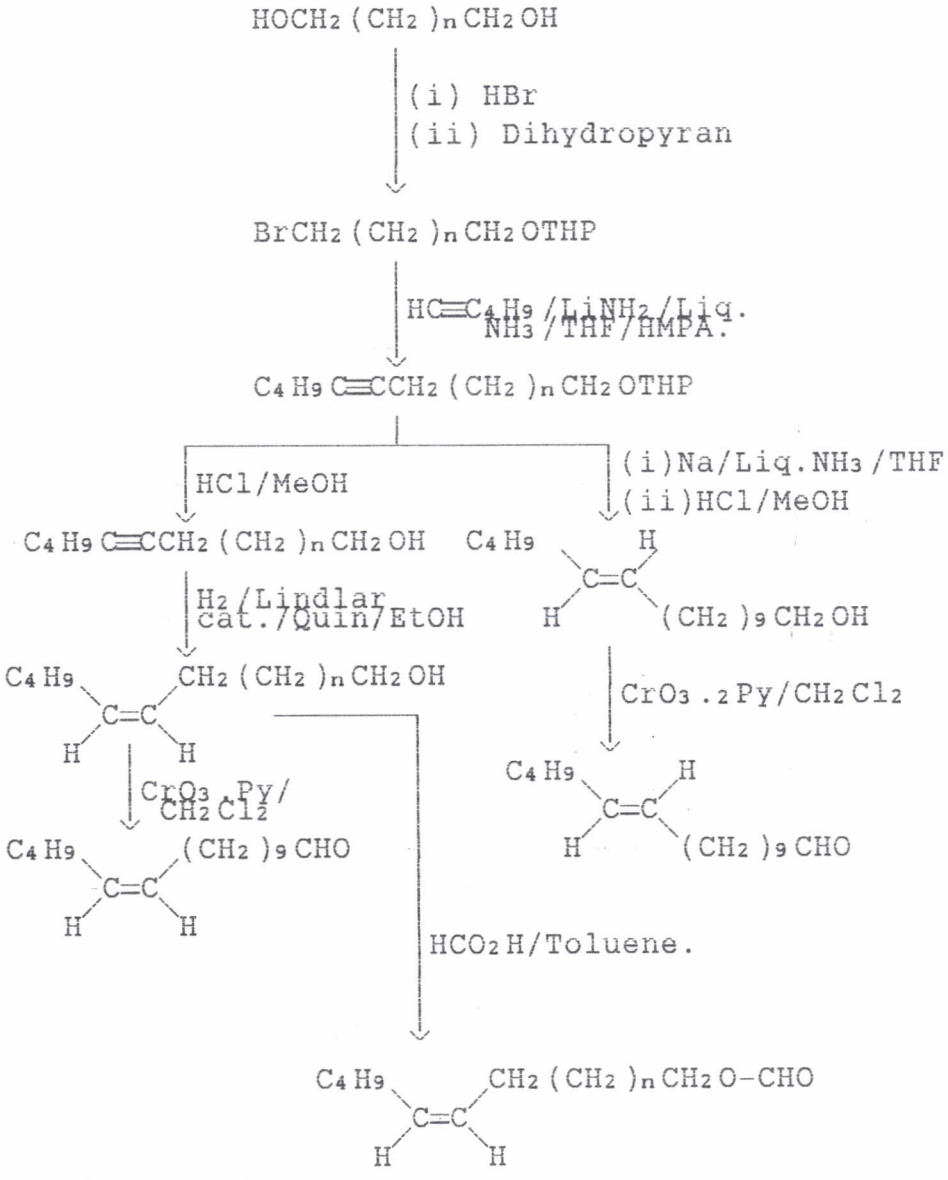
CHAPTER 2

RESULTS AND DISCUSSION.

2.1 Synthesis.

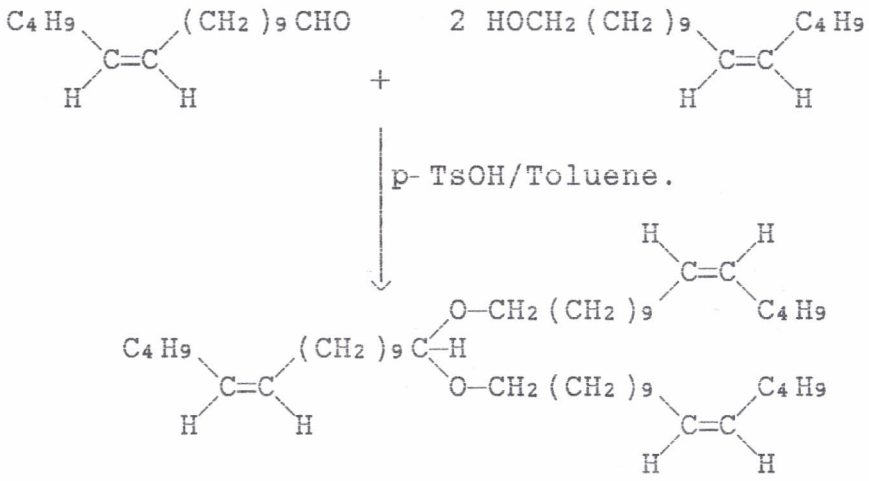
The synthetic compounds and their analogues were synthesized through the schemes 1, 2, and 3 shown below.

Scheme 1. *Synthetic pathway for pheromones and analogues.*

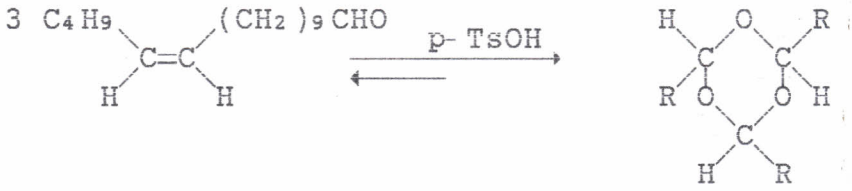


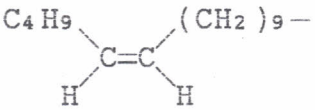
Where n= 6,8.

Scheme 2. *Synthesis of acetal from pheromonal compounds.*



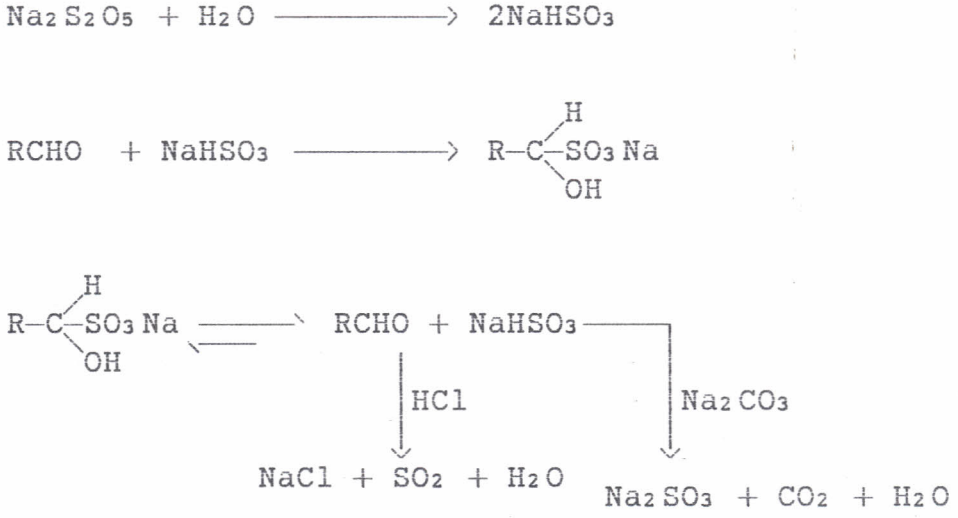
Scheme 3. *Synthesis of trimer from pheromonal aldehyde.*



Where R is 

Scheme 4 was a reaction characteristic of the purification of the aldehydal compounds formed. Addition of $NaHSO_3$, employed in saturated 40% aqueous solution to an aldehyde resulted in an equilibrium, in which the carbonyl component was converted almost entirely into addition product by use of excess bisulfite (Nesbit. et. al., 1975b). The addition product was crystalline salt and had the usual characteristic of an ionic metal compound. It was very soluble in water but subject to salting out by common-ion effect and it was insoluble in ether, infusible and non volatile. Since the reaction was reversible the aldehyde was regenerated by adding aqueous solution of the product on an amount of sodium carbonate or hydrochloric acid sufficient to destroy the free bisulfite present in equilibrium.

Scheme 4. *A reaction for precipitation of aldehydes.*



2.1.1 (Z)-11-Hexadecenal.

The Gas chromatography (GC) profile (Fig 3) shows that the compound was 98% pure with a retention time of 14.5 min.

The mass spectrum (MS) (Fig 4) of Z-11-hexadecenal shows the molecular ion peak m/z 238 which is discernible as expected for the aldehyde (Silverstein et al., 1981). Cleavage of the C-H bond next to the oxygen atom resulted in an M-1 peak m/z 237, which is a good diagonistic peak for long chain aldehydes (Ewing, 1985). The prominent peak at M-18 (loss of water) m/z 220, and the peaks at M-43 (loss of CH_2=CH-O·), m/z 195, M-44 (loss of CH_2=CH-OH), m/z 194 are also good diagonistic peaks for the aldehyde. The peaks at m/z 41, 55, 69, 83, 97, 111, 125, and 139, correspond to the formula C_nH_{2n-1} with n= 3, 4, 5, 6, 7, 8, 9, and 10, respectively, involving the olefinic bond. Double bonds favour allylic cleavage and give resonance-stabilized allylic carbonium ions (Silverstein et al., 1981). The peaks at m/z 44, 58, 72, 86, and 100 are due to the McLafferty cleavage of the αß C-C bond of the aldehyde.

The IR spectrum (Fig 5) shows the following absorptions: 2920 cm^{-1} (3.42 µm) methyl (CH_3) C-H asymmetrical stretch (V_{as}); 2845 cm^{-1} (3.51 µm) carbonyl (CHO) C-H asymmetrical stretch (V_{as}); 2962 cm^{-1} (3.38 µm) methylene (CH_2) C-H asymmetrical stretch (V_{as}); 2950 cm^{-1} (3.39 µm) methyl (CH_3) C-H symmetrical stretch (V_s); 1455 cm^{-1} (6.87 µm) methylene (CH_2). C-H symmetrical bending vibration (δ_s); 1465 cm^{-1} (6.83 µm) methyl (CH_3) C-H asymmetrical bending vibration (δ_{as}); 1690 cm^{-1} (5.92 µm) C=C asymmetrical stretch (V_{as}); 1735 cm^{-1} (5.76 µm) normal aldehydic C=O stretch.

The proton nuclear magnetic resonance (^{1}H n.m.r) spectrum (Fig 6) shows resonance at δ 0.85 ppm appearing as a triplet for three methyl protons. The singlet complex at δ 1.2 ppm is due to resonance of eighteen equivalent methylenic protons, and the four allylic protons resonates at δ 2.00 ppm with overlapping doublet and triplet. The resonance of the two methine protons on the olefinic bond occur at δ 5.49 ppm as a triplet, the two α-methylenic protons attached to the aldehyde resonates at δ 2.39 ppm with an overlapping doublet and triplet, and the resonance occurring downfield at δ 9.7 ppm as a triplet is due to the single aldehydic proton.

The ^{13}C n.m.r spectrum were as far as possible tentatively interpreted by comparing with published ^{13}C n.m.r spectral data (Silverstein et al., 1981). The assignment of the (Z)-11-hexadecenal of the ^{13}C n.m.r (Fig 7) is given below,

$C^{a}H_3C^{b}H_2C^{i}H_2C^{g}H_2C^{k}H=C^{k}HC^{h}H_2C^{d}H_2(C^{f}H_2)_4C^{c}H_2C^{e}H_2C^{j}H_2C^{l}HO$

a-13.8 ppm, b-22.0 ppm, c-22.2 ppm, d-26.8 ppm, e-27.1 ppm, f-29.1 ppm, g-29.3 ppm, h-29.7 ppm, i-31.9 ppm, j-43.8 ppm, k-129.7 ppm, l-202.0 ppm. The ^{13}C n.m.r spectrum confirms the presence of the carbonyl carbon atom at δ 202.0 ppm and the olefinic groups at δ 129.7 ppm.

2.1.2 (Z)-11-Hexadecen-1-ol

The gas chromatography (GC) profile (Fig 8) for (Z)-11-hexadecenol shows that the compound was above 99% pure, with a retention time of 15.0 min. The alcohol had a higher retention

temperature compared to its analogous pheromonal aldehyde component.

The mass spectrum (MS) (Fig 9) shows the molecular ion peak m/z 240 is quite small, but detectable implying that the compound is a primary alcohol (Willard et al., 1986; Silverstein et al., 1981; and Ewing, 1985). A prominent peak m/z 222 found at (M-18) is due to loss of water. Elimination of water together with elimination of an olefin from the Z-11-hexadecen-1-ol accounts for the presence of peaks at M-(olefin + H_2O), that is at M-46, M-74, M-102, M-130, M-158 and M-186 i.e at m/z 194, 166, 138, 110, 82, and 54 respectively. The peaks at m/z 41, 55, 69, 83, 97, 111, 125, and 139 correspond to the formula C_nH_{2n-1} with n=3, 4, 5, 6, 7, 8, 9, and 10 respectively, involving the olefinic bond.

The IR spectrum (Fig 10) shows absorptions at 3355 cm^{-1} (2.98 µm), O-H stretching vibration, broad peak due to intermolecular hydrogen bonding; 2920 cm^{-1} (3.42 µm) methyl (CH_3) C-H asymmetrical stretching vibration (V_{as}); 2850 cm^{-1} (3.39 µm) methylene (CH_2) C-H symmetrical stretch (V_s); 1660 cm^{-1} (6.02 µm) C=C stretching vibration; 1460 cm^{-1} (6.85 µm) methylene (CH_2.) C-H symmetrical bending vibration (δ_s); 1440 cm^{-1} (6.94µm) methyl (CH_3) C-H asymmetrical bending vibration (δ_{as}); 1045 cm^{-1} (9.57µm) C-O alcoholic stretch.

The ^1H n.m.r spectrum (Fig 11) shows resonance at δ 0.85 ppm appearing as a triplet for the three methyl protons and the singlet complex at δ 1.2 ppm is due to the eighteen equivalent methylenic protons. The four allylic protons resonates at δ 2.00 ppm and the

triplet at δ 3.6 ppm resonance is due to one alcoholic proton, while the two methine protons on the olefinic bond resonates at δ 5.35 ppm (triplet).

The assignment of the ^{13}C n.m.r (Fig 12) of Z-11-hexadecen-1-ol is given below,

$C^aH_3C^bH_2C^iH_2C^gH_2C^lH=C^lHC^hH_2C^dH_2(C^fH_2)_4C^eH_2C^cH_2C^jH_2C^kH_2OH$

a-13.8 ppm, b-22.3 ppm, c-25.8 ppm, d-26.9 ppm, e-27.2 ppm, f-29.3 ppm, g-29.5 ppm, h-29.7 ppm, i-31.9 ppm, j-32.8 ppm, k-62.9 ppm, l-129.8 ppm. The ^{13}C n.m.r confirms the presence of the alcohol carbon at 62.9 ppm and the olefinic carbons at 129.8 ppm.

2.1.3 (E)-11-Hexadecenal.

The GC profile (Fig 13), shows that E-11-hexadecenal was above 98% pure with a retention time of 14.7 min. This is slightly higher than the corresponding Z- isomer.

The mass spectrum (MS) (Fig 14) of the trans isomer shows the molecular ion peak m/z 238, is discernible as expected for the aldehyde (Silverstein et al., 1981). Cleavage of the C-H bond next to the oxygen atom resulted in an M-1 peak m/z 237, which is very weak, but it is a good diagonistic peak for long chain aldehydes (Ewing, 1985). The prominent peak at M-18 (loss of water) m/z 220, and the peaks at M-43 (loss of CH_2=CH-O·) m/z 195, M-44 (loss of CH_2=CH-OH), m/z 194 are also good diagonistic peaks for this aldehyde. The peaks at m/z 41, 55, 69, 83, 97, 111, 125, and 139 correspond to the formula C_nH_{2n-1} with n= 3, 4, 5, 6, 7, 8, 9 and 10 respectively, involving the olefinic bond. Double bonds favour

allylic cleavage and give resonance-stabilized allylic carbonium ions (Silverstein et al., 1981). The peaks at m/z 44, 58, 72, 86, and 100 are due to McLafferty cleavage of the αß C-C bond of the aldehyde.

The IR spectrum (Fig 15) has the following absorption peaks: 2960 cm^{-1} (3.38 µm) methyl (CH_3) C-H asymmetrical stretch (V_{as}); 2930 cm^{-1} (3.41 µm) methyl (CH_3) C-H symmetrical stretch (V_s); 2870 cm^{-1} (3.41 µm) methylene (CH_2.) C-H asymmetrical stretch (V_{as}); 2845 cm^{-1} (3.51 µm) aldehydic C-H asymmetrical stretch (V_{as}); 1685 cm^{-1} (5.93µm) and 1691 cm^{-1} (5.91 µm) are due to asymmetrical and symmetrical C=O coupled stretching vibrations respectively. 1645 cm^{-1} (6.08 µm) C=C asymmetrical stretch (V_{as}); 985 cm^{-1} (10.15 µm) C=C asymmetrical bending vibration of the trans isomer distinctive band of the (E) isomer. 1160 cm^{-1} (8.62 µm) and 1110 cm^{-1} (9.01 µm) are due to C-CO-C symmetrical stretching (V) and bending (δ) vibrations respectively.

The proton nuclear magnetic resonance (^1H n.m.r) spectrum (Fig 16) shows resonance at δ 0.85 ppm as a triplet for the three methyl protons. The singlet complex at δ 1.2 ppm is due to resonance of eighteen equivalent methylenic protons, and the four allylic protons resonates at 2.00 ppm. The resonance of the two methine protons on the olefinic bond occurs at δ 5.49 ppm as a triplet, the two α-methylenic protons attached to the aldehyde resonates at 2.39 ppm with an overlapping doublet and triplet, and the resonance occurring downfield at δ 9.7 ppm as a triplet is due to a single aldehydal proton.

The assignment of the (E)-11-hexadecenal of the ^{13}C n.m.r (Fig 17) is given below,

$C^aH_3C^bH_2C^cH_2C^fH_2C^jH=C^jHC^gH_2C^dH_2(C^eH_2)_5C^cH_2C^iH_2C^kHO$

a-13.9 ppm, b-22.1 ppm, c-22.3 ppm, d-26.9 ppm, e-27.1 ppm, f-29.3 ppm, g-29.7 ppm, h-32.0 ppm, i-43.9 ppm, j-129.8 ppm, k-202.4 ppm. The ^{13}C n.m.r spectrum (Fig 17) confirms the presence of the carbonyl carbon (δ 202.0 ppm) and the olefinic carbons (δ 129.8 ppm).

2.1.4 (E)-11-Hexadecen-1-ol.

The GC profile (Fig 18) shows that the trans isomer had a retention time of 15.2 min, higher than that of the Z- isomer with a purity of above 98%.

The MS (Fig 19) has the molecular ion peak m/z 240, quite small but detectable implying that the compound is a primary alcohol as expected (Willard et al., 1986; Silverstein et al., 1981; and Ewing 1985). A prominent peak m/z 222 found at (M-18), is due to loss of water. Elimination of water together with elimination of an olefin from E-11-hexadecen-1-ol accounts for the presence of peaks at M-(olefin + H_2O), that is at M-46, M-74, M-102, M-130, M-158, and M-186, i.e at m/z 194, 166, 138, 110, 82, and 54 respectively. The peaks at m/z 41, 55 69, 83, 97, 111, 125, and 139 correspond to the formula C_nH_{2n-1} with n= 3, 4, 5, 6, 7, 8, 9, and 10 respectively, involving the olefinic bond.

The IR spectrum (Fig 20) shows a broad band at 3560 cm^{-1} (2.81 μm) O-H stretch coupled with intermolecular hydrogen bonding, 2960

cm^{-1} (3.38 µm) methyl (CH_3) C-H asymmetrical stretch (V_{as}); 2930 cm^{-1} (3.41 µm) methyl (CH_3) C-H symmetrical stretch (V_s); 2830 cm^{-1} (3.46 µm) methylene (CH_2.) C-H asymmetrical stretch (V_{as}); 2865 cm^{-1} (3.49 µm) methylenic (CH_2.) C-H symmetrical stretch (V_s); 1640 cm^{-1} (6.10 µm) weak C=C asymmetrical stretch (V_{as}); 1030 cm^{-1} (9.71 µm) alcoholic C-O stretch; 960 cm^{-1} (10.42 µm) distinctive (E) band asymmetrical bending vibration .

The ^{1}H n.m.r spectrum (Fig 21) shows resonance at δ 0.85 ppm appearing as a triplet for the three methyl protons and a singlet complex at 1.2 ppm is from the resonance of eighteen equivalent methylenic protons. The four allylic protons resonates at δ 2.00 ppm. The triplet at δ 3.6 ppm resonance is due to a single alcoholic proton, while the two methine protons on the olefinic bond resonates at δ 5.35 ppm (triplet).

The assignment of the ^{13}C n.m.r (Fig 22) of E-11-hexadecen-1-ol is given below,

$C^{a}H_3C^{b}H_2C^{i}H_2C^{g}H_2C^{l}H{=}C^{l}HC^{h}H_2C^{d}H_2(C^{f}H_2)_4C^{e}H_2C^{c}H_2C^{j}H_2C^{k}H_2OH$

a-13.8 ppm, b-22.3 ppm, c-25.8 ppm, d-26.9 ppm, e-27.2 ppm, f-29.3 ppm, g-29.5 ppm, h-29.7 ppm, i-32.0 ppm, j-32.8 ppm, k-62.9 ppm, l-129.8 ppm

The ^{13}C n.m.r confirms the presence of the alcoholic carbon (62.9 ppm) and the olefinic carbons (129.8 ppm).

2.1.5 (Z)-11-Hexadecenyl Formate.

The GC profile (Fig 23) shows that the compound, an analogue, had a retention time of 15.7 min and a purity of above 95%.

The MS (Fig 24) of (Z)-11-hexadecenyl formate, reveals the elimination of formic acid in the same manner that the primary alcohols eliminate water with a mechanism involving a hydride transfer to the carbonyl oxygen (McLafferty rearrangement) (Silverstein et al., 1981). This accounts for the prominent peak at M-46 i.e m/z 222. The preceding loss of formic acid is so facile that no detectable molecular ion peak is observed (Silverstein et.al. 1981). Elimination of formic acid, together with elimination of an olefin from the ester accounts for the presence of a peak at M-(olefin + HCO_2H), at M-74, M-102, M-130, M-158, M-186, M-204 i.e at m/z 194, 166, 138, 110, 82, and 54 respectively. The peaks at m/z 41, 55, 69, 83, 97, 111, 125, 139 correspond to the formula C_nH_{2n-1} with n= 3, 4, 5, 6, 7, 8, 9 and 10 respectively involving the olefinic bond.

The IR spectrum (Fig 25) has the following absorption bands: 2950 cm^{-1} (3.39 µm) methyl (CH_3) C-H asymmetrical stretch (V_{as}); 2920 cm^{-1} (3.42 µm) methyl (CH_3) C-H symmetrical stretch (V_s); 2850 cm^{-1} (3.51 µm) methylenic (CH_2.) C-H asymmetrical stretch (V_{as}); 1734 cm^{-1} (5.77 µm) C=O asymmetrical stretching vibration of the formate. This occurs at a higher frequency (shorter wavelength) than that of the normal aldehyde due to inductive effect of oxygen atom (Silverstein et.al. 1980); 1460 cm^{-1} (6.85 µm) methylene C-H bending vibration; 1170 cm^{-1} (8.55 µm) broad band C-O stretching vibration, consisting of two asymmetrical coupled vibrations: C-C(=O)-O and O-C-C. The absorption of the olefin is weak and coupled with the noise and is not visible at 1667-1640 cm^{-1} (6.00-6.10 µm) (Silverstein et al., 1980)

The proton nuclear magnetic resonance (^1H n.m.r) spectrum (Fig 26) shows resonance at δ 0.85 ppm (triplet), for the three methyl protons. The resonance at δ 1.29 ppm is due to eighteen equivalent methylenic protons attached to methylene groups, the four allylic protons resonates at δ 2.00 ppm. The

resonance at δ 4.12 ppm (triplet) is due to two α-methylenic protons, a triplet at δ 5.4 ppm are the two olefinic protons and a singlet at δ 8.0 ppm is the methine proton of the formate (-O-CHO).

The assignment of the ^{13}C n.m.r (Fig 27) of Z-11-hexadecenyl formate is as given below,

$C^{a}H_3C^{b}H_2C^{k}H_2C^{h}H_2C^{m}H{=}C^{m}HC^{j}H_2C^{c}H_2C^{d}H_2(C^{i}H_2)_3C^{g}H_2C^{e}H_2C^{f}H_2C^{l}H_2{-}O{-}C^{n}HO$

a-13.8 ppm, b-22.3 ppm, c-25.8 ppm, d-26.9 ppm, e-27.1 ppm, f-28.5 ppm, g-29.1 ppm, h-29.2 ppm, i-29.4 ppm, j-29.7 ppm, k-31.9 ppm, l-64.0 ppm, m-129.7 ppm, n-160.7 ppm. The ^{13}C n.m.r confirms the presence of olefinic carbon (129.7 ppm) and formate carbon (160.7 ppm).

2.1.6 (Z)-9-Tetradecenyl Formate

The Gas chromatography (GC) profile (Fig 28) shows the Z-9-tetradecenyl formate was above 96% pure, with a retention time of 14.1 which is lower than that of the analogous aldehyde (Fig 1).

The IR spectrum (Fig 29) shows the absorptions at : 2950 cm^{-1} (3.39 μm) methyl (CH_3) C-H asymmetrical stretch (V_{as}); 2920 cm^{-1} (3.42 μm) methyl (CH_3) C-H symmetrical stretch (V_s); 2850 cm^{-1} (3.51 μm) methylenic (CH_2.) C-H asymmetrical stretch (V_{as}); 1730 cm^{-1} (5.78μm) C=O stretching vibration of the formate which occurs at higher frequency (shorter wavelength) than that of normal aldehyde due to inductive effect of the oxygen atom (Silverstein et.al. 1980). 1460 cm^{-1} (6.85 μm) methylene (CH_2.) C-H bending (δ) vibration; 1170 cm^{-1} (8.55 μm) broad band, C-O stretching vibration, consisting of two asymmetrical coupled vibrations C-C(=O)-O and O-C-C. The absorption of the olefinic stretch is weak coupled with the noise and is not visible at the range 1667-1640 cm^{-1} (6.00-6.10 μm) (Silverstein at al., 1980).

The proton n.m.r spectrum (Fig 30) has resonance at δ 0.85 ppm (triplet) due to the three methyl, the fourteen equivalent protons resonates at δ 1.29 ppm and the overlapping triplet and doublet at δ 2.00 ppm is due to the allylic protons. The α-methylene protons (triplet) resonate at δ 4.12 ppm , the two olefinic protons (triplet) at δ 5.40 ppm and at δ 8.1 ppm (singlet) is the methine proton of the formate.

The assignment of the ^{13}C n.m.r (Fig 31) of Z-9-tetradecenyl formate is as given below,

$C^aH_3C^bH_2C^jH_2C^gH_2C^lH{=}C^lHC^iH_2C^eH_2C^dH_2(C^hH_2)_2C^eH_2C^fH_2C^kH_2{-}O{-}C^mHO$

a-13.9 ppm, b-22.3 ppm, c-25.8 ppm, d-26.9 ppm, e-27.2 ppm, f-28.6 ppm, g-29.2 ppm, h-29.5 ppm, i-29.7 ppm, j-32.0 ppm, k-64.0 ppm, l-129.8 ppm, m-160.9 ppm. The ^{13}C n.m.r confirms the presence of olefinic carbons (129.8 ppm) and formate carbon (160.9 ppm).

2.1.7 8-Bromooctanyl Formate.

The GC profile (Fig 32) indicates that the compound was above 96% pure with a retention time of 11.9 min, below that of the corresponding olefinic formate (Fig 28).

The Mass spectrum of 8-bromooctanyl formate (Fig 51) has a prominent peak at m/z 191 which is due to elimination of methanoic acid (M-46), and at m/z 157 due due elimination of bromine. The peaks at m/z 41, 55, 69, 83, 97, 111 and 125 are cleavages corresponding to the formular C_nH_{2n+2}.

The IR spectrum (Fig 33) has the following absorptions 2930 cm^{-1} (3.41 μm) methylenic (CH_2.) C-H symmetrical stretch (V_s); 2850 cm^{-1} (3.51 μm) methylenic asymmetrical stretch (V_{as}); 1730 cm^{-1} (5.78 μm) C=O stretching vibration of the formate; 1460 cm^{-1} (6.85 μm) methylenic (CH_2.) C-H asymmetrical bending vibration

(δ); 1170 cm^{-1} (8.55 μm) broad band for the C-O stretching vibration consisting of two asymmetrical coupled vibrations C-C(=O)-O and O-C-C.

The proton n.m.r spectrum (fig 34) has resonance at δ 1.4 ppm for the twelve equivalent methylenic protons, and the ramp between δ (1.5-2.0 ppm) is due to the impurities. The two protons of the carbon atom attached to bromine resonates at δ 3.4 ppm (triplet), the triplet at δ 4.15 ppm are the α-methylenic protons to the formate, and the singlet at δ 8.05 ppm is due to the formate proton.

The assignment of ^{13}C spectrum (Fig 35) is as given below,

$BrC^{e}H_2C^{b}H_2C^{c}H_2(C^{d}H_2)_3C^{d}H_2C^{f}H_2-O-C^{g}HO$

a-25.7 ppm, b-28.1 ppm, c-28.53 ppm, d-28.9 ppm, e-32.8 ppm, f-63.9 ppm, g-161.0 ppm.

The ^{13}C n.m.r spectrum shows the presence of the formate ester but with no methyl carbon, and no olefinic carbons.

2.1.8 11-Hexadecynal.

The GC profile (Fig 36) indicates a purity of above 90% with a retention temperature of 9.5 min, which is far much lower than the corresponding analogous olefinic functional pheromone (Fig 1). 11-Hexadecynal was prepared by oxidizing the analogous acetylenic alcohol.

2.1.9 10-Bromodecanal.

The GC profile (Fig 37) had the compound with a purity of above 96% with the retention temperature of 12.1 min. This was prepared by oxidizing 10-bromodecanol (halohydrin) directly.

2.1.10 Trimer of Pheromonal Aldehyde

In the trimerization experiments 1%, 5%, 10%, 20%, 50% hexane solutions of freshly prepared Z-11-hexadecenal and a 20 mg neat sample were exposed to the following conditions: (1) kept in clean untreated vials; (2) kept in vials which were washed with ethanolic KOH and then with distilled water; (3) kept in vials and *p*-toluenesulfonic acid (1 mg) was added. The samples were kept at room temperature or in the refrigerator for more than three weeks (Dunkelblum et al., 1984), while the trimerization process was monitored by TLC. The trimer of Z-11-hexadecenal was only noticed in (3), with traces in (1), and none in (2).

The presence of the trimer was deduced from the thin layer chromatography (TLC) which was performed on "Merk" precoated silica-gel 60 F_{254} plates. Analysis of the trimer on the capillary GC gave only a sharp peak corresponding to Z-11-hexadecenal (Fig 3), due to thermal decomposition (Dunkelblum et al., 1984).

Separation of the aldehyde from its trimer was only achieved on the TLC, eluting with a mixture of hexane and ethyl acetate (8:1), which gave an excellent separation with an R_f of 0.69 and 0.75 for Z-11-hexadecenal and its trimer respectively. Thus the process of trimerization of Z-11-hexadecenal was enhanced by addition of *p*-toluenesulfonic acid even in the refrigerator. The process was faster with neat pheromone material compared with hexane solutions. To eliminate any possible effect of acid, storage vessels were base-washed, although it seems that external acid, might be involved in the trimerization of Z-11-hexadecenal. One of them could have originated from oxidizing agent pyridinium chlorochromate (Hall et al., 1980; Klug et al., 1982) which is mildly acid or from its decomposition products. Accordingly it was useful to purify the crude reaction product either by washing with mild base or column chromatography prior to

distillation.

The IR spectrum of the a freshly prepared trimer from the aldehyde is shown in Fig 50. The most distinguishing feature was the absence of any absorption peak at carbonyl absorption region. The presence of an intense peak at 1215 cm^{-1} which was the stretching vibration from C-O-C bond. This peak tends to diminish on storage, giving the original IR spectrum of the aldehyde (Fig 5) with time.

2.1.11 Acetal from Pheromonal Aldehyde and Alcohol

The acetal was prepared by refluxing freshly prepared pheromone components Z-11-hexadecenal (Z-11-HDAL) and Z-11-hexadecen-1-ol (Z-11-HDOL) in the ratio of 1:2 in toluene(solvent) under a stream of nitrogen.

The nitrogen atmosphere was necessary to minimize any oxidation of the aldehyde. The extent of acetalization was monitored on the TLC and the capillary GC. The acetal could be decomposed to its monomers by refluxing in the presence of water and an acid. Accordingly, it was useful to use a clavenger apparatus to eliminate water and push the equilibrium forward.

The percentage yield of the acetal was low (20%) by GC analysis and this was attributed to the steric hindrance. The significant peaks on the GC profile were those of the monomers, Z-11-hexadecenal and Z-11-hexadecen-1-ol. Further purification using the flash chromatography afforded some significant amount of the acetal for spectral analysis.

GC profile (Fig 38) shows a purity of above 90% of the acetal and a retention time of 20.9 min.

The mass spectrum (Fig 39) has significant peaks at m\z 41, 55, 69, 83, 97, 111, 125 and 139 which are due to the olefinic bond cleavage. They correspond to the formular C_nH_{2n-1} with n= 3, 4, 5, 6, 7, 8, 9, and 10 respectively.

The IR spectrum (Fig 40) has the following absorption peaks: 2920 cm^{-1} (3.42μm) methyl (CH_3) C-H asymmetrical stretching vibration; 2850 cm^{-1} (3.39μm) methylenic C-H symmetrical stretch; 1440 cm^{-1} (6.94μm) C=C stretching vibration.

2.2 Electrophysiology

The electroantennographic response (Arn et al., 1975) tests showed that the synthetic pheromones and their analogues differed in their ability to evoke EAG responses.

EAG responses confirmed that Z11-16:CHO was a better stimulant than the corresponding alcohol Z11-16:OH. A total of ten compounds were tested on the electroantennographic detector (EAD) using preparations of one day old male *C. partellus*. The female sex pheromones and their analogues were synthesized and isolated in good yields. The compounds that were tested are given in Table 3 below.

Table 3. *Synthetic compounds tested with electroantennograph using preparations of male C. partellus antennae.*

Compound	Structural notes
(Z)-11-hexadecenal	Z11-16:CHO
(Z)-11-hexadecen-1-ol	Z11-16:OH
(E)-11-hexadecenal	E11-16:CHO
(E)-11-hexadecen-1-ol	E11-16:OH
(Z)-11-hexadecenyl formate	Z11-16:OCHO
(Z)-9-tetradecenyl formate	Z9-14:OCHO
11-Hexadecynal	≡11-16:CHO
8-Bromooctanyl formate	BrC_8-OCHO
10-Bromodecanal	BrC_9CHO
2,4,6-Tripentadec-10-enyl-1,3,5-trioxane	3(Z11-16:CHO-)

Table 4. *EAG olfactory response (mV) of the synthetic sex pheromones and their analogues at different concentrations.*

	Concentrations (ng/10 µl of hex)						
Compound	1	5	10	20	40	80	100
Z11-16:CHO	3.39±.05	4.11±.05	4.65±.09	5.70±.12	6.59±.04	8.36±.02	7.35±.03
E11-16:CHO	2.34±.09	2.97±.03	3.57±.09	4.14±.17	3.33±.10	2.84±.18	2.54±.16
Z11-16:OH	1.41±.07	1.94±.04	2.31±.08	2.61±.05	2.81±.02	2.91±.02	3.61±.30
E11-16:OH	1.44±.03	1.61±.06	1.72±.03	2.03±.03	2.70±.02	2.88±.01	3.21±.05
Z11-16:OCHO	1.61±.18	1.81±.02	2.76±.16	3.00±.18	3.29±.19	4.05±.14	2.97±.04
Z9-14:OCHO	1.52±.06	2.20±.13	2.78±.08	5.22±.07	4.03±.45	2.97±.03	2.55±.23
$Br(CH_2)_8OCHO$	2.29±.32	2.10±.21	4.72±1.12	5.14±1.26	4.98±1.21	4.83±1.21	4.77±1.2
$Br(CH_2)_9CHO$	1.30±.14	1.55±.11	3.60±.81	3.60±.69	3.69±.76	3.50±.66	3.54±.66
≡11-16:CHO	2.14±.06	2.43±.05	2.70±.02	2.72±.14	3.11±.06	3.35±.03	3.51±.03
Trimer	0.99±.01	1.34±.02	1.42±.02	1.60±.01	1.94±.03	2.50±.03	1.97±.03
7:1 Ratio	1.04±.02	2.28±.07	2.85±.12	3.17±.09	3.40±.13	3.02±.10	2.89±.04

Where Trimer is 3(Z11-16:CHO-) and 7:1 ratio is the pheromonal components Z11-16:CHO and Z11-16:OH in the ratio emitted naturally by the female *C. partellus*.

The standard used in this responses was commercial (Z)-11-hexadecenal at 80 ng/10 µl of hex which gave the response 7.99±0.01 mV

Antennal responses to the synthetic sex pheromones and their analogues showed that the sensillum was particularly sensitive to the Z11-16:CHO component while the alcohol component, Z11-16:OH did not evoke a response of the same magnitude (Fig 1a). The E11-16:CHO also evoked a higher response than its

corresponding analogous alcohol. The olfactory responses at different concentrations of the synthetic samples is shown in Table 4.

Although Z11-16:CHO alone had a high olfactory EAG antennal responses to the male *Chilo partellus*, this compound as well as its blend with Z11-16:OH were later found to be ineffective in inducing substantial catches of male *C. partellus* in the field (Unithan and Saxena, 1990). However it has been shown that even a small separation between the release points of *C. partellus* pheromone components substantially decreases trap efficiency (Lux et al., in Press). Although at the sensory level, stimulation due to each pheromone component is received independently, successive steps of a behavioural sequence are evoked by relevant biological signals perceived as an integral whole rather than as a component stimuli. Thus the effects of the pheromonal aldehyde (Z11-16:CHO) and its alcohol (Z11-16:OH) were not complimentary in their EAG responses. Addition of the alcohol to the aldehyde in the ratio (1:7) of the volatiles found in "calling" females reduced the response significantly. The E11-16:CHO also gave higher EAG responses as compared to the corresponding alcohol. Since, however efficient the oxidation reagent used, small amount of the alcohol would be expected to be present in the aldehyde. Thus it seems possible that the alcohol found in the volatiles of a "Calling" female (Nesbitt et al., 1979) should be a biosynthetic precursor for the preceding aldehyde.

The dose response curves (Fig 1) were obtained from the percentage difference between the response to the standard and other concentrations of each sample and plotted against $\log_{10}$concentration.

Fig 1a. EAG response curves of Aldehydes and Alcohols

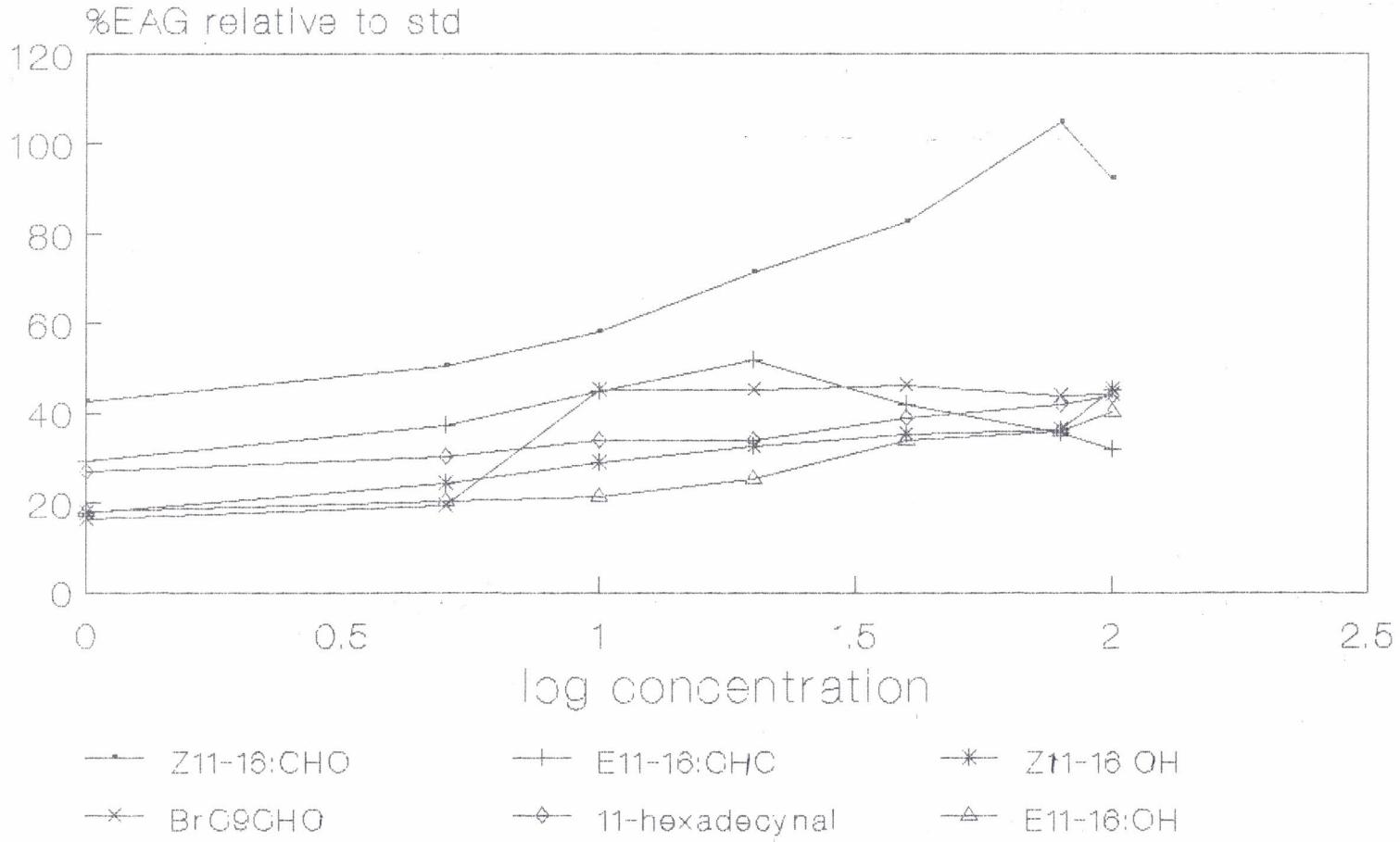
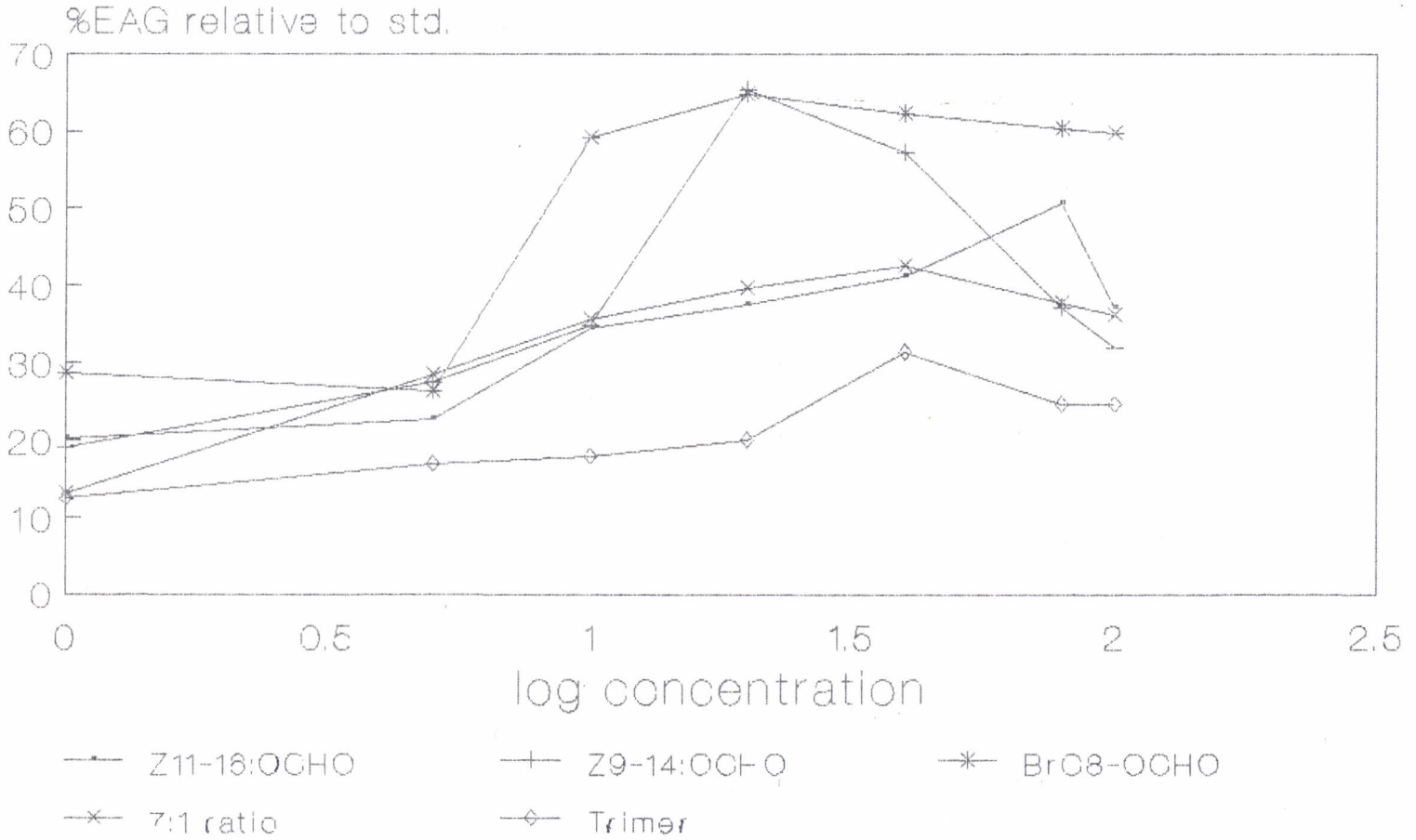


Fig 1b. EAG response curves of formates, trimer and natural ratio.



EAG responses to nanogram amounts of the synthetic pheromone compounds and their analogues indicated that (Z)-11-hexadecenal (Z11-16:CHO) was most potent olfactory stimulant (Fig 1a) followed by Z9-14:OCHO, $Br(CH_2)_8OCHO$, Z11-16:OCHO, (Fig 1b) E11-16:CHO (Fig 1a), $Br(CH_2)_9CHO$ (Fig 1c), ≡11-16:CHO (Fig 1d), Z11-16:OH (Fig 1a), E11-16:OH (Fig 1c), 7:1 ratio (Fig 1d) and the trimer (Fig 1c).

The formate derived by replacing ß-methylene group of the pheromonal aldehyde by oxygen was a potent olfactory stimulant than its homologous formate with two methylene groups longer, but lower than the aldehyde (Z11-16:CHO). Although the majority of lepidopterous female sex pheromones identified to date have C_{12} to C_{18} acetates, alcohols and/or aldehydes (Campion and Nesbitt, 1983), further field work and studies should be focused on the formates of the corresponding aldehydes.

It is interesting that in some lepidoptrous e.g the *H. viriscens* and *C. suppressalis* the pheromone complex consist of two homologous compounds differing in chain length by two carbon atoms (Roelofs et al., 1974; Nesbitt et al., 1975b; and Ohta et al., 1976) although with differing potent olfactory stimulancy. Where the attractant is composed of two homologues, the less volatile, higher molecular weight component must have a relatively close-range effect (unless it is more potent or more abundant than the lower homologue) (Nesbitt et al., 1975b).

The geometric isomer (E11-16:CHO) (Fig 1a) elicited some EAG response, lower than the pheromonal aldehyde (Z isomer) and the formates. Multi-component pheromone systems in lepidoptera identified to date have been mixtures of positional or geometric isomers of a single or combination of compounds of identical chain length with different degrees of unsaturation (Jacobson et al., 1970; Nesbitt et al., 1973; Sower et al., 1974), and there has been several

reports of pheromones which consist of two positional or geometric isomers of a single compound (Tamaki et al., 1971a,b; Klun et al., 1973; Beroza et al., 1973; Hummel et al., 1973).

The brominated formate and the aldehyde also elicited EAG responses which could be attributed to the reactivity of the halogen with the receptor molecules on the antennae of *C. partellus*. Subsequently further field work and studies should be centered on the halogen (especially fluorine) substituted analogues because of its small size and high reactivity.

The high molecular weight of the 2,4,6-tripentadec-10-enyl-1,3,5-trioxane makes it less volatile thus the low EAG response elicited by the trimer of the pheromonal aldehyde. The red bollworm sex pheromone complex is unusual in being a mixture of different compounds, in which the major component exists in two isomeric forms. It is becoming increasingly apparent that relatively differences in ratios of components can have a marked effect on biological activity. For sex pheromones to be used effectively for insect survey and control it is essential that the exact composition of the attractive scent should be carried out to elucidate the precise role of each component.

CHAPTER 3

MATERIALS AND METHODS.

3.1 Chromatography.

Thin layer chromatograph (TLC) was performed on "Merck" precoated silica-gel 60 F_{254} plates (0.25 mm thickness), with 10% ethyl ethanoate in petroleum ether as the eluting solvent, and developed in iodine chamber. Column chromatography was carried out on silica-gel 60 (0.063-0.200 mm, 230-240 mesh ASTM) using a quickfit column with application of nitrogen pressure. The Gas chromatography (GC) analyses were carried out on a Hewlett Packard model HP5890A series Gas chromatography equipped with a split / splitless injector and a flame ionization detector (F.I.D) at a temperature of 280^0 C. The column was Ultra-1 (cross-linked methyl silicone) of dimensions (50 m x 0.32 mmID, 0.17 µm. film thickness). White spot nitrogen (3.25 cm^3/sec) was the carrier gas, hydrogen (45 cm^3/sec) and medical air (360 cm^3/sec) were the fuel gases. All the GC analyses were performed in the splitless mode with the injector temperature at 280^0 C, the oven temperature was programmed to stay at 60^0 C for 3 minutes and a rise at a rate of 15^0/min to 280^0 C at which it was maintained for 13 minutes. The peak areas, the percentage composition and the retention time (retention temperatures) were calculated on a Hewlett Packard model HP 3393A integrator.

3.2 Spectroscopy.

The Mass spectra (MS) a gas chromatography linked Mass spectrometric electron impact analysis, a Hewlett packard model 5790A series gas chromatograph coupled to a V.G Mass lab 12-250 analytical organic mass spectrometer equipped

with a data system was used. Chromatographic separations were achieved on an Ultra-1 cross linked methyl silicone column (50 m x 0.2 mmID, 0.33 μm film thickness). All the GC-MS analysis were performed in a splitless mode with helium as the carrier gas. The column temperatures were programmed as stated earlier and the ion source temperature (180^0 C) and the integrator temperature (240^0 C). The Infrared (IR) analyses of the samples was by a Beckman Acculab[I.R]2 Spectrophotometer and the spectra recorded on a Beckman spectrophotometer chart paper. The samples were liquids and thus were analysed as neat samples with NaCl cells (window). The samples were pressed between flat plates producing a film of 0.01 mm or less in thickness, the plates being held together by capillary. Between 3-10 mg of each sample was required and the spectrophotometer was scanned between 4000-600 cm^{-1} and the peaks interpreted according their range of absorption. Proton (^1H) Nuclear Magnetic Resonance (NMR) were recorded on 90 MHz perkin Elmer R-24A and Varian EM 360 spectrometers and were specified on 100 MHz Jeol FX-100 instrument, with $CDCl_3$ as the solvent and ^{13}C NMR specified on 100 MHz Jeol FX instrument.

3.3 Electroantennograph.

The moths of *Chilo partellus* used throughout this work were obtained from the International Centre of Insect Physiology and Ecology (I.C.I.P.E), insect mass rearing unit. The pupae were kept to emerge 1-2 days after picking. Moths antennae for scanning electron microscopy were fixed in 70% alcohol. The specimen were then mounted on stups coated with carbon and palladium electrodes and observed in a Jeol, AC/DC preamplifier instrument.

The Electroantennogram (EAG) a Grass P16 AC/DC preamplifier was used to record the antennal response of the moth to the synthetic pheromones and the

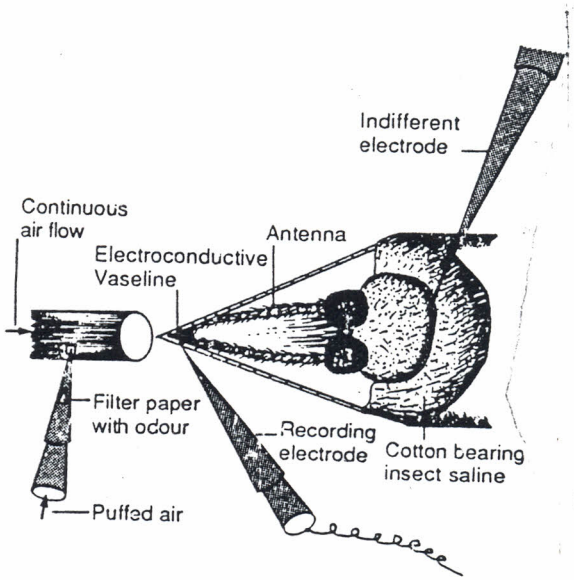
related compounds. Electroantennogram (EAG) responses to the synthetic pheromones and the related compounds were recorded using intact antennae as demonstrated by Waladde et.al.(1990). The moth was anaesthetized with carbon dioxide, wings cut off and the head capsule was punctured to damage the brain in order to eliminate antennal movements. The moth was placed upside down on the specimen stage, and the antennae were straightened taking care to ensure that their ventral surfaces bearing the sensilla faced upwards. The distal tips of the antennae were cut off and brought close to each other with a tiny blob of electroconductive vaseline. The recording electrode was placed in the vaseline blob to pick up the EAG signals while the indifferent electrode was placed in the moth body. This arrangement exposed the antennal sensilla to either light or continuous air stream bearing pheromone or analogous odour stimuli as shown in the Figure 2. It was possible to get EAG signals, from both antennae simultaneously using standard electrophysiological procedures with a Gas P16 AC/DC preamplifier and a magnetic tape recorder as well as a chart recorder to record the EAG responses. EAG amplitudes were converted to millivolts and the data was used to draw response curves. Ten male *C.partellus* were used for each concentration of a sample and the response (mV) obtained on average. Puffing was done at an interval of 30 sec between each concentration.

3.4 Solvents.

Dichloromethane, hexane, toluene, methanol and ethanol were purified by distillation, passed through a column of Woelm neutral alumina to remove any polar contaminants and stored on activated molecular sieves. Diethylether, petroleum ether and ethyl ethanoate were analar grade and they were passed through a column of Woelm neutral alumina before storing on activated molecular

sieves. Tetrahydrofuran (THF), hexamethylphosphoramide (HMPA) and the quinoline were analar grade.

Fig 2. *Diagram showing the moth antennal preparation and stimulus delivery system.*



3.5 Preparation of Test Samples for Bioassays.

The samples used to test the olfactory responses of the *Chilo partellus* were made by successive dilutions of the stock solution for each sample.

3.5.1 Preparation of the Stock Solution.

The stock solution was made in the concentration of 100 ng/10 µl. This was achieved by dissolving 1 mg of sample in 100 mls of hexane. The following concentrations were prepared from the stock solution for each synthetic pheromone and their related compounds, in 10 µl, 0 ng, 1 ng, 5 ng, 10 ng, 20 ng, 40 ng, 80 ng, and 100 ng. The separate concentrations were analysed on the EAG and the

responses converted to millivolts, where the data was used to draw response curves for each sample. Mixtures of the pheromones and their analogues were also prepared and analysed in a similar manner.

3.6 Synthesis of Pheromones and Related Compounds.

The *Chilo partellus* female sex pheromones and their analogues were prepared from 1,10-decanediol, 1,8-octanediol and 1-hexyne as described by Nesbitt et.al. (1975b) and the tetradecenyl formates were prepared by an analogous acetylenic route.

The diols were converted to the corresponding bromohydrins through the substitution of one of the hydroxyls and these were protected as tetrahydropyranyl ethers. Coupling with 1-hexyne using a freshly prepared lithium amide in liquid ammonia/tetrahydrofuran/hexamethylphosphoramide and acid catalyst deprotection of the crude products gave the acetylenic alcohols, 11-hexadecynol and 9-tetradecynol. Semi-hydrogenation over lindlar catalyst (palladium on calcium carbonate poisoned with lead) in ethanol/quinoline mixture gave the corresponding (Z)-olefinic alcohols which were oxidized with trioxide-pyridinium complex formed in *'situ'* in dichloromethane. The corresponding (E) isomers were prepared by sodium/liquid ammonia reduction of the corresponding protected acetylenic tetrahydropyranyl ethers described above, followed by deprotection and oxidation as illustrated in the Scheme 1. The formates were prepared by refluxing the alcohols with formic acid catalysed by p-TsOH. The trimer was prepared from the freshly prepared pheromonal aldehyde in the presence of *p*-toluenesulphonic acid as a catalyst in a neat sample (Scheme 3) and the acetal from the corresponding pheromonal aldehyde and alcohol, the reaction being catalysed by *p*-toluenesulphonic acid (Scheme 2).

3.7 Procedures for the Synthetic Reactions.

3.7.1 Formation of Bromohydrins.

An equimolar (0.046 mol) mixture of α,ω-alkane diols and hydrobromic acid were refluxed (Teng et.al., 1988) in toluene for 24 hrs. The cold mixture was washed with aqueous sodium bicarbonate ($NaHCO_3$), followed with excess of distilled water. The organic layer was dried on anhydrous sodium sulphate (Na_2SO_4), filtered, the solvent removed on a rotary evaporator and the sample analysed on the silica gel TLC plates, purified by column chromatography and distilled under reduced pressure (102^0 C/0.07 mmHg). The reaction yielded ω-bromoalkanols with a high reaction selectivity and yield of 97-99%. Fig 41, 42, 43, and 44 shows the IR, 1H n.m.r, 13C n.m.r and GC profiles of 10-bromodecanol (halohydrin) confirming the structure of this compound. 8-Bromooctanol was also obtained by the same procedure.

3.7.2 Tetrahydropyranyl (THP)- Ether Linkage Formation.

This was accomplished by adapting the methods described by Vogel, 1989; Fieser and Fieser, 1967 and Henrick, 1977, as follows.

Concentrated hydrochloric acid (0.1 ml) was added to a mixture of 2,3-dihydropyran (0.05 mol) and the alcohol (0.036 mol). The reaction commenced immediately on stirring and was moderated by cooling in an ice-water bath. The mixture was stirred for a further 30 minutes, allowed to stand overnight, diluted with diethylether and the solution washed twice with aqueous $NaHCO_3$ solution. The ethereal solution was dried on anhydrous Na_2SO_4, filtered, the solvent evaporated on a rotary evaporator and analysed on silica gel TLC plates. The residue was purified on a silica gel packed column and distilled under reduced

pressure (120^0 C/0.09 mmHg) to yield a pure product with percentage yield of 95%. Fig 45 shows the GC profile of protected bromodecanol [$Br(CH_2)_{10}OTHP$], with a retention time of 16.7 min.

3.7.3 Coupling Reaction to Lengthen the Carbon Chain.

The coupling reaction was accomplished by adapting the methods described by Schwarz and Waters, 1972; Ando et.al. 1972; Chi-chu and Pei-min,1989; Ames et.al. 1963 and Klug et.al. 1982, as follows. 1-Hexyne (0.15 mol) was added to a stirred suspension of lithamide (0.15 mol) from lithium in liquid ammonia and the mixture stirred under reflux for 1 hr. After addition of w-bromo-$(CH_2)_x$OTHP (0.036 mol) in tetrahydrofuran (THF) and hexamethylphosphoramide (HMPA) [strong activating solvent], the mixture was refluxed for 8 hrs and then allowed to evaporate overnight. Addition of dilute hydrochloric acid and isolation with diethylether gave the product. The product was washed with excess water, and dried over anhydrous Na_2SO_4, filtered, evaporated, and the residue analysed on TLC plates, purified through a silica gel packed column and distilled under reduced pressure (110^0 C/0.09 mmHg) giving the product (yield 70-75%). Fig 46 shows the GC profile, with retention time of 15.2 min which was lower than the corresponding briminated compound and Fig 47 shows the Mass spectrum of $C_4H_9C{\equiv}C(CH_2)_{10}OTHP$, the product of coupling reaction with the peaks at m/z 40, 54, 68, 82, 96, 110 and 124 corresponding to the cleavage of the acetylenic group (C_nH_{2n-2}), where n= 3, 4, 5, 6, 7, 8 and 9 respectively.

3.7.4 Deprotection of the THP-Ether Linkage.

The deprotection of ether linkages was described by Nesbitt et.al., 1977. The THP-ether (0.025 mol) was dissolved in methanol (200 mls) and concentrated hydrochloric acid (30 mls), and the mixture heated under reflux for 4 hrs. After cooling, the solution was neutralized by addition of an excess of $NaHCO_3$, diluted with ether (200 mls), and washed thoroughly with excess of water. The residue was dried on Na_2SO_4, filtered, evaporated, analysed on the TLC plates. It was then purified through a silica gel packed column and distilled under reduced pressure (b.p 96-98^0 C/0.04 mmHg) (yield 85%). Fig 48 and 49 are the GC and Mass spectrum of $C_4H_9C{\equiv}C(CH_2)_{10}OH$ a deprotected product. The GC profile has a retention time of 13.1 min which is lower than the corresponding pheromonal alcohol (Fig 8), and the MS shows a prominent peak at m/z 220 which is due to loss of water (M-18). The peaks at m/z 40, 54, 68, 82, 96, 110 and 124 are due to cleavage corresponding to C_nH_{2n-2} where n= 3, 4, 5, 6, 7, 8 and 9 respectively.

3.7.5 Partial Hydrogenation of the Alkyne.

In a 50 ml hydrogenation flask, 100 mg of Lindlar catalyst and a solution of 0.025 mol of the alkyne in 20 mls of ethanol. 20 mls of quinoline was added and hydrogen bubbled through at room temperature and pressure. The extent of hydrogenation was monitored on the TLC plates. Yields of the desired product (95% of the Z- isomer) were obtained. The residue was diluted with ether and washed with aqueous hydrochloric acid, to remove the quinoline, followed by 3 portions of water and dried on Na_2SO_4, then distilled under reduced pressure (b.p 75^0 C/0.01 mmHg). The spectral data of the Z-11-hexadecen-1-ol is given by Figures 9, 10, 11, and 12.

3.7.6 Reduction on Sodamide in Ammonia.

Reduction on sodamide in ammonia was described by Lo et.al. 1988.

To a stirred suspension of sodamide made from sodium (0.02 mol) by use of ferric nitrate catalyst in liquid ammonia (20 mls), cooled to -40^0 C, (using dry ice), a solution of the alkyne 0.025 mol in 20 mls of diethylether was added in portions for 10 minutes and stirring was continued for a further hour. Sodium (0.04 mol, excess) was then added in small pieces for a period of 15 minutes, then ammonium chloride (0.04 mol) was added. The ammonia was allowed to evaporate overnight through a water-cooled condenser, water and ether were then added to the residue, washed twice with water, dried on $MgSO_4$, filtered, evaporated and distilled under reduced pressure (82^0 C/0.04 mmHg) giving 89-91% of the trans isomer. Figures 19, 20, 21 and 22 are the spectra of the E- isomer of hexadecen-1-ol.

3.7.7 Oxidations

Oxidations were accomplished by adapting methods described by Correy and Suggs, 1975; Collins et.al. 1968; Ratcliffe and Rodehorst, 1970, as follows.

In a 500 ml round bottomed flask fitted with a reflux condenser pyridinium chlorochromate [Pcc, 150 mmol], prepared in "*situ*", was suspended in 200 mls of anhydrous dichloromethane. The alcohol (100 mmol) in 20 mls of dichloromethane was added in one portion to the magnetically stirred solution. After 1.5 hrs, 200 mls of dry ether was added to the supernatant solution and then decanted from the black gum. The insoluble residue was washed thoroughly with 50 ml portions of anhydrous ether whereupon it became black granular solid. The combined organic solutions were passed through a short pad of florosil and the solvent removed by evaporation. Distillation of the residue (75^0 C/0.01 mmHg) gave 78%

of the product. The aldehydes were extracted from the crude products by washing with excess aqueous sodium metabisulfite ($Na_2S_2O_5$) (Scheme 4). This converted the aldehydes to solid sodium bisulfite derivative. Removal of non-aldehydic material was by washing with diethylether and regeneration of the aldehyde was by sodium carbonate or an acid. This provided a convenient method for the purification of the aldehyde.

3.7.8 Preparation of Pyridinium Chlorochromate. (PCC)

Correy and Suggs,1975 method of formation of PCC was used with some modifications.

To 2 mls 6 M HCl (0.03 mol), (0.02 mol) of chromium trioxide (CrO_3) was added rapidly with stirring. After 5 minutes the homogenous solution was cooled to 0^0 C and (0.02 mol) of pyridine was carefully added over 5 minutes. Recooling to 0^0 gave a yellow orange solid which was collected on a sintered glass funnel and dried for 1 hr in a vacuo. (yield 84%). The solid was not appreciably hydroscopic and could be stored for extended periods at room temperature.

3.7.9 Formation of Formates.

The method of Morrison and Boyd, 1988 was followed with the following modifications.

In a 250 ml round bottomed flask provided with a reflux condenser were put (0.02 mol) of formic acid 98% and 0.01 mol of the alcohol in toluene. Catalytic amount of P-TsOH was added, and the mixture refluxed for 10 hrs. The cold mixture was washed with saturated sodium bicarbonate until effervescence ceased and dried on Na_2SO_4. The residue was purified through a column and distilled

through a short fractionating column (yield 95%). The spectral data of Z-11-hexadecenyl formate are given in Figures 24, 25, 26, and 27.

3.7.10 Trimerization of the Aldehyde.

This was accomplished as described by Dunkelblum et.al. 1984.

To a freshly prepared aldehyde, pheromone sample (Z-11-HDAL) in a vial P-toluenesulphonic acid was added, and the sample sealed under nitrogen and kept at room temperature for 3 weeks. The extent of trimerization was monitored on alumina TLC plates. The trimer could not be analysed on a capillary GC column because it underwent thermal degradation. The residue was then washed with $NaHCO_3$ to remove the acid then with water and $NaHSO_3$ to remove the aldehyde.

3.7.11 Formation of the Acetal.

In a 250 ml round bottomed flask provided with a reflux condenser and clavenger apparatus (1 part, 40 mg) of freshly prepared pheromone (Z-11-HDAL) aldehyde and (2 parts, 80 mg) of freshly prepared (Z-11-HDOL) alcohol and a catalytic amount of P-TsOH in toluene were put. The mixture was heated under reflux under the nitrogen atmosphere for 24 hrs. The cold mixture was washed with excess $NaHCO_3$ to remove the acid and with $NaHSO_3$ to remove the excess aldehyde. The residue was washed with water and dried on sodium sulphate. Flash chromatography was used to purify the acetal off the alcohol (yield, 20-25%). Figures 38,39, and 40 are the GC profile, Mass spectrum and IR spectrum respectively of the acetal.

SPECTRA

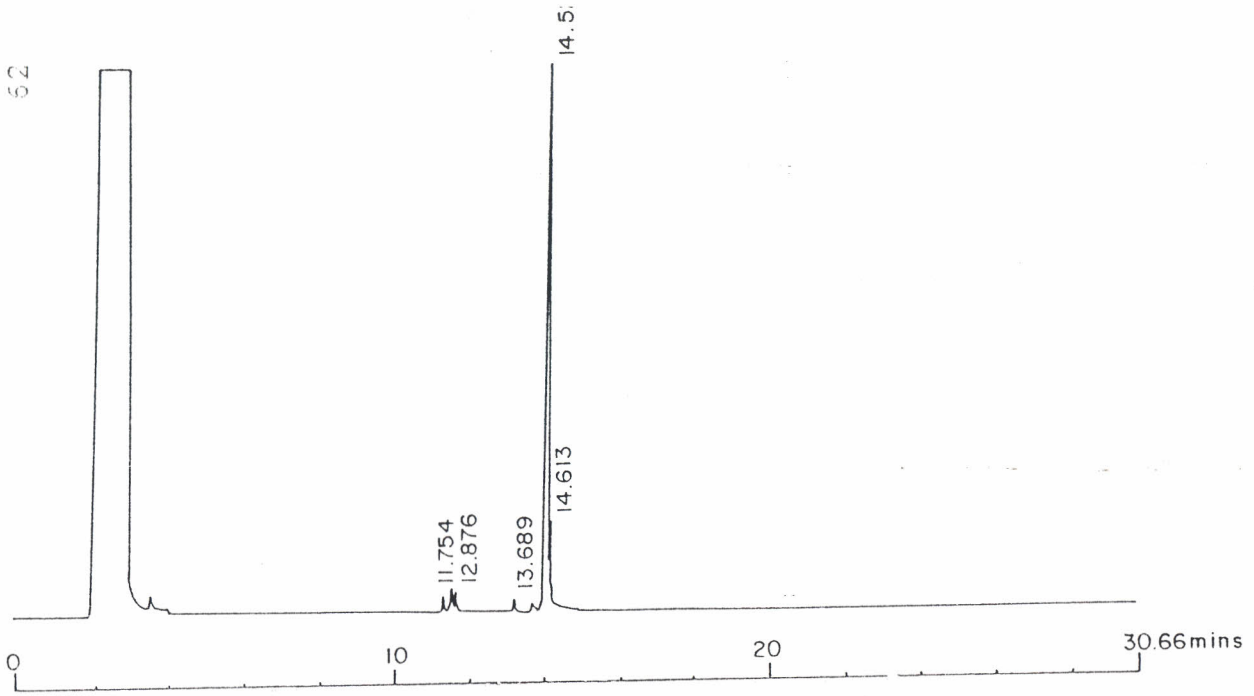


Fig 3 *GC profile of (Z)-11-hexadecenal.*

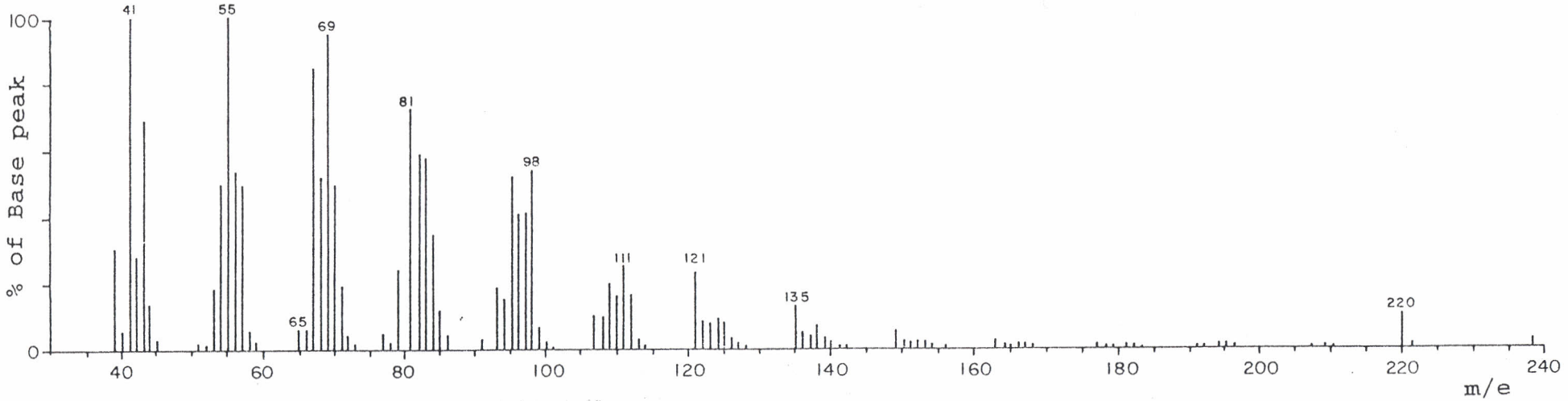


Fig 4 *Mass spectrum of (Z)-11-hexadecenal.*

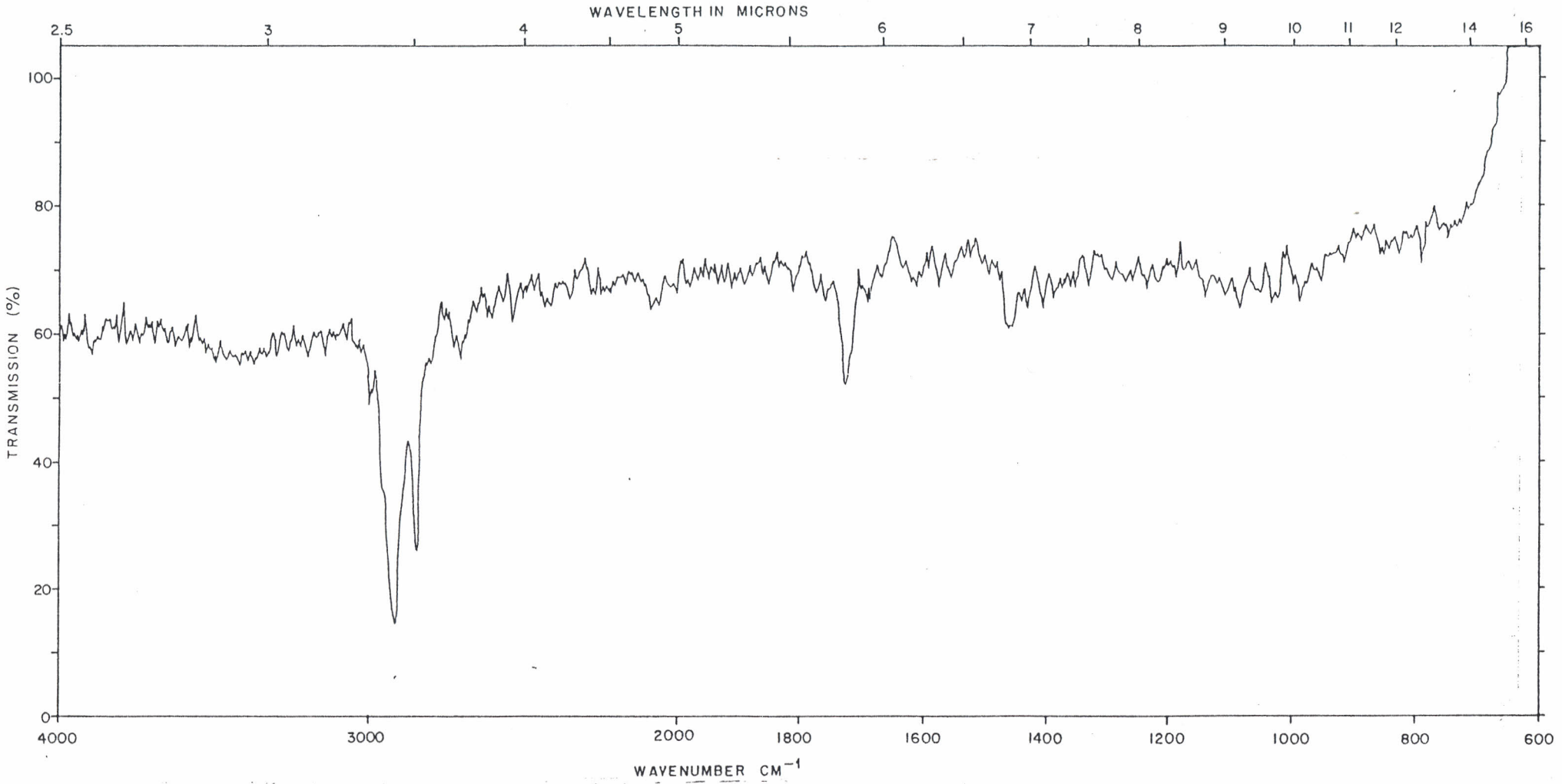


Fig 5 *Infra-red spectrum of (Z)-11-hexadecenal.*

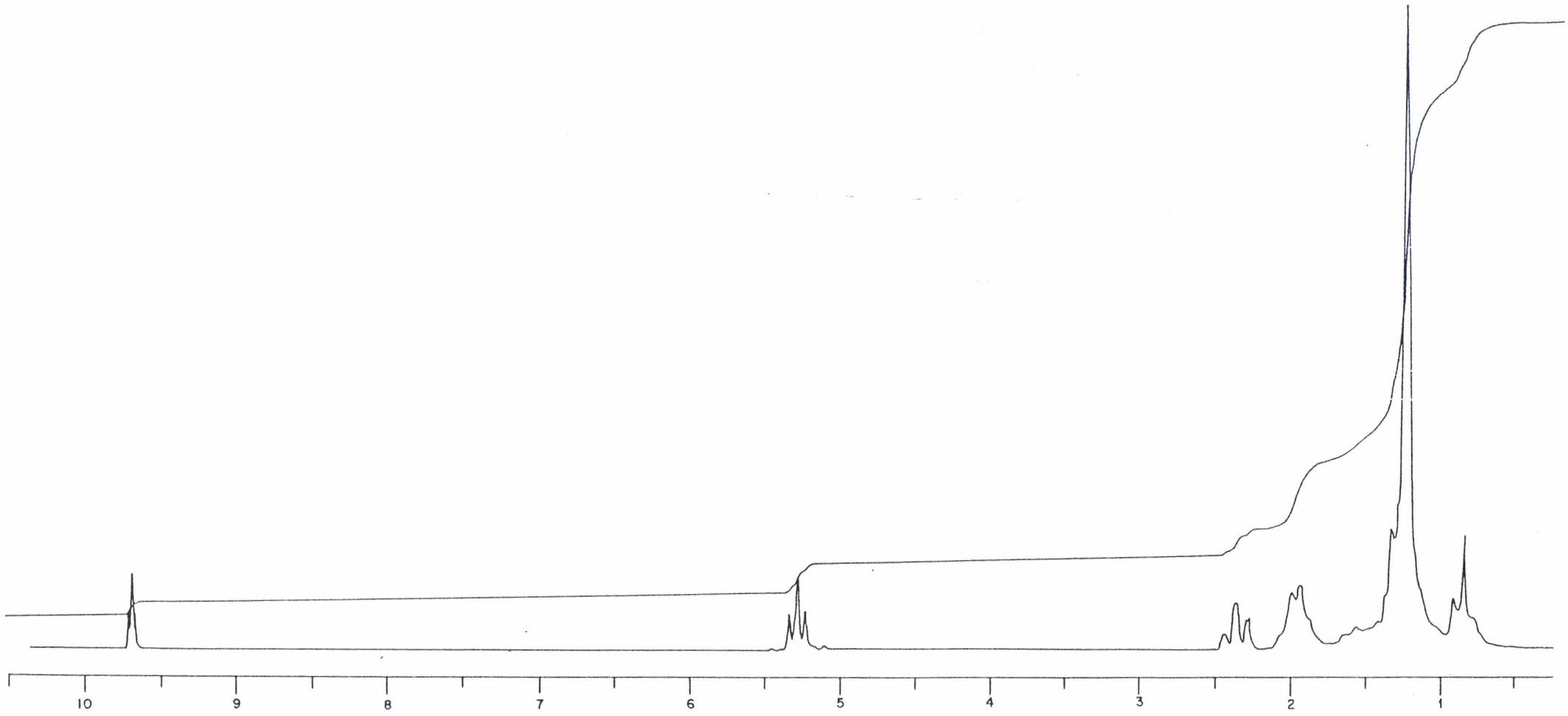


Fig 6 *Proton n.m.r spectrum of (Z)-11-hexadecenal.*

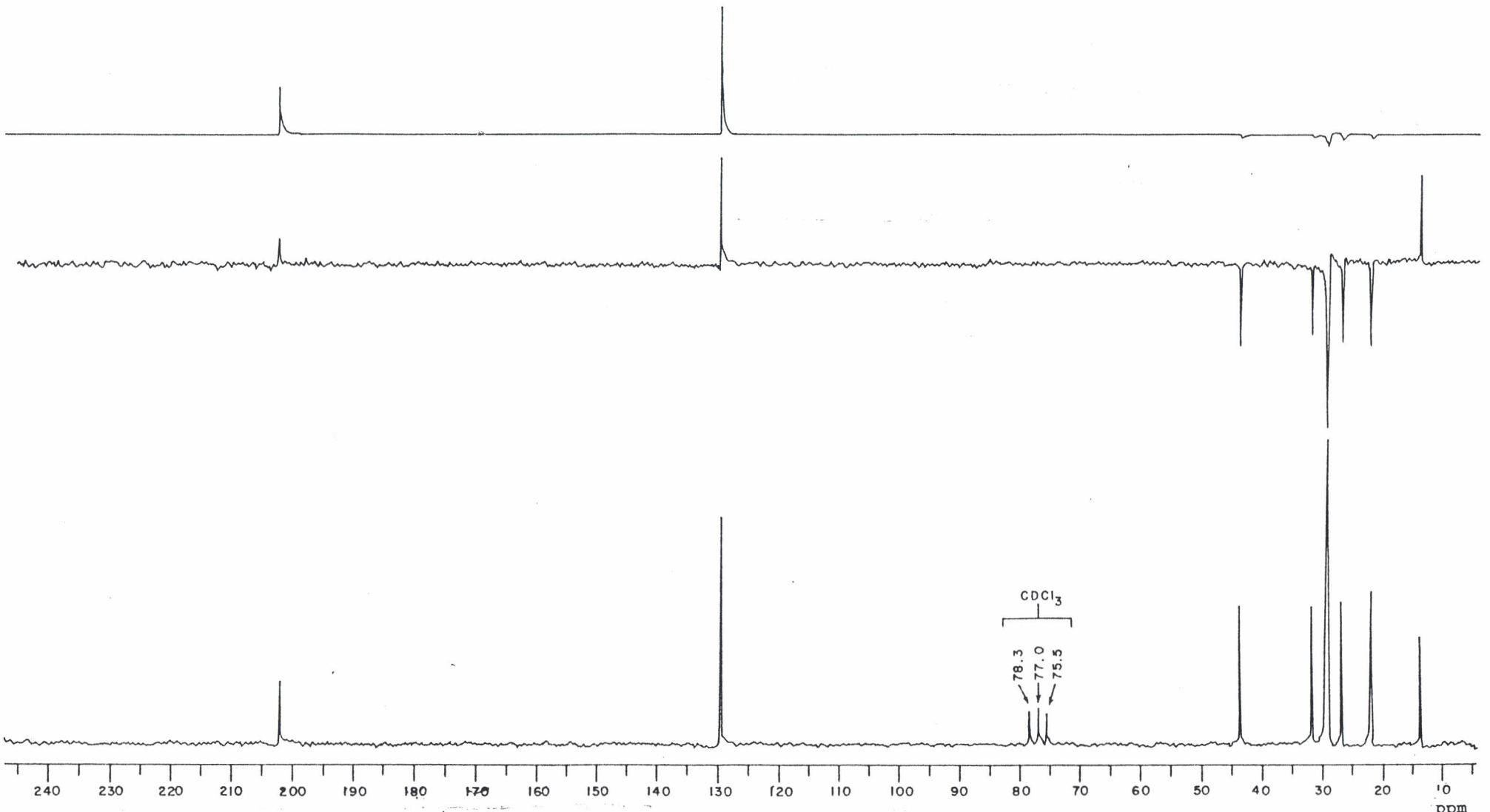


Fig 7 ^{13}C n.m.r spectrum of (Z)-11-hexadecenal.

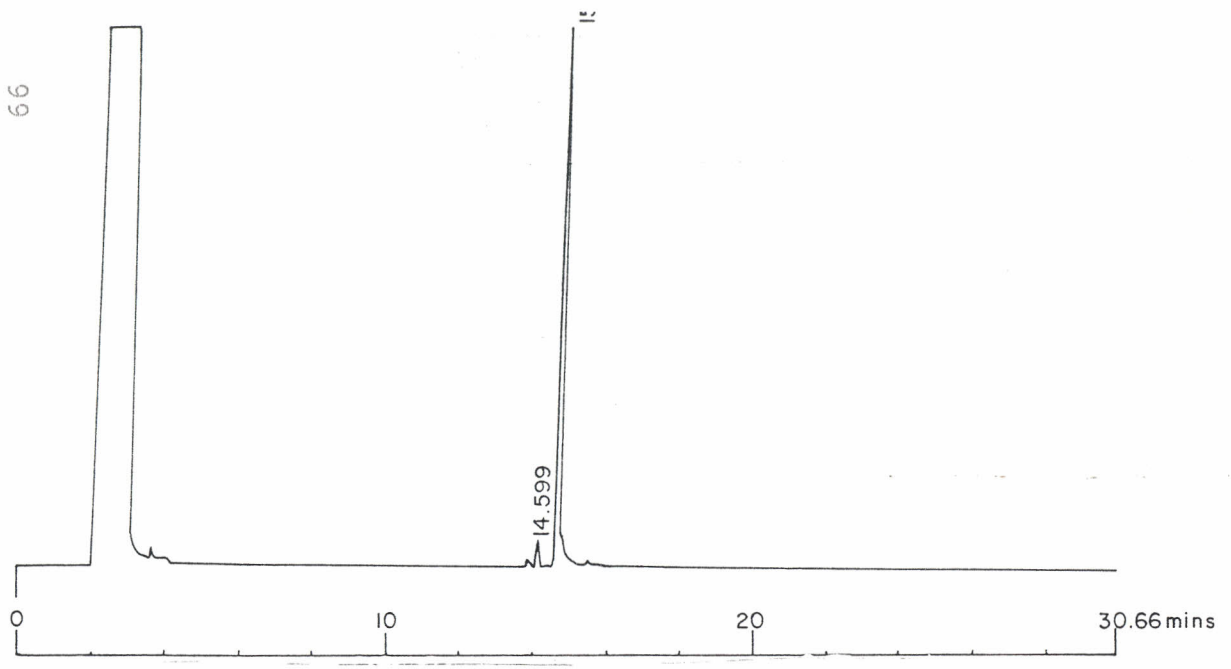
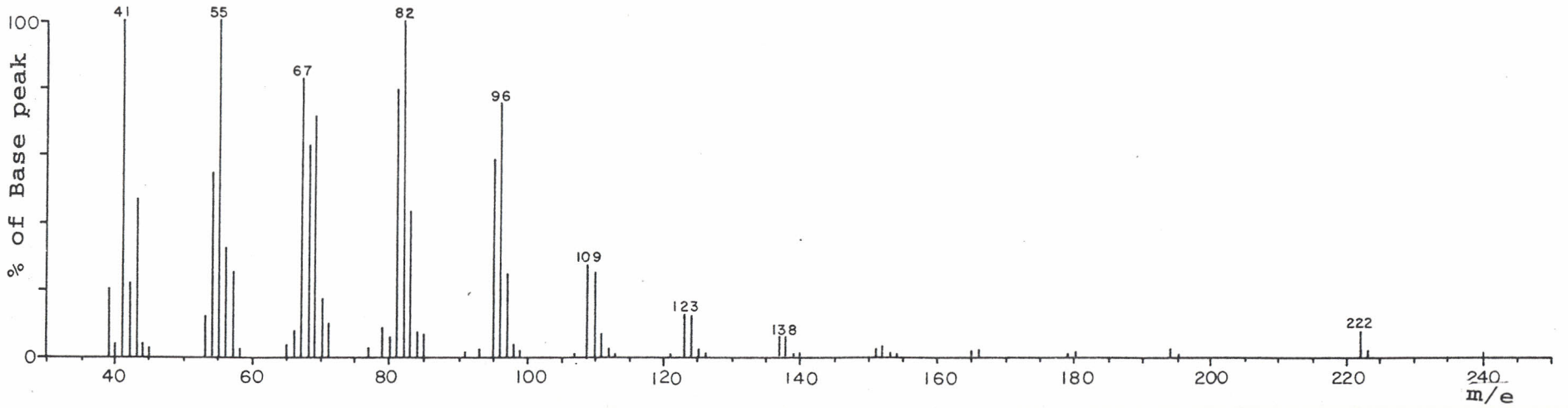


Fig 8 *GC profile of (Z)-11-hexadecen-1-ol.*



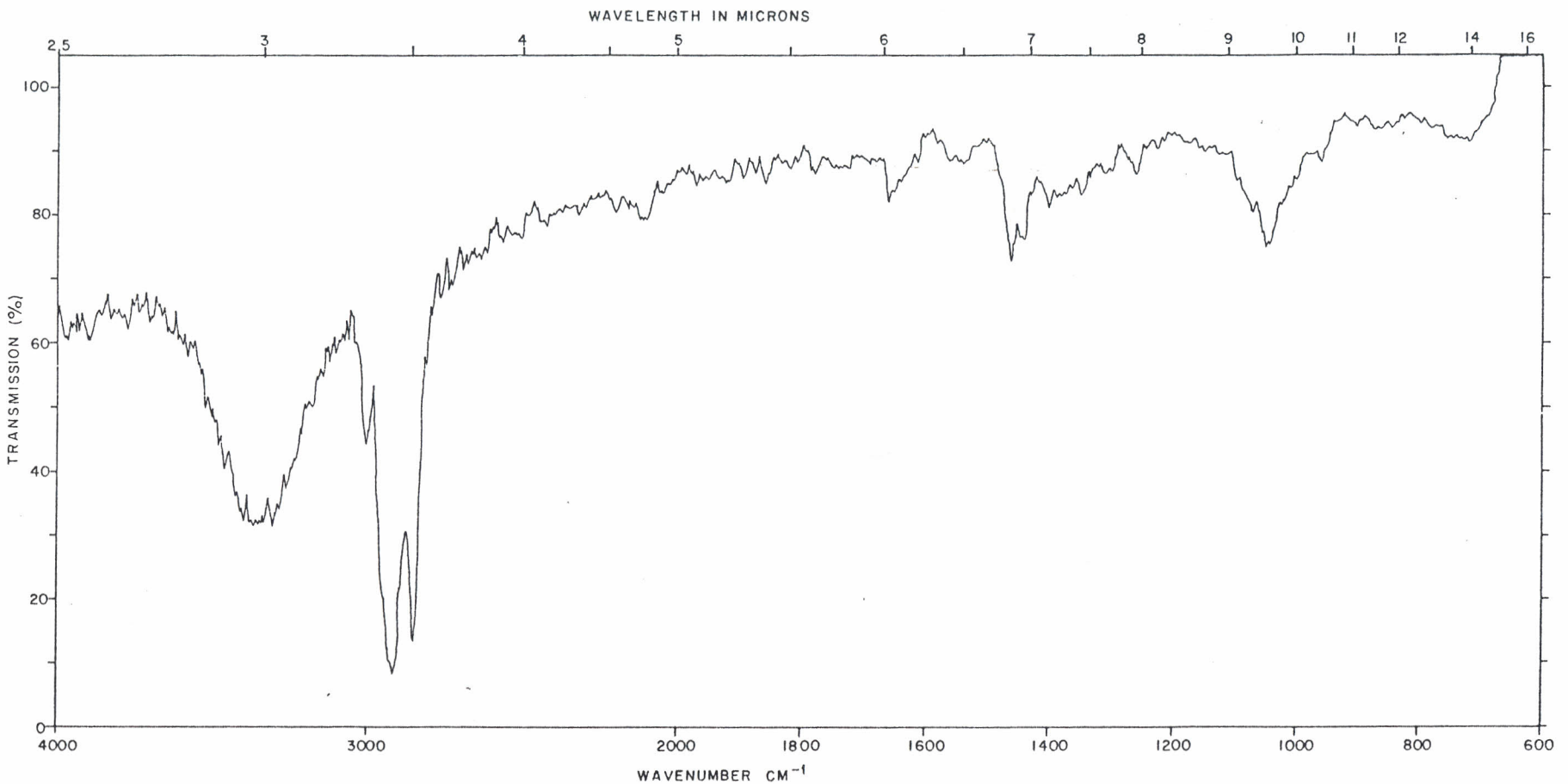


Fig 10 *Infra-red spectrum of (Z)-11-hexadecen-1-ol.*

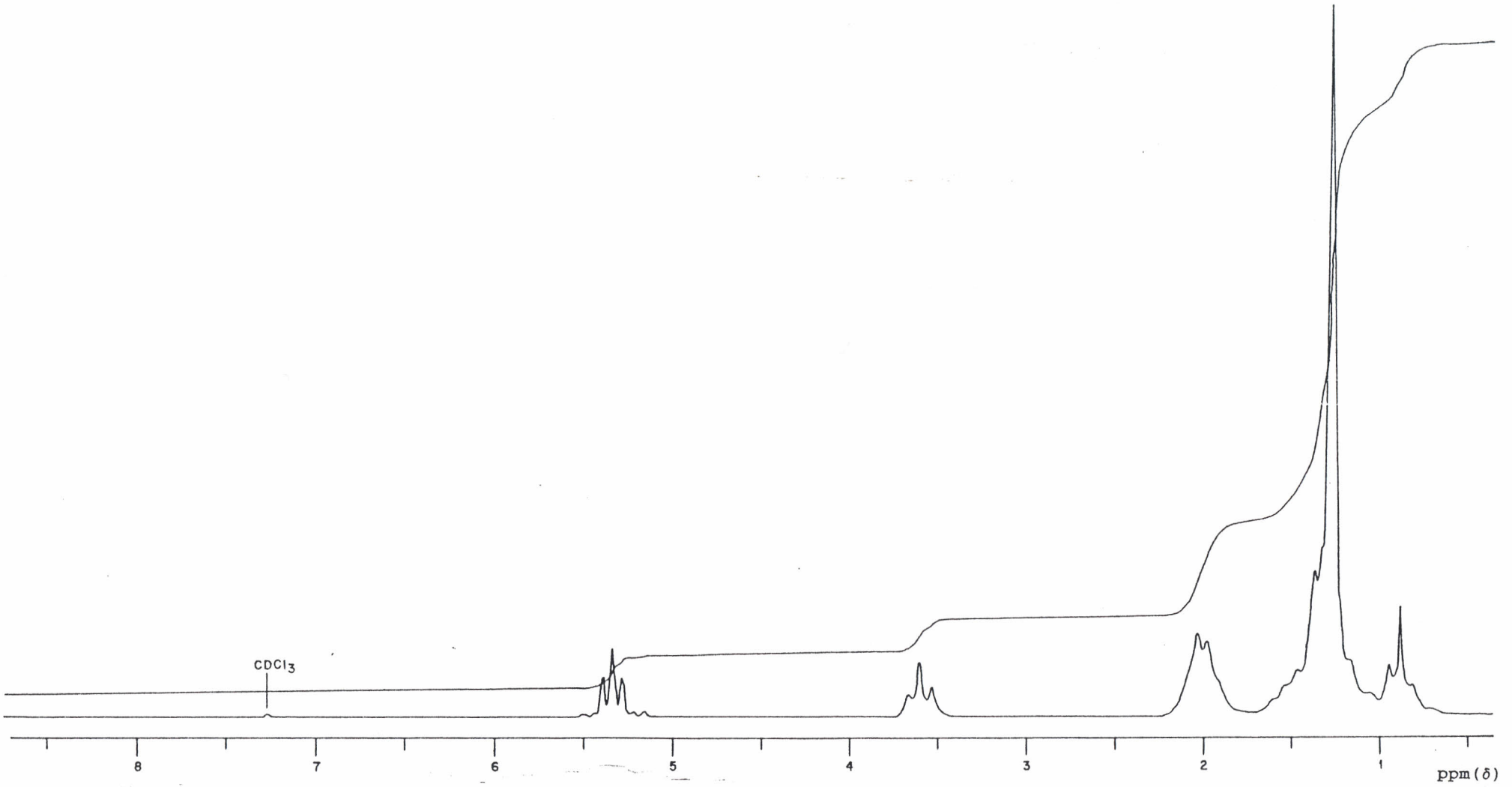


Fig 11 *Proton n.m.r spectrum of (Z)-11-hexadecen-1-ol.*

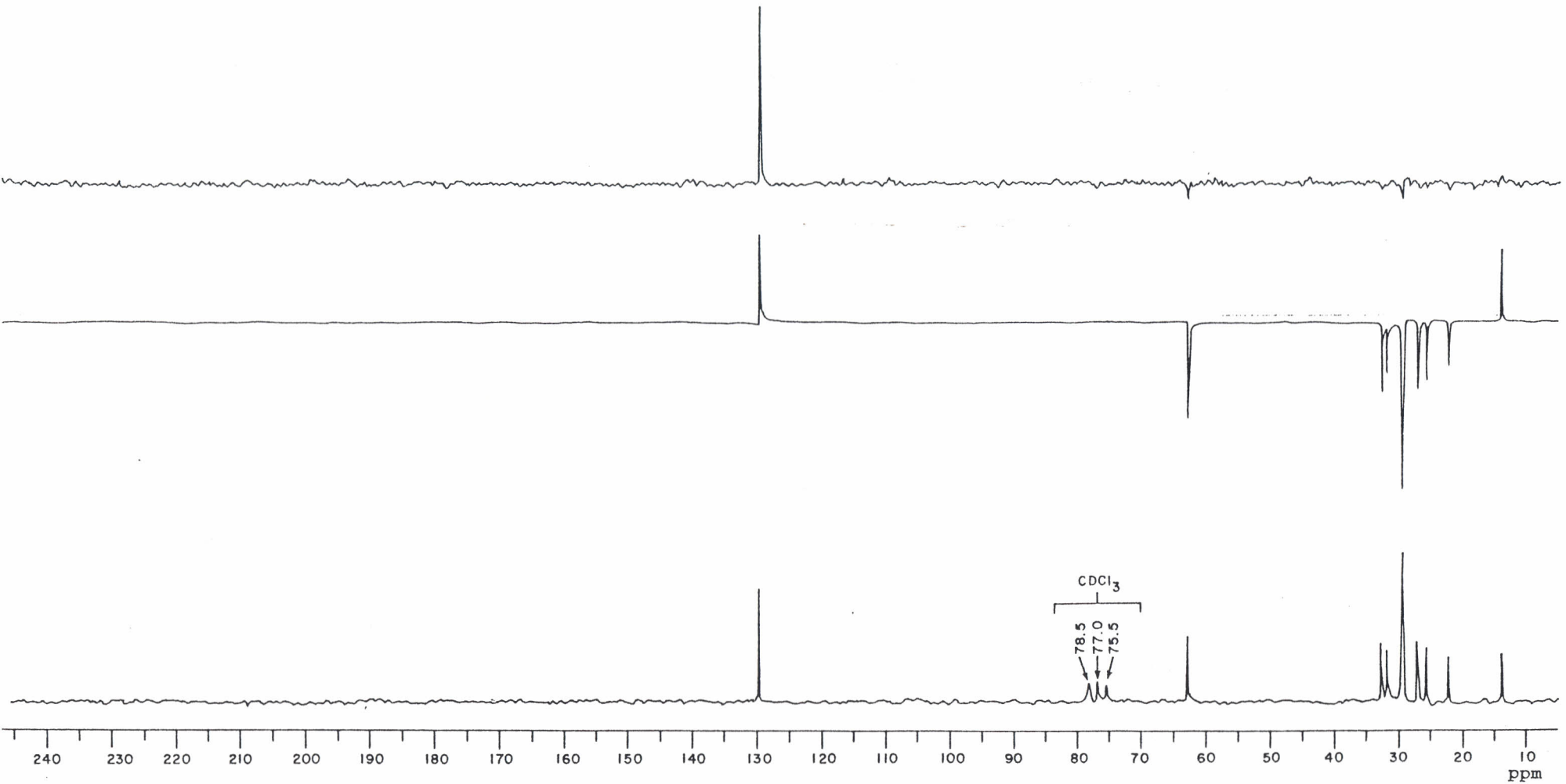


Fig 12 ^{13}C n.m.r spectrum of (Z)-11-hexadecen-1-ol.

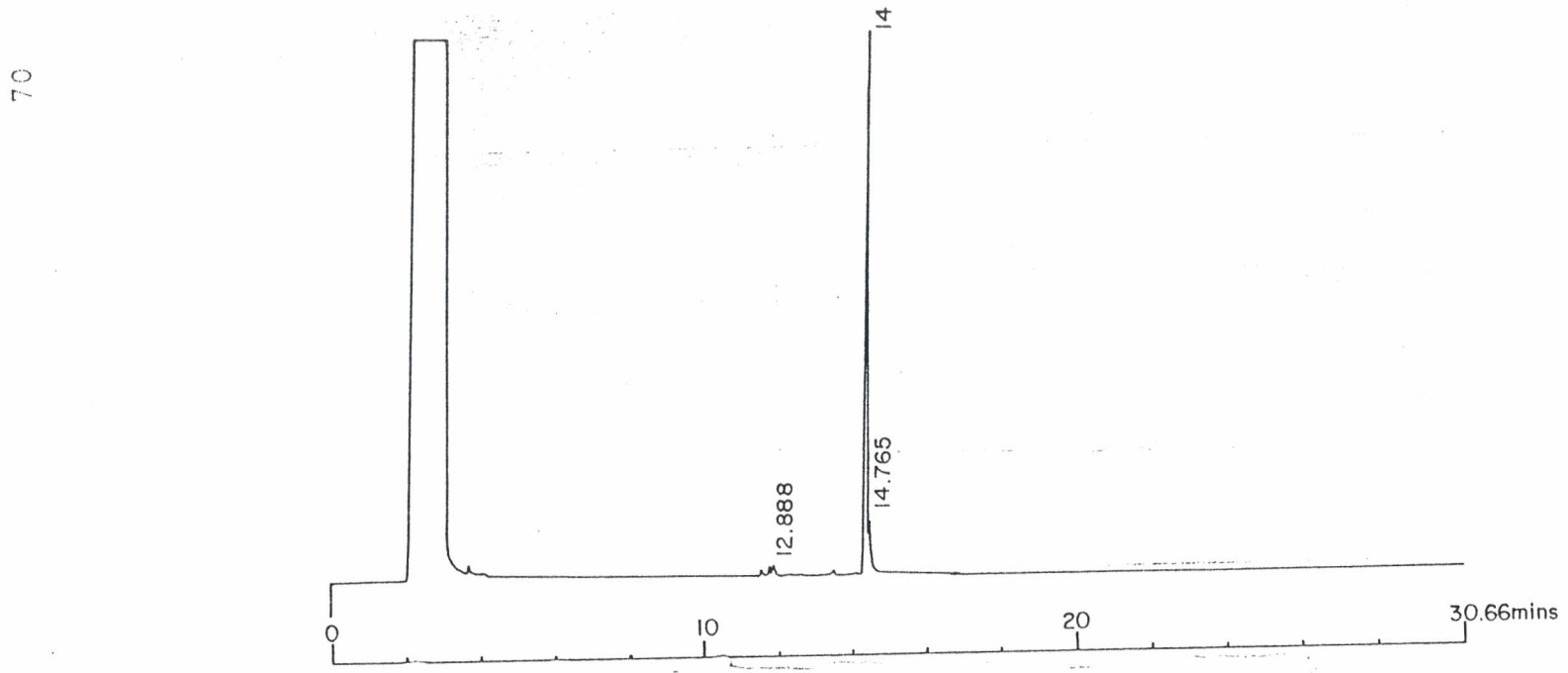
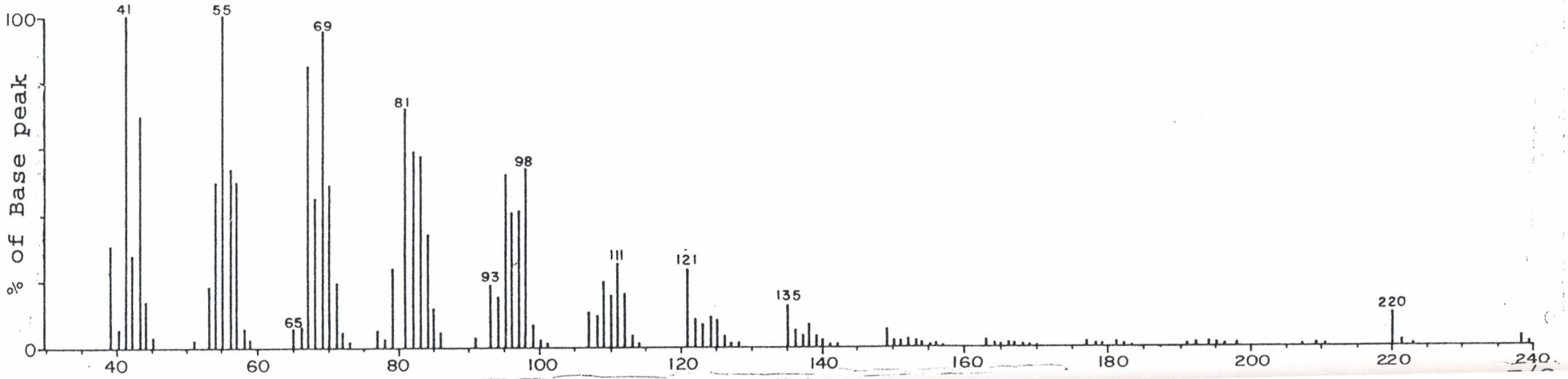


Fig 13 *GC profile of (E) -11-hexadecenal.*



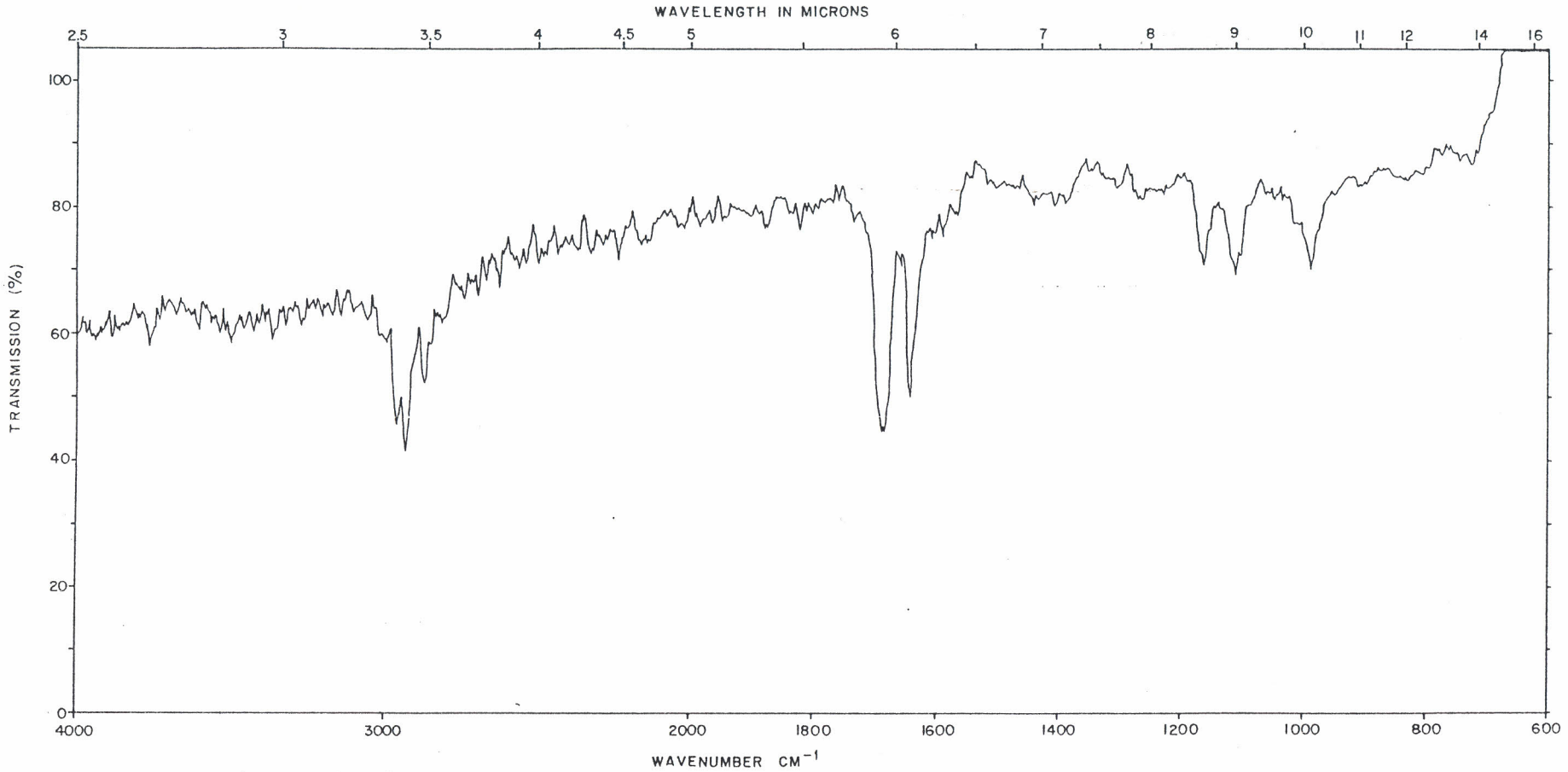


Fig 15 *Infra-red spectrum of (E)-11-hexadecenal.*

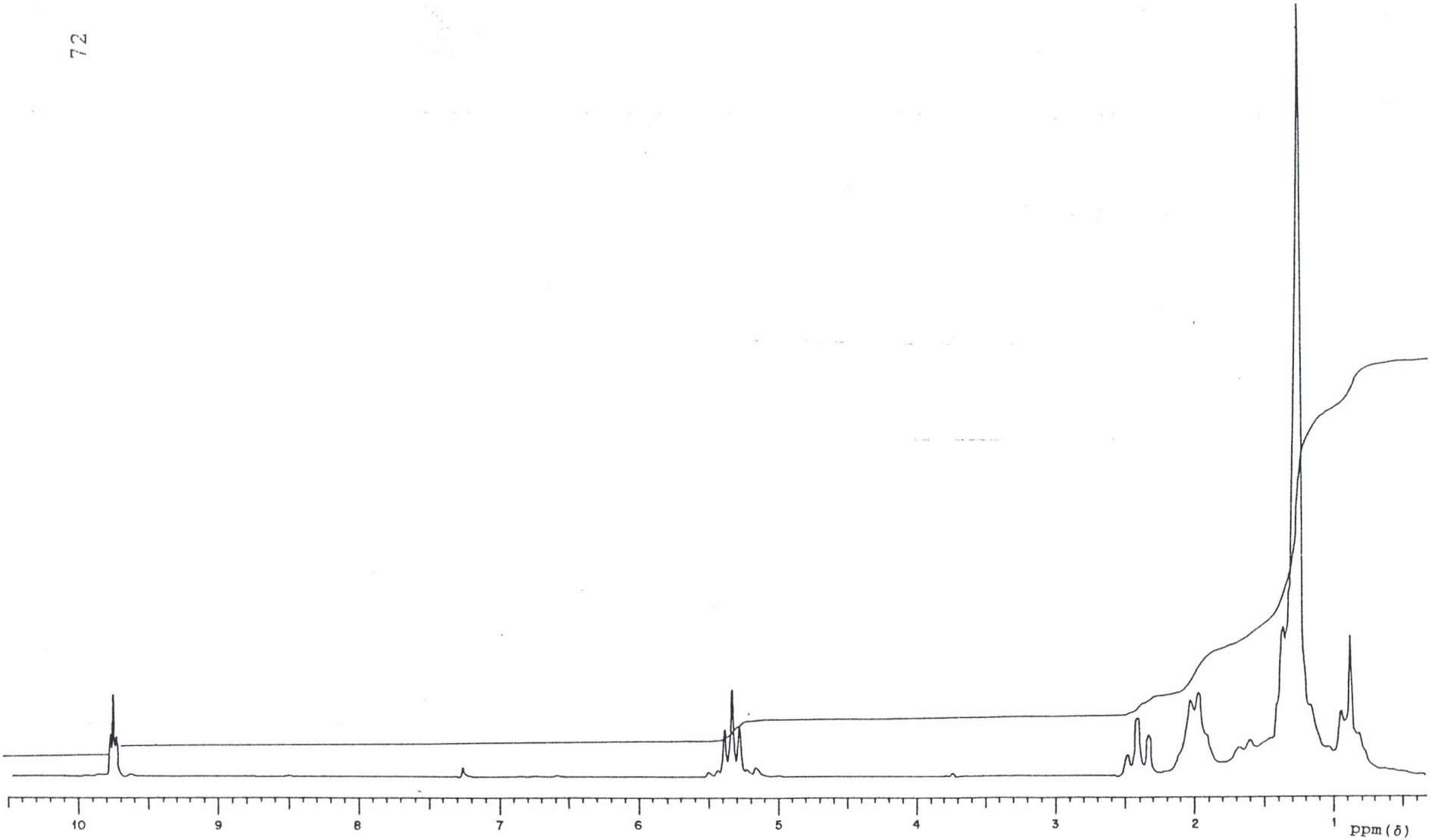


Fig 16 *Proton n.m.r spectrum of (E)-11-hexadecenal.*

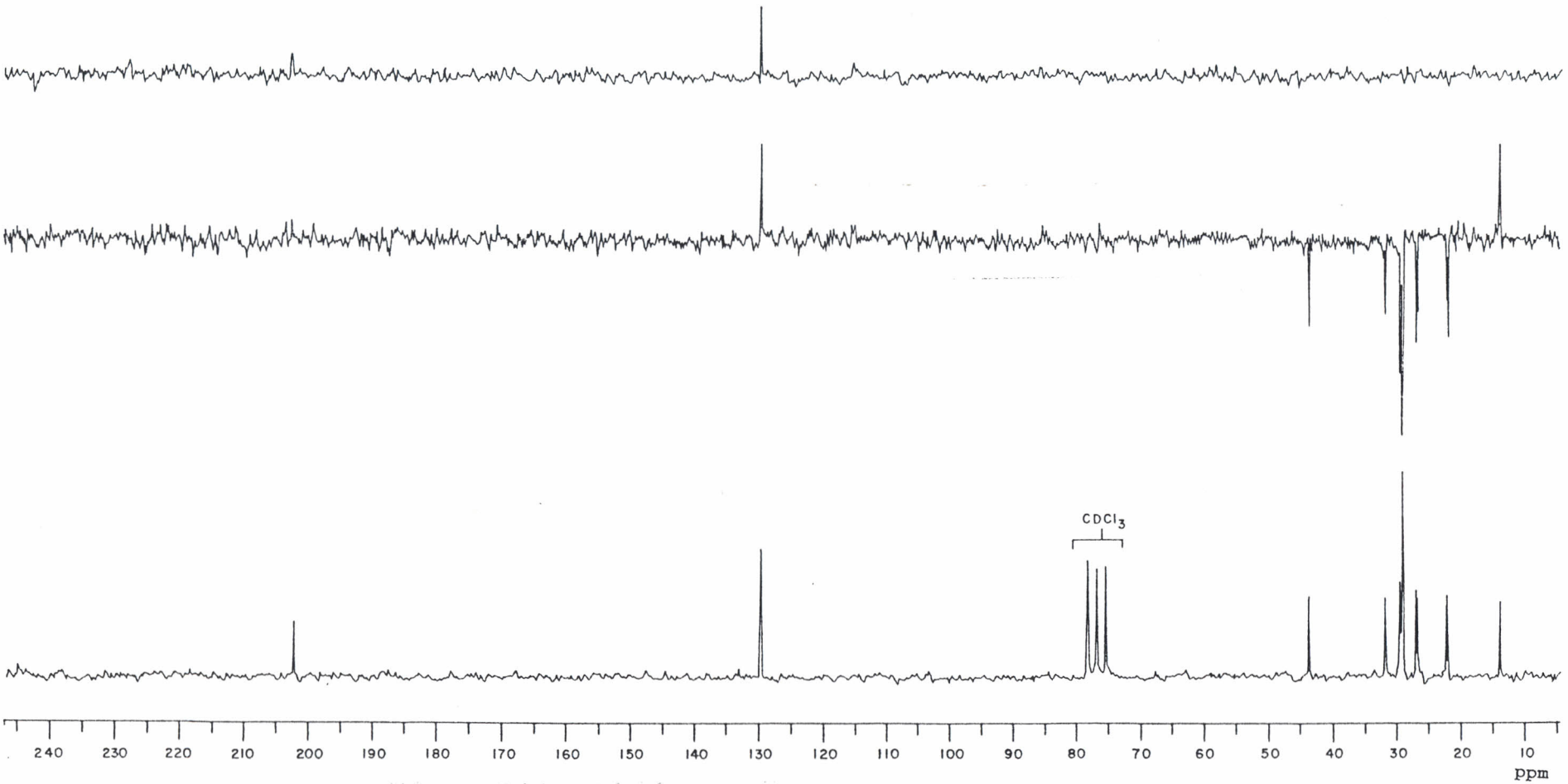


Fig 17 ^{13}C n.m.r spectrum of (E)-11-hexadecenal.

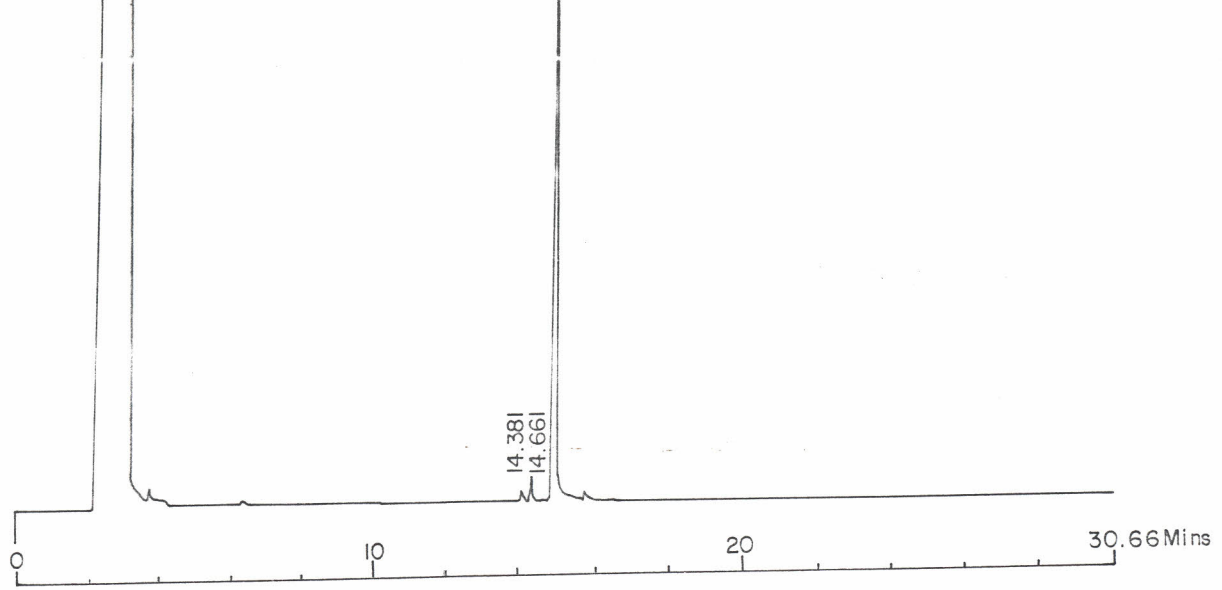


Fig 18 *GC profile of (E) -11-hexadecen-1-ol.*

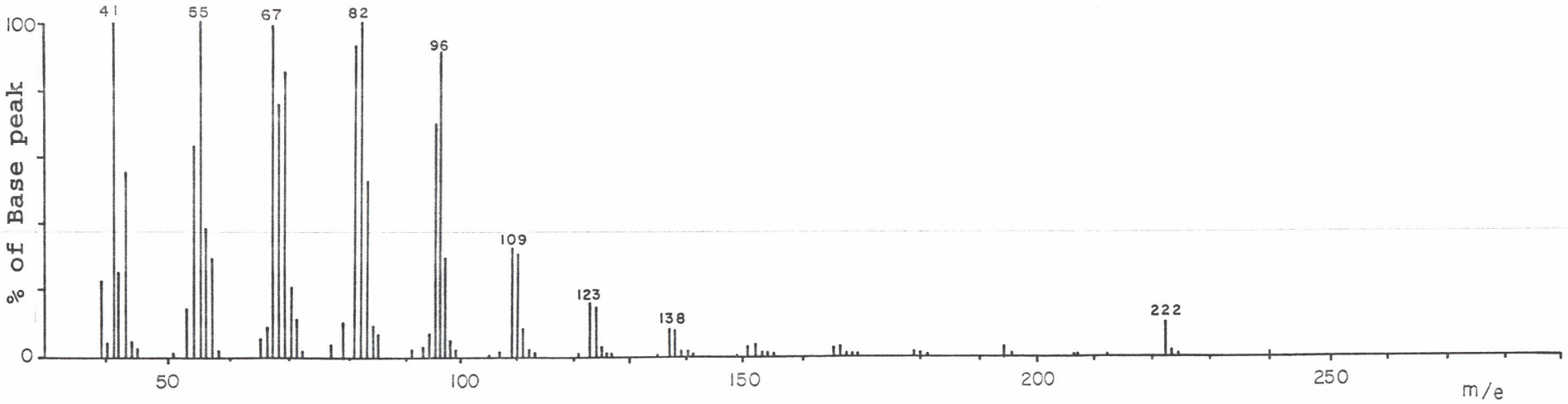


Fig 19 *Mass spectrum of (E)-11-hexadecen-1-ol.*

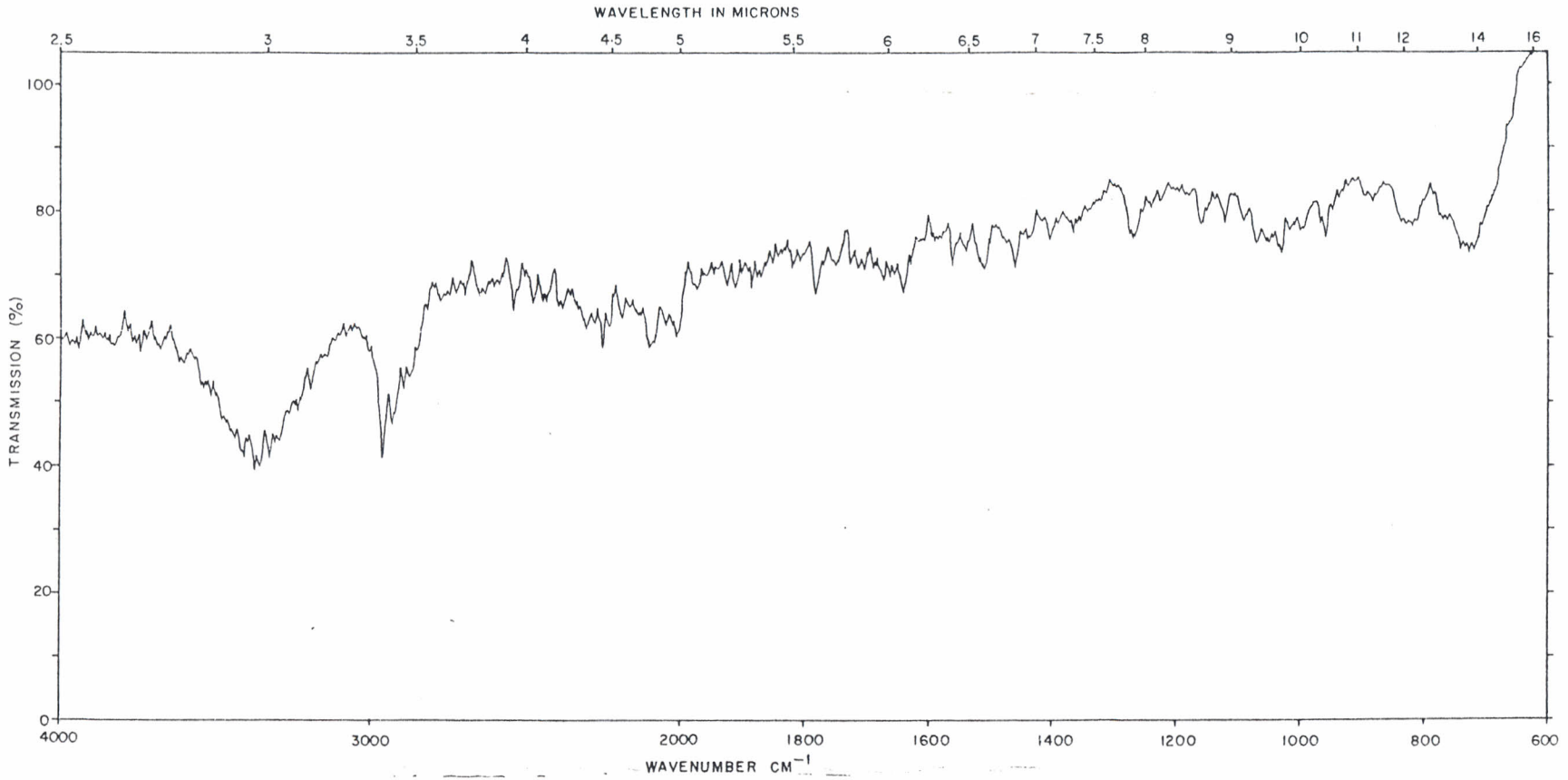


Fig 20 *Infra-red spectrum of (E)-11-hexadecen-1-ol.*

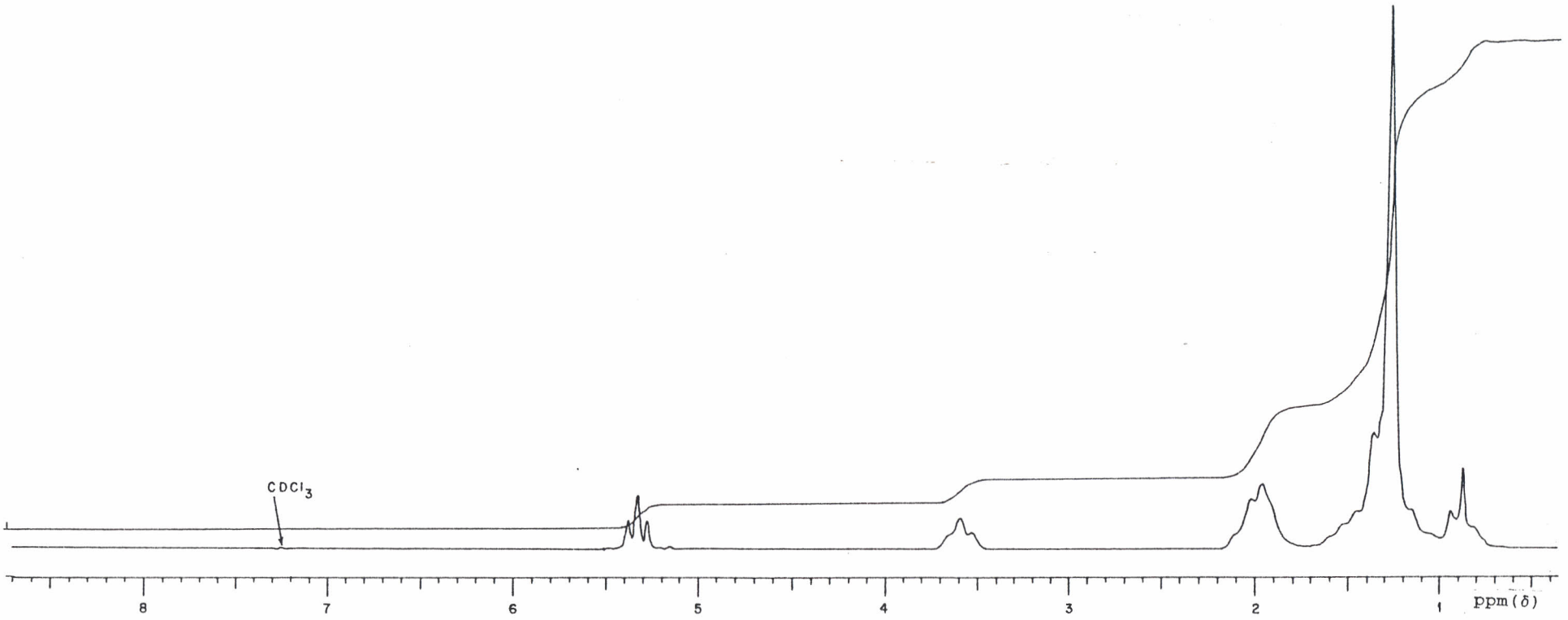


Fig 21 *Proton n.m.r spectrum of (E)* -11-hexadecen-1-ol.

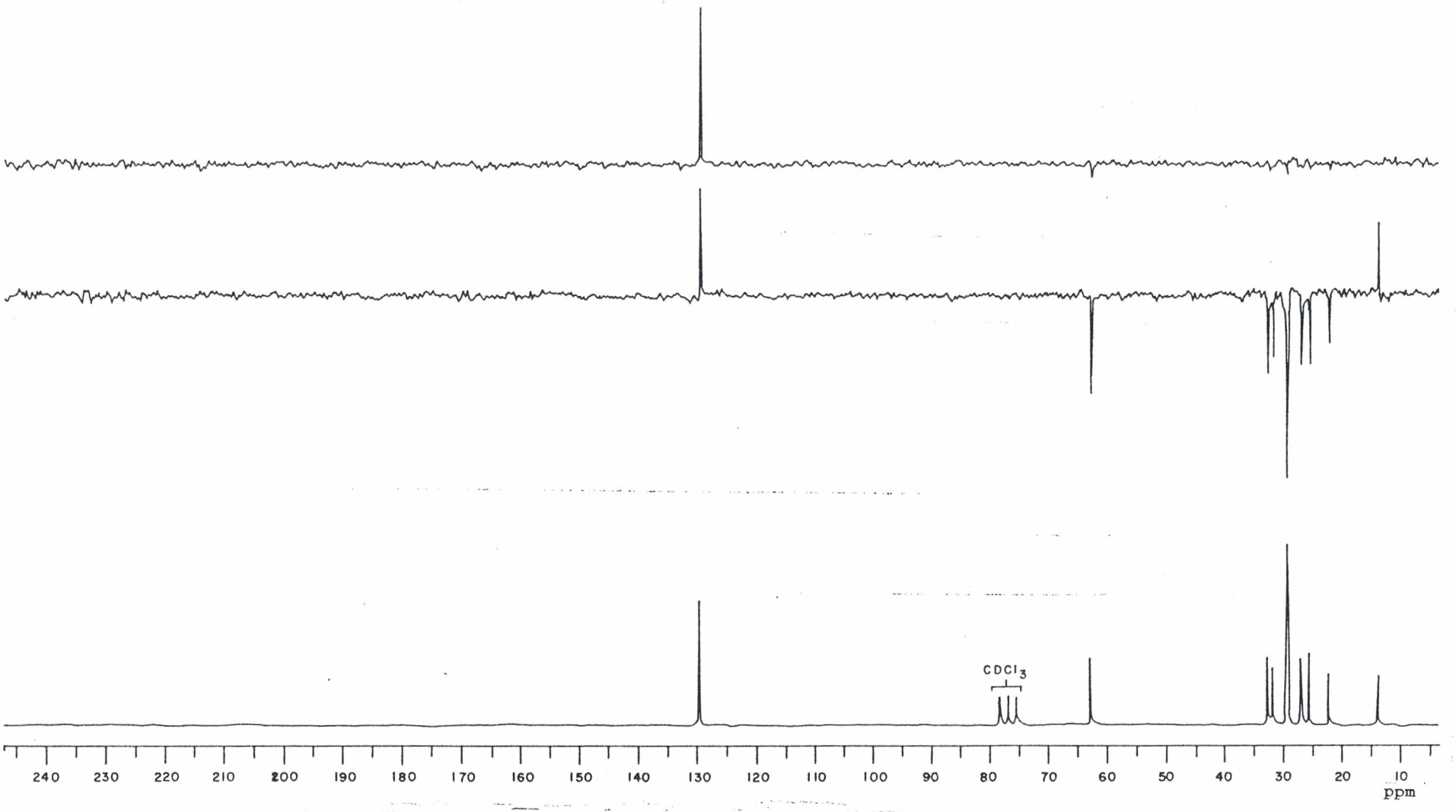


Fig 22 ^{13}C n.m.r spectrum of (E)-11-hexadecen-1-ol.

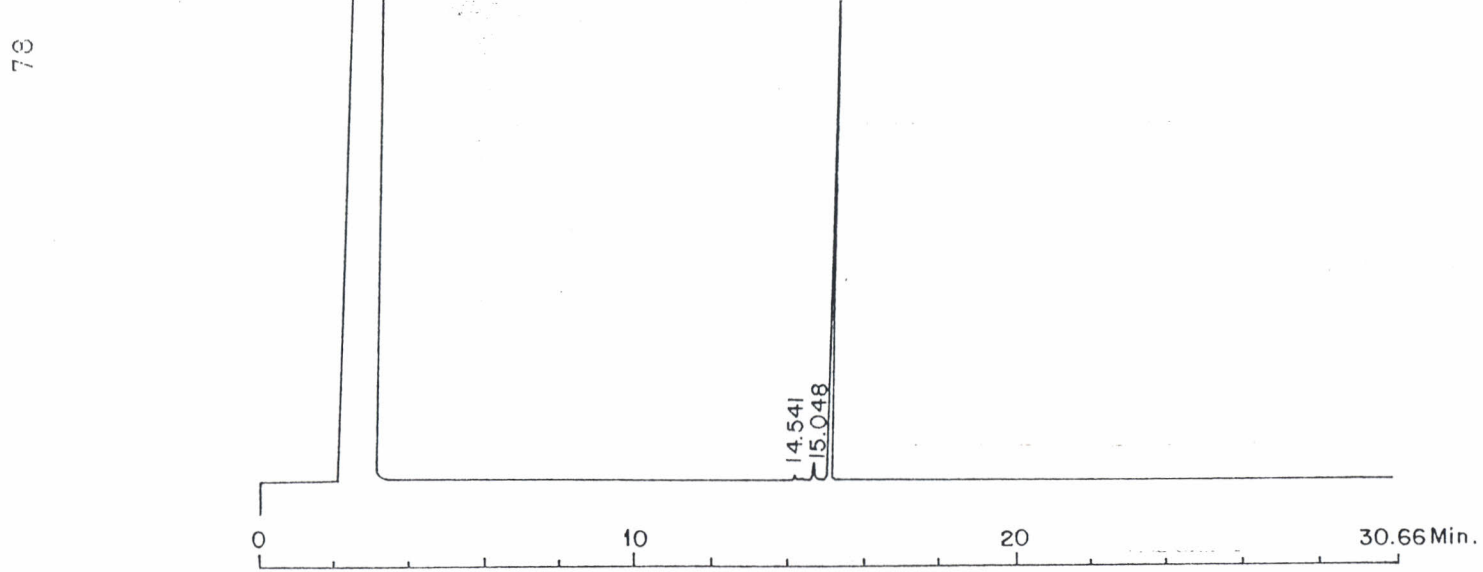


Fig 23 *GC profile of (Z)-11-hexadecenyl formate.*

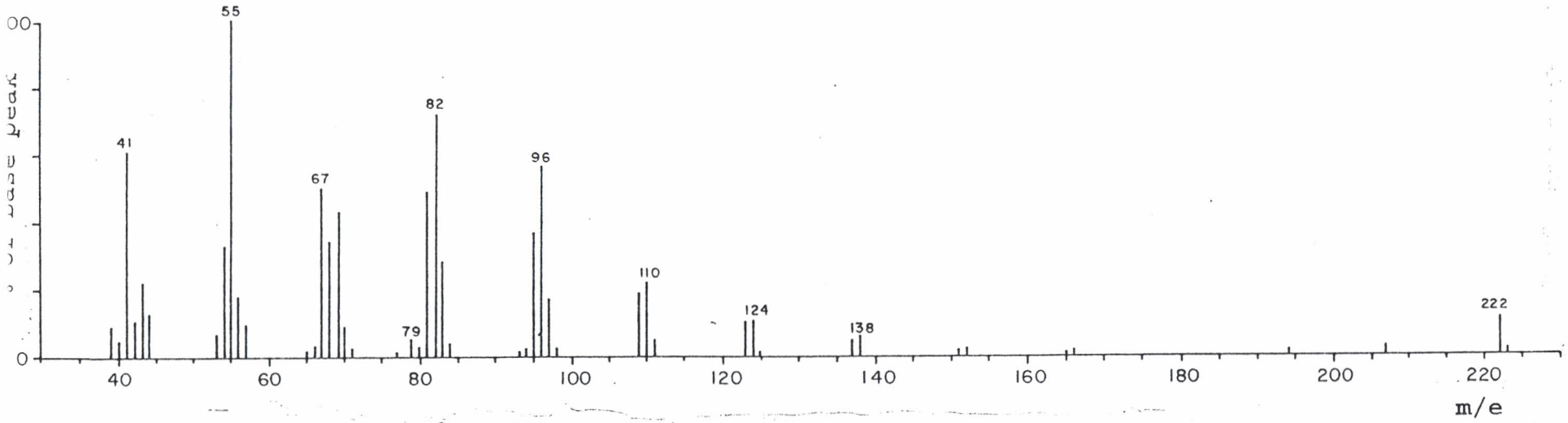


Fig 24 *Mass spectrum of (Z)-11-hexadecenyl formate.*

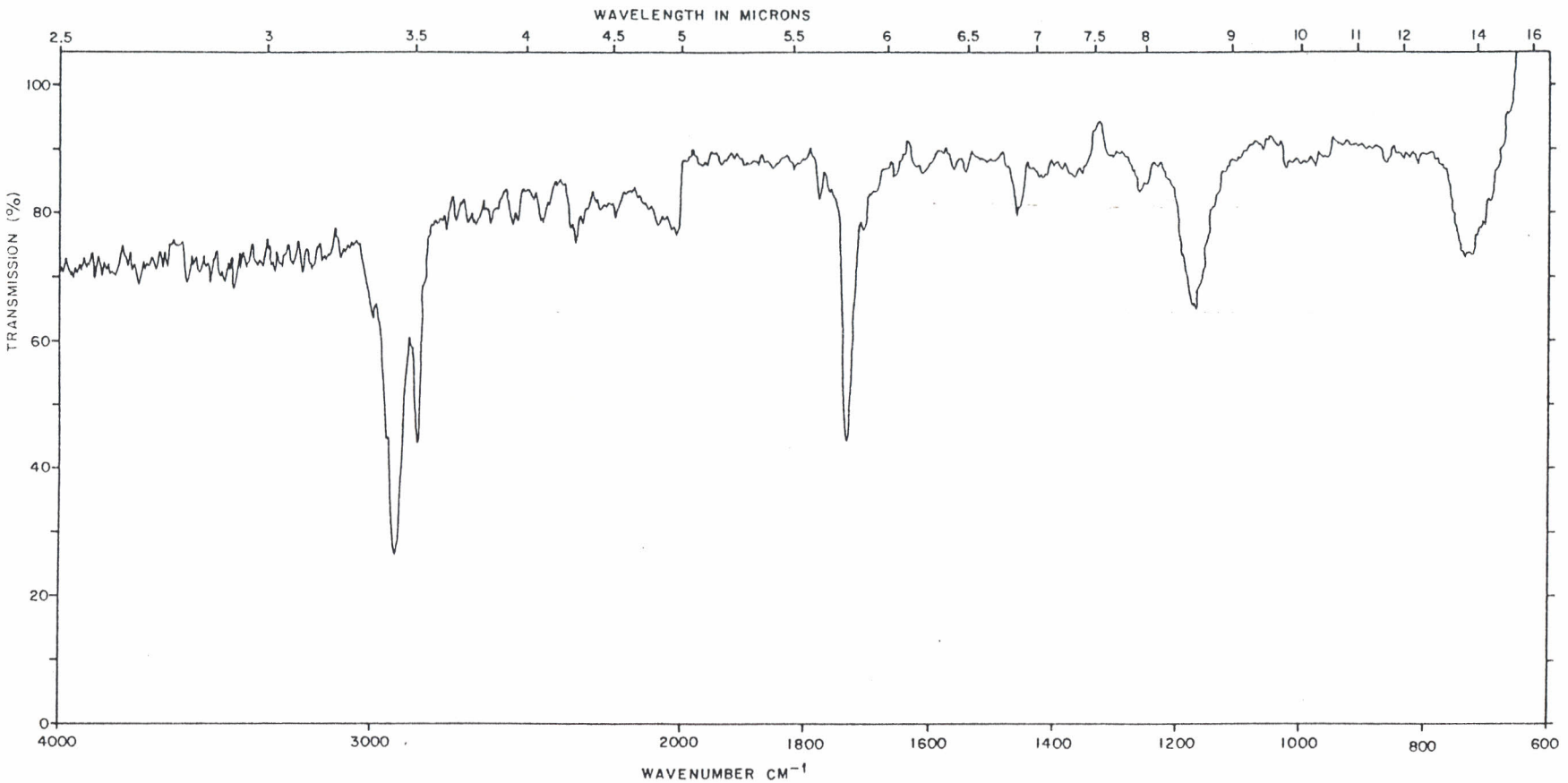


Fig 25 *Infra-red spectrum of (Z)-11-hexadecenyl formate.*

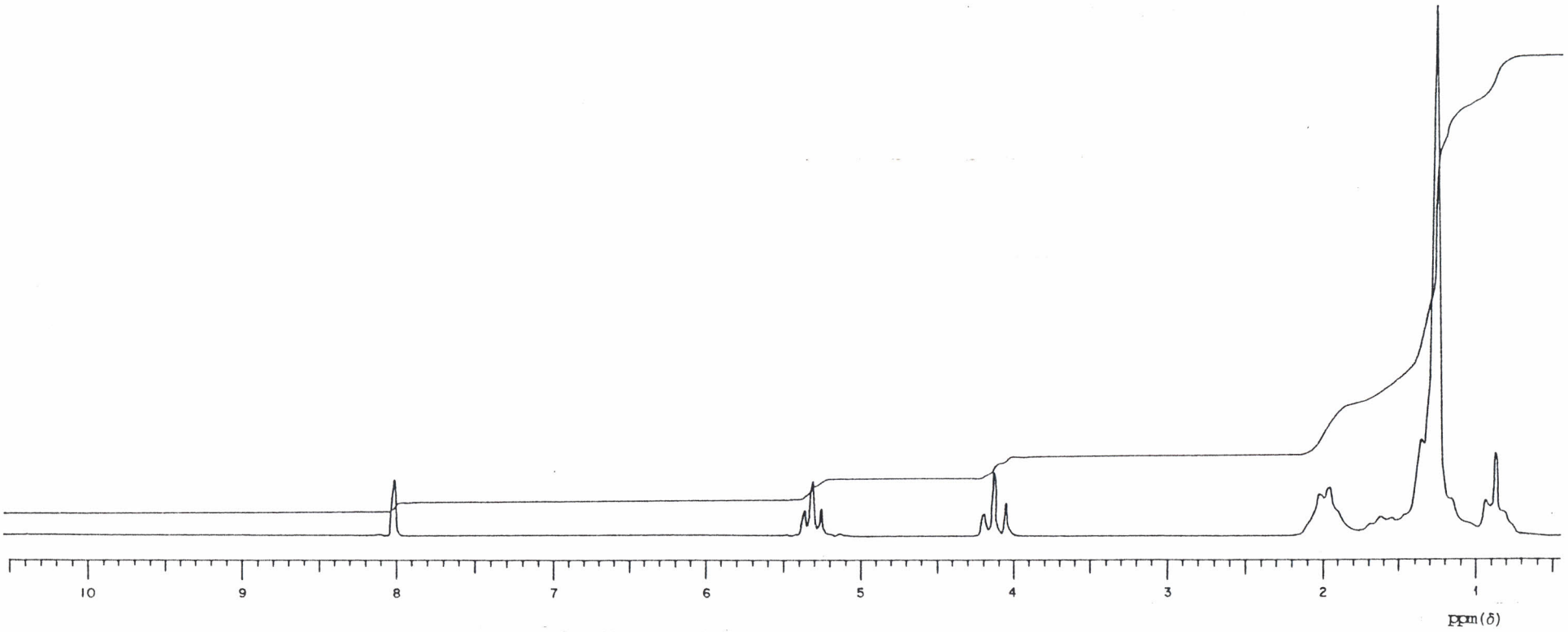


Fig 26 *Proton n.m.r spectrum of (Z)-11-hexadecenyl formate.*

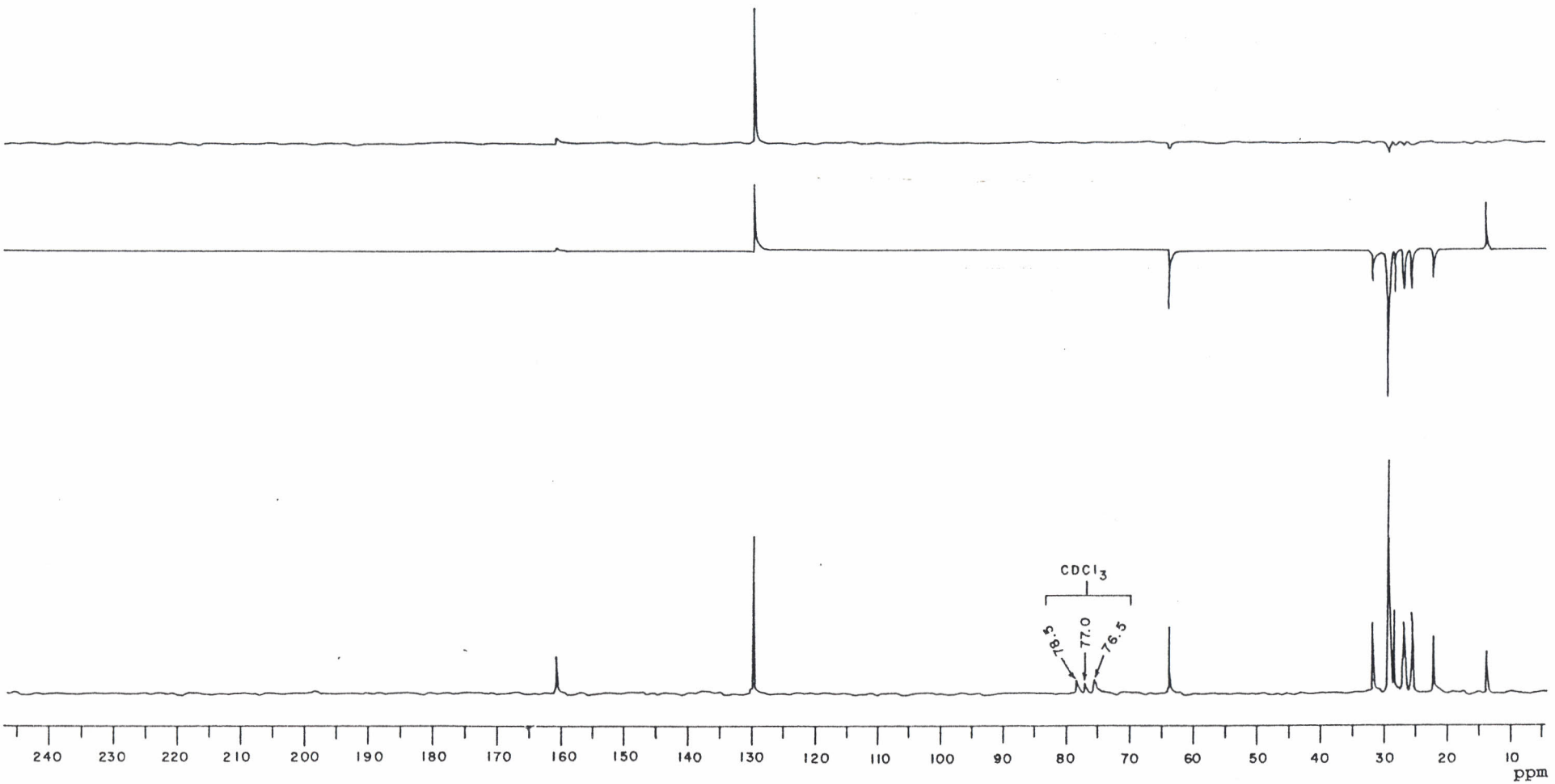


Fig 27 ^{13}C n.m.r spectrum of (Z)-11-hexadecenyl formate.

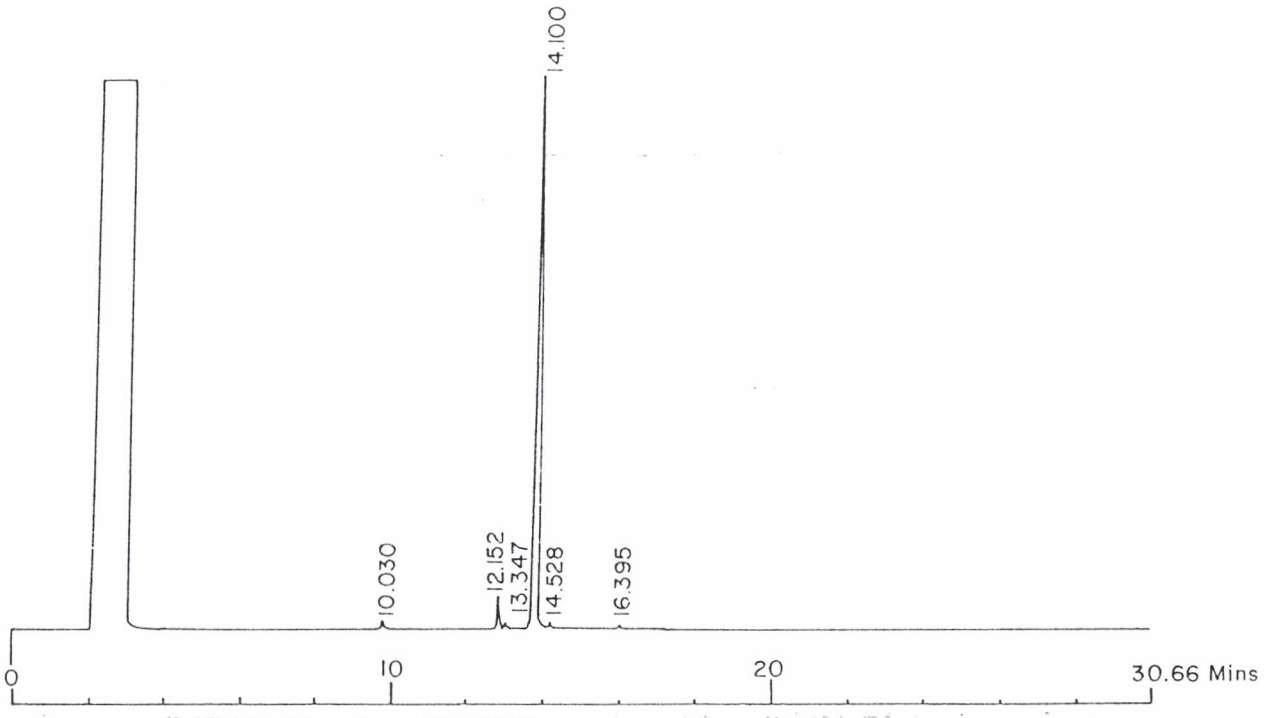


Fig 28 *GC profile of (Z)-9-tetradecenyl formate.*

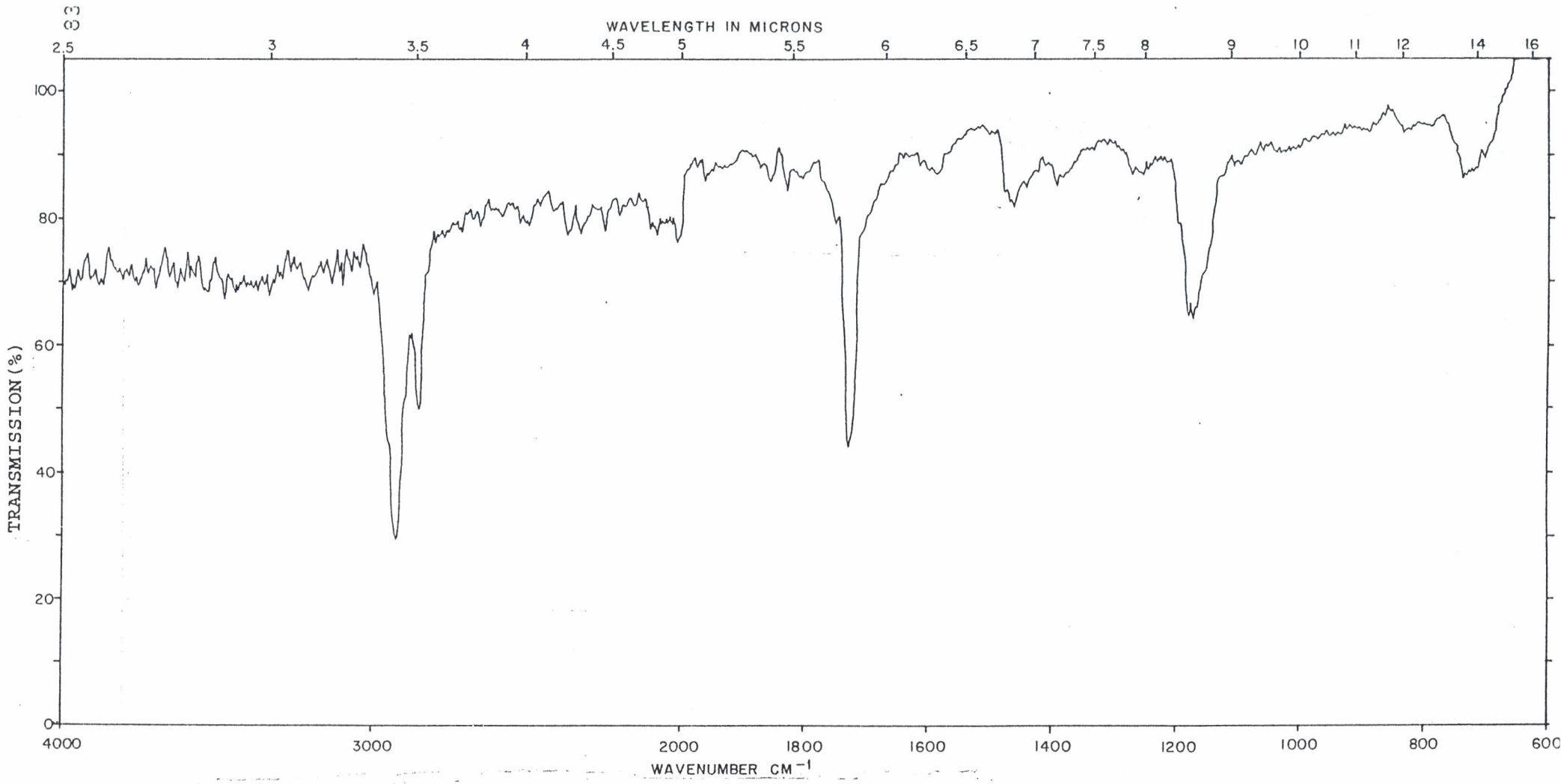


Fig 29 *Infra-red spectrum of (Z) -9-tetradecenyl formate.*

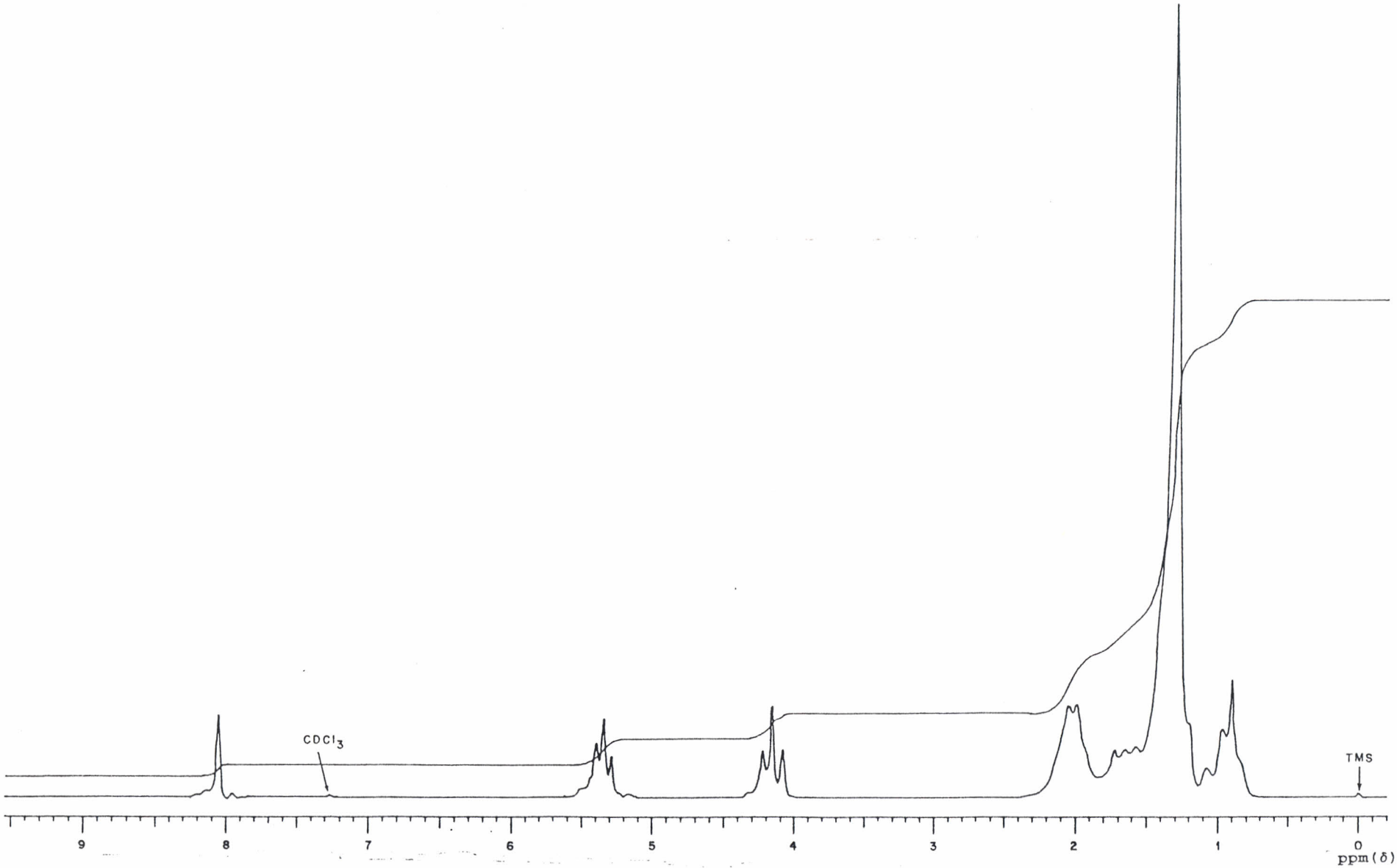


Fig 30 *Proton n.m.r spectrum of (Z)-9-tetradecenyl formate.*

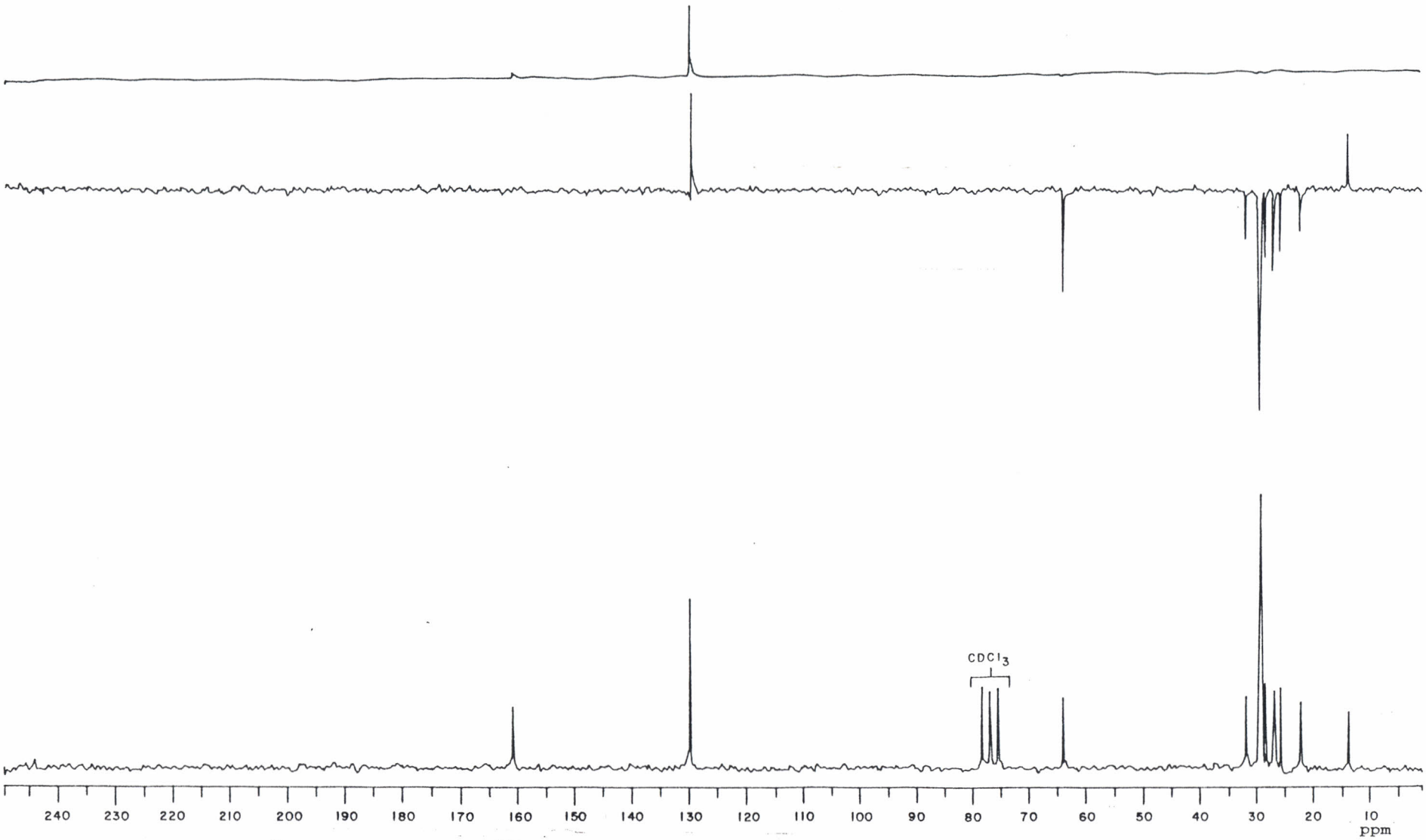


Fig 31 ^{13}C *n.m.r spectrum of (Z)-9-tetradecenyl formate.*

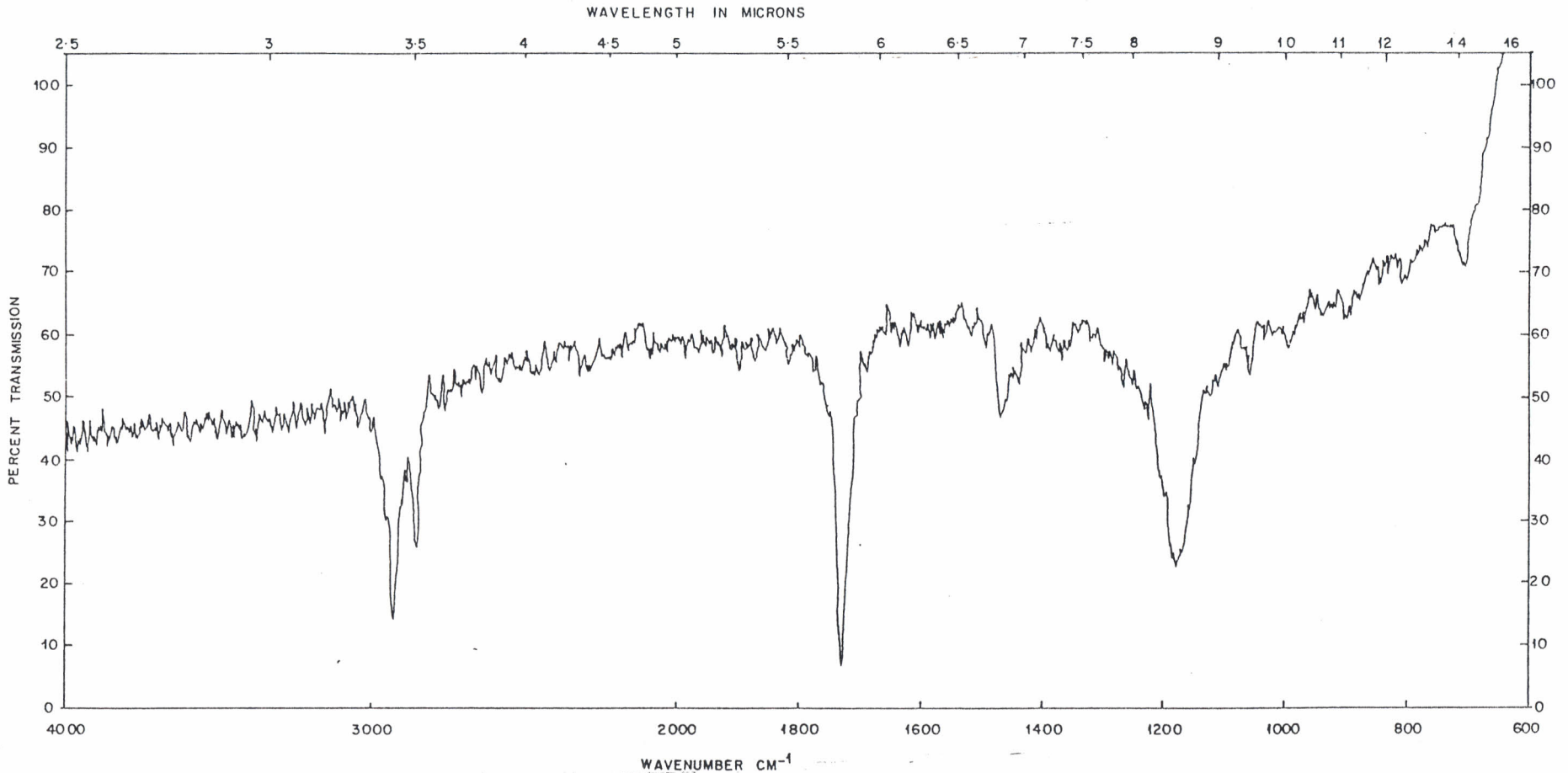


Fig 33 *Infra-red spectrum of 8-bromooctanyl formate.*

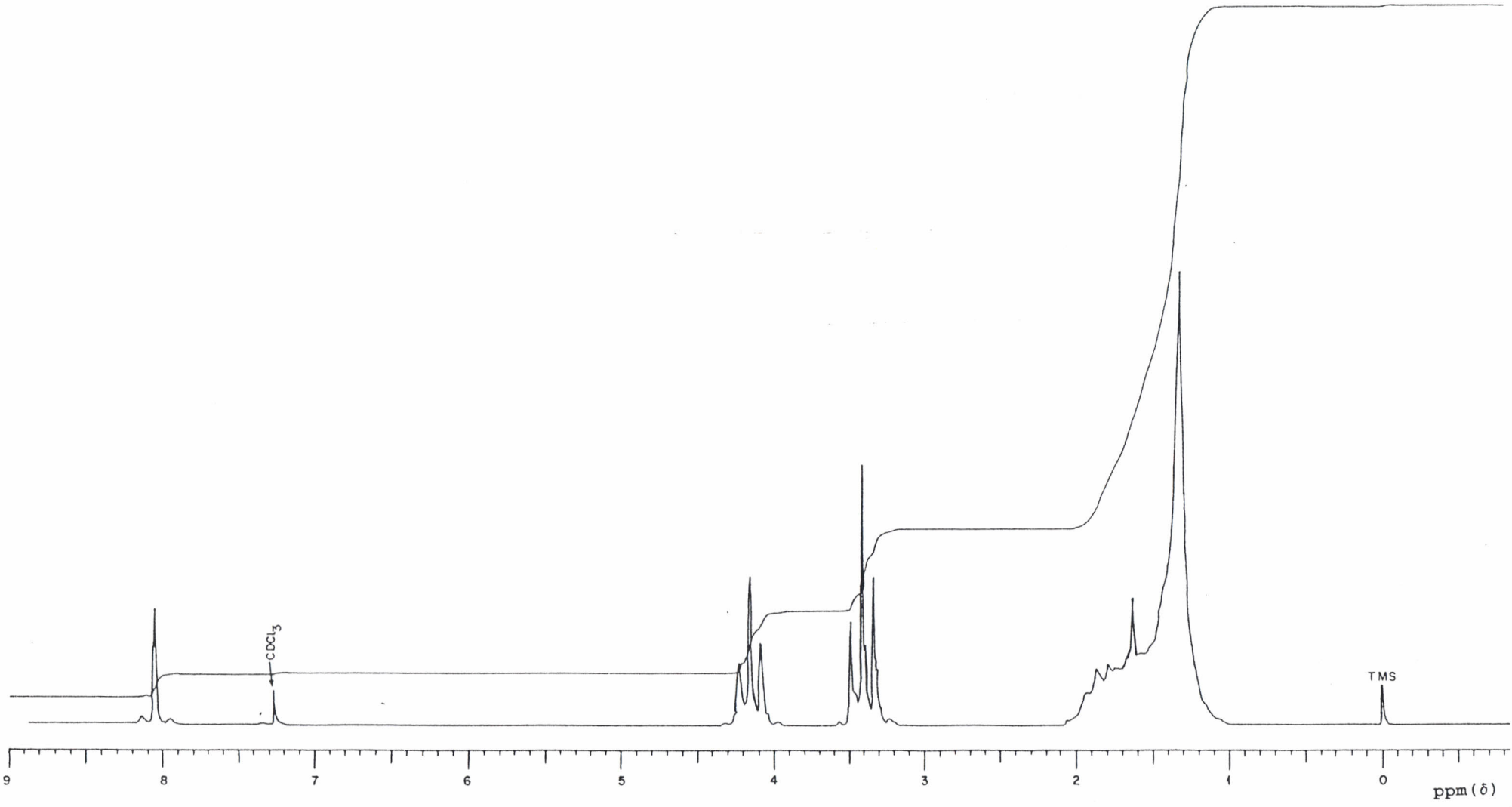


Fig 34 *Proton n.m.r spectrum of 8-bromooctanyl formate.*

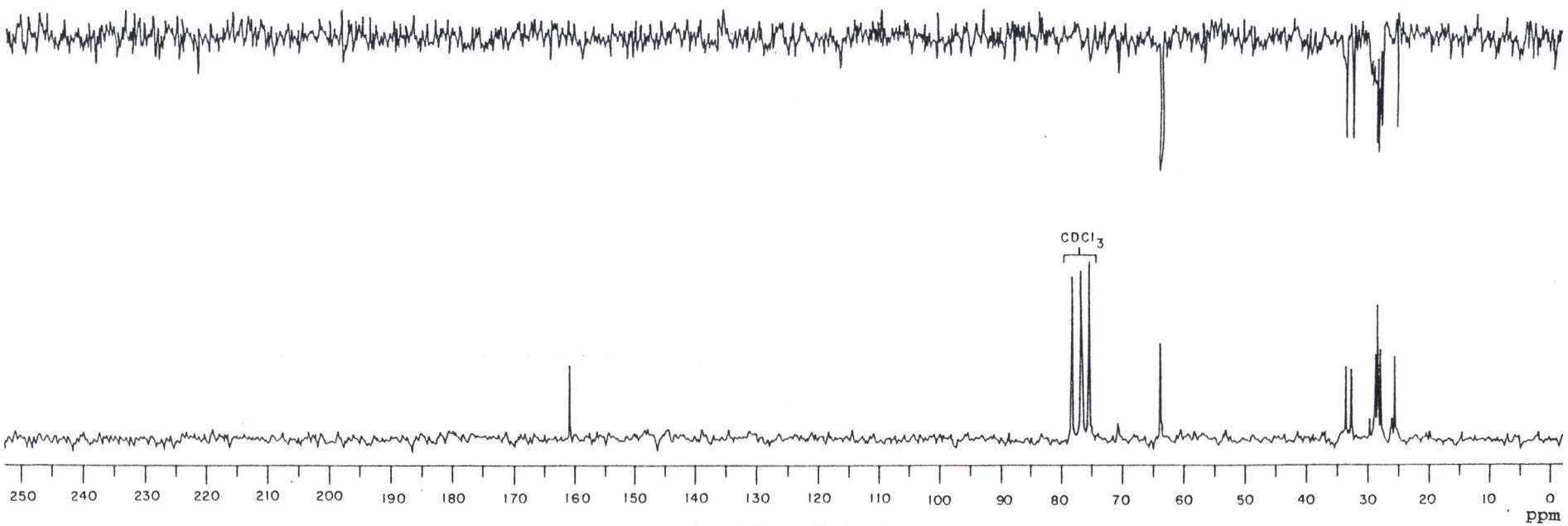


Fig 35 *^{13}C n.m.r spectrum of 8-bromooctanyl formate.*

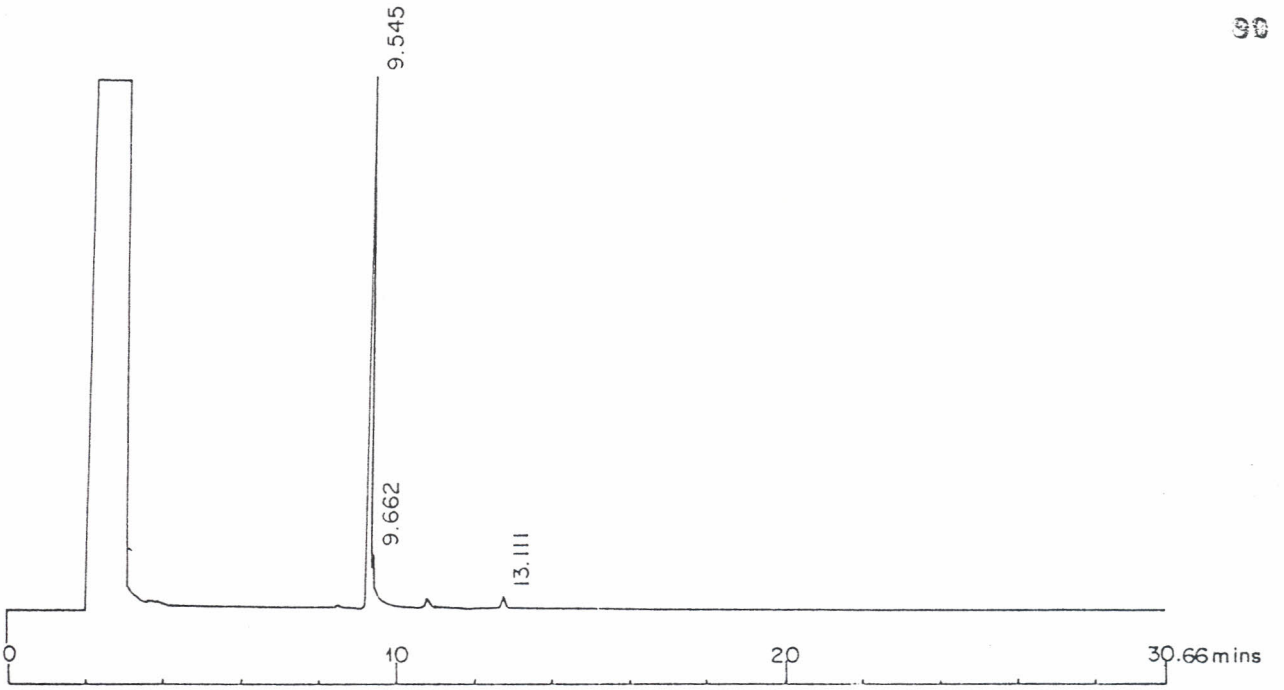


Fig 36 *GC profile of 11-hexadecynal.*

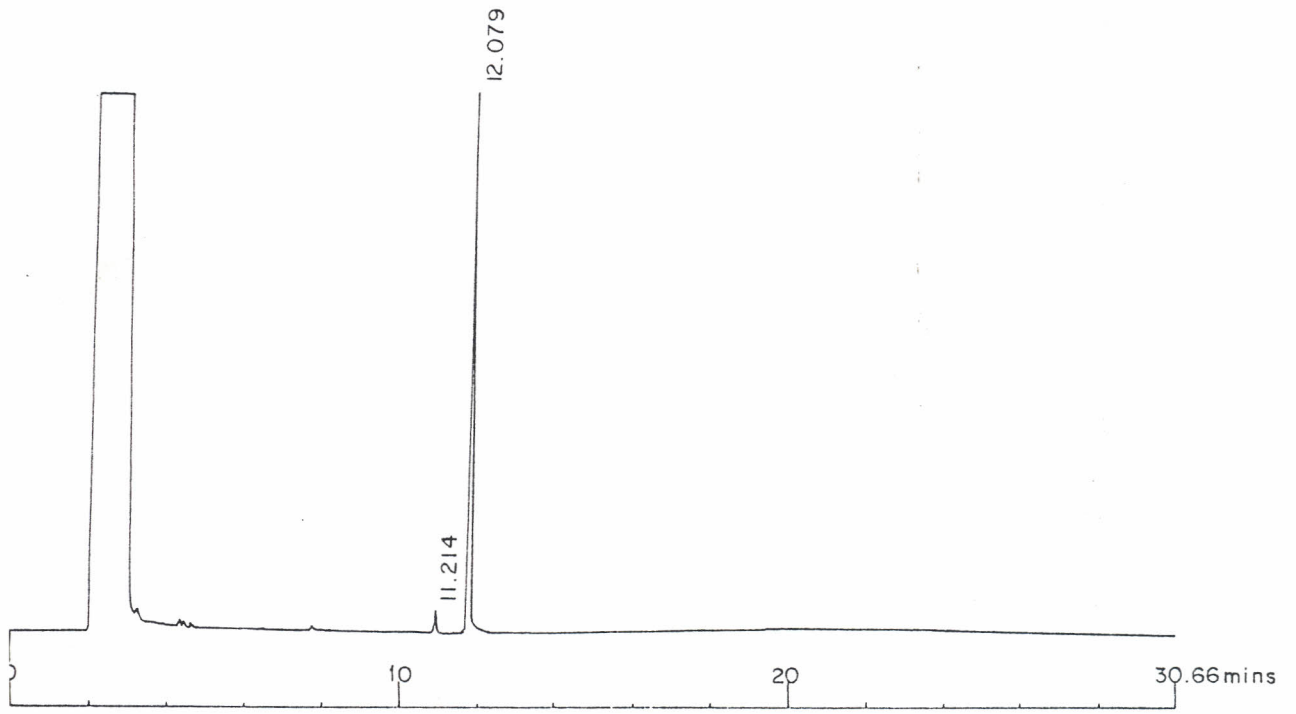


Fig 37 *GC profile of 10-bromodecanal.*

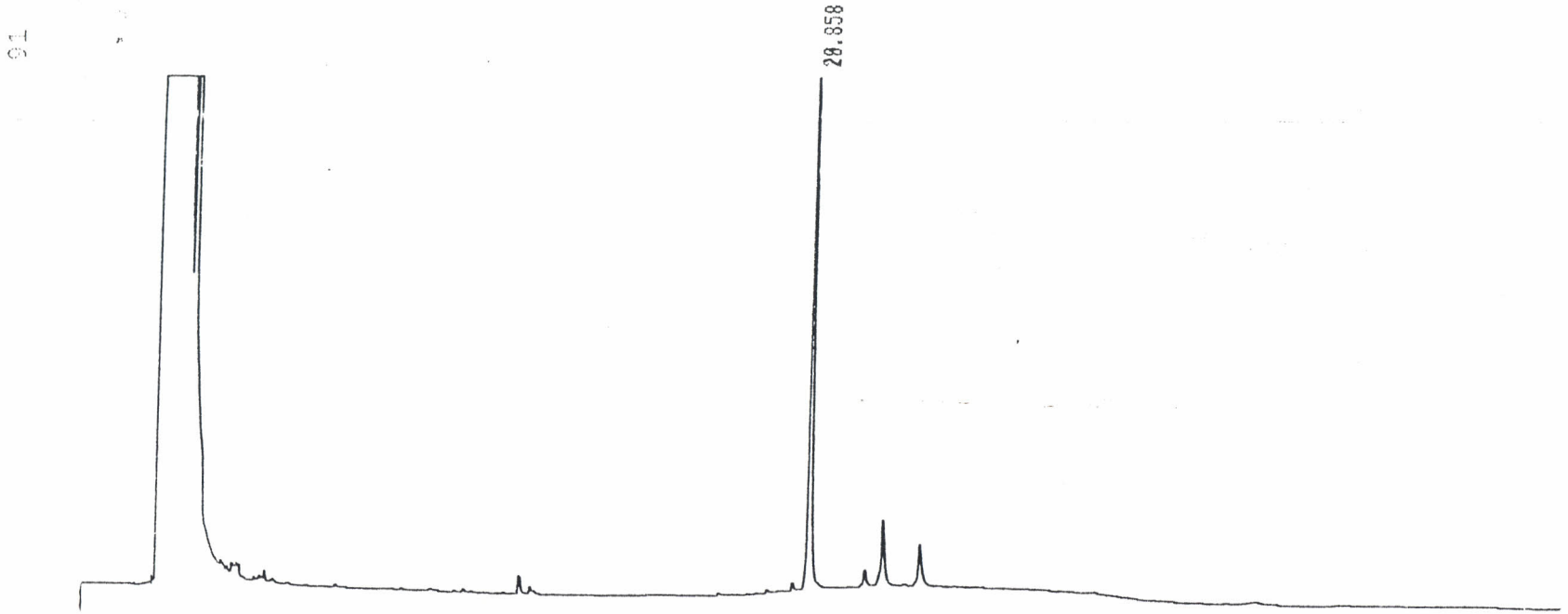


Fig 38. GC profile of acetal from pheromonal components.

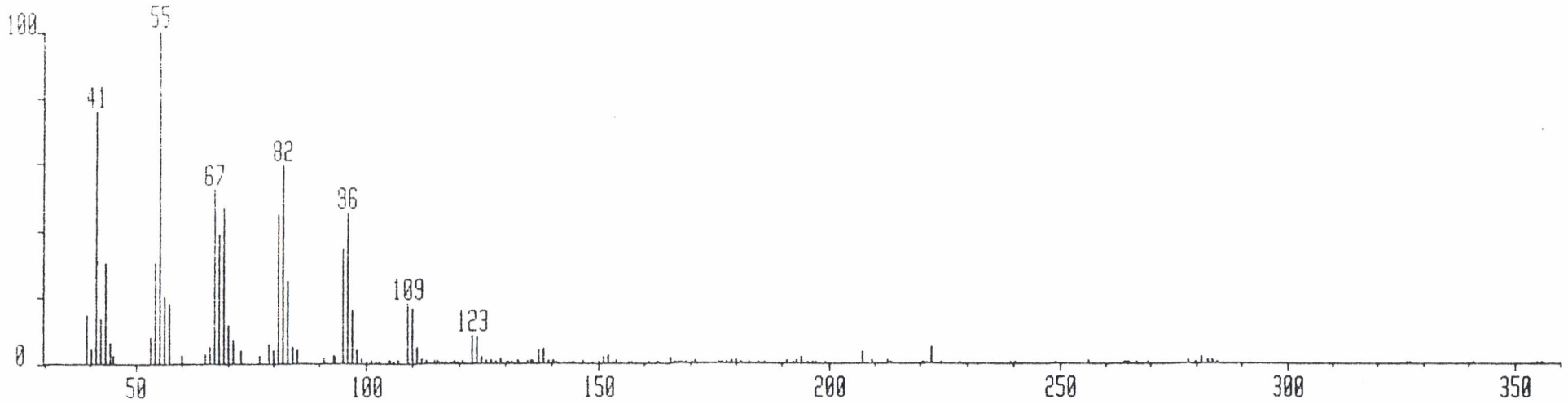


Fig 39. Mass spectrum of acetal from pheromonal components.

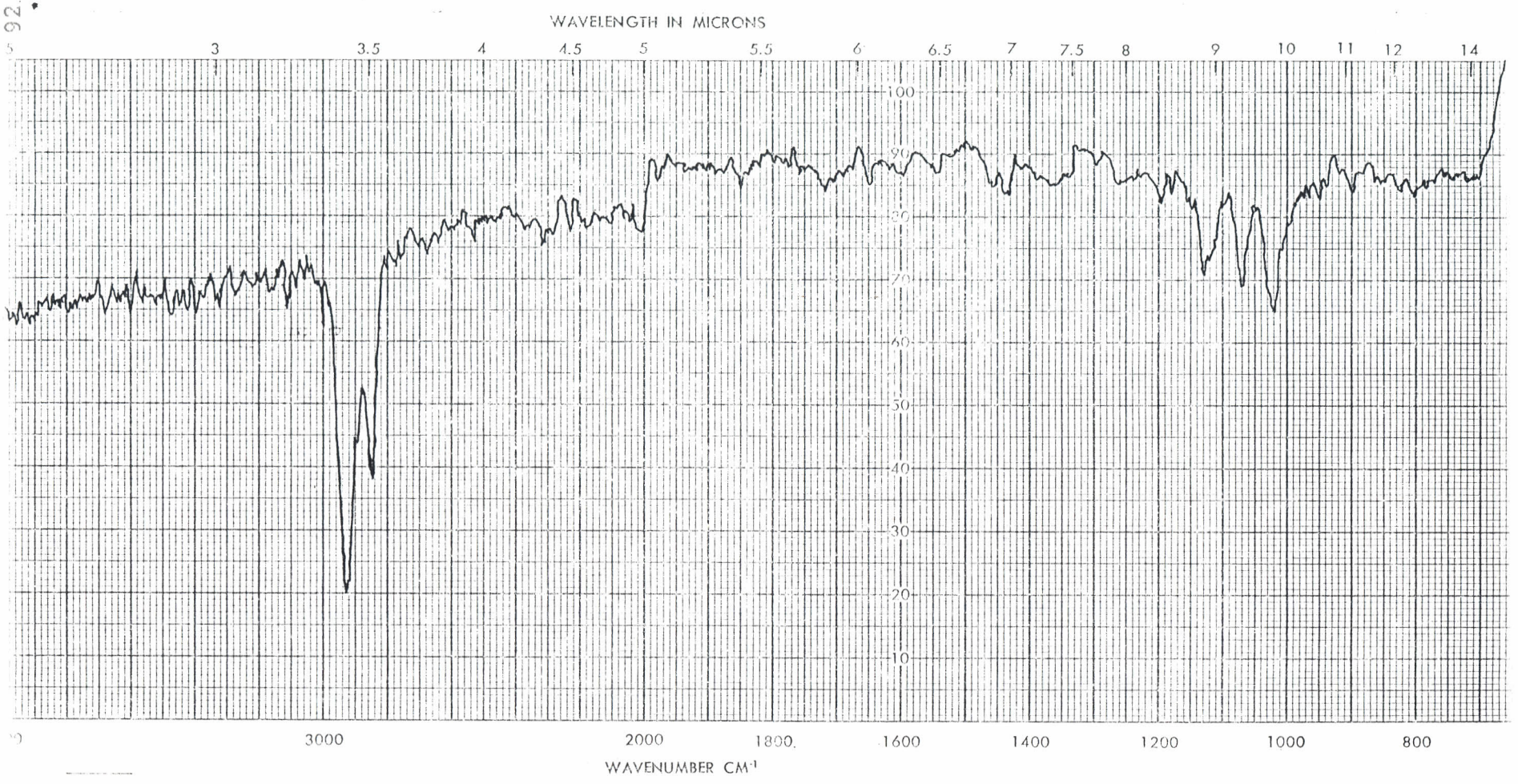


Fig 40. IR spectrum of acetal from pheromonal components.

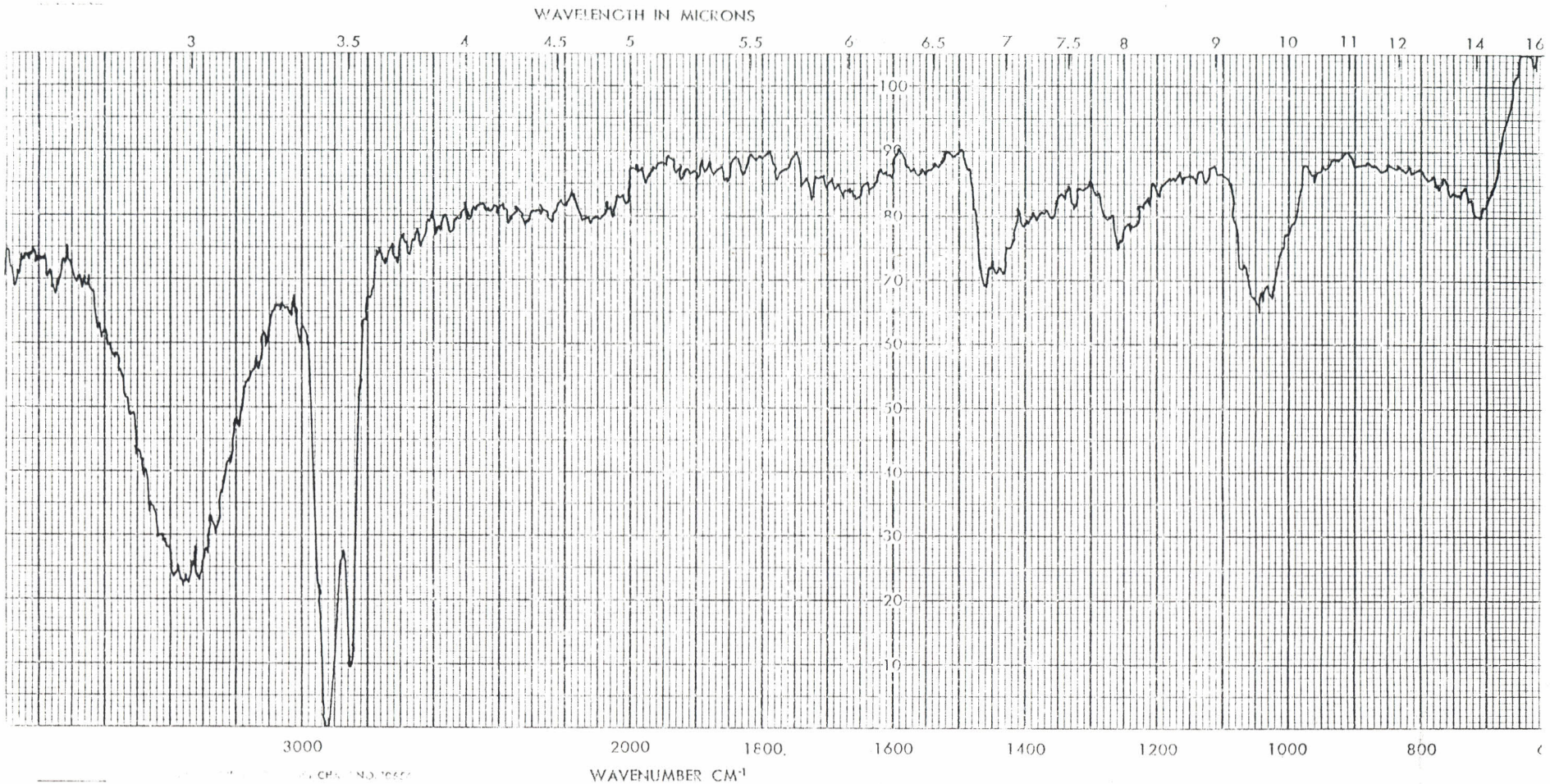


Fig 41. IR spectrum of 10-Bromodecanol [$Br(CH_2)_{10}OH$]

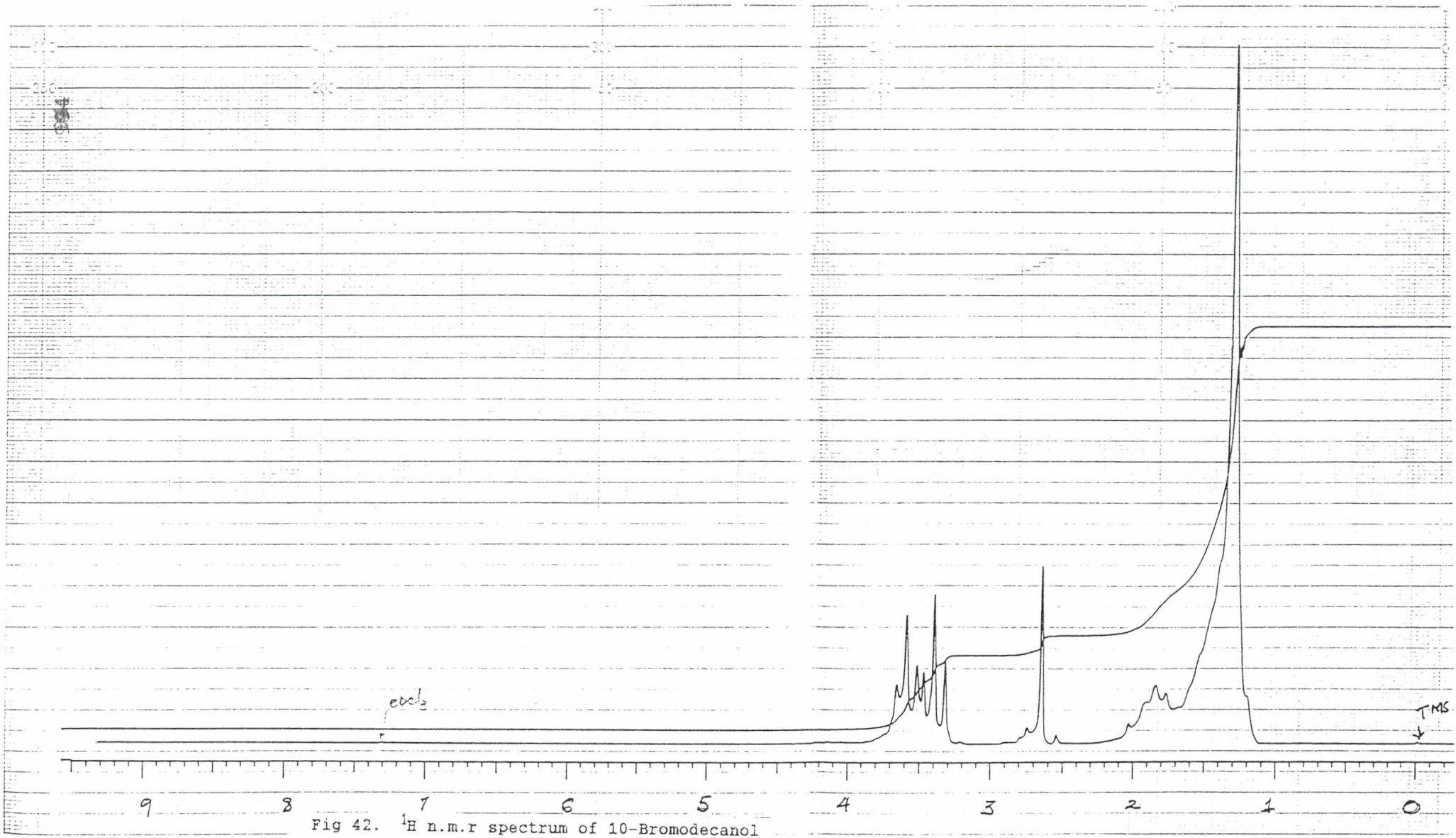


Fig 42. ^{1}H n.m.r spectrum of 10-Bromodecanol

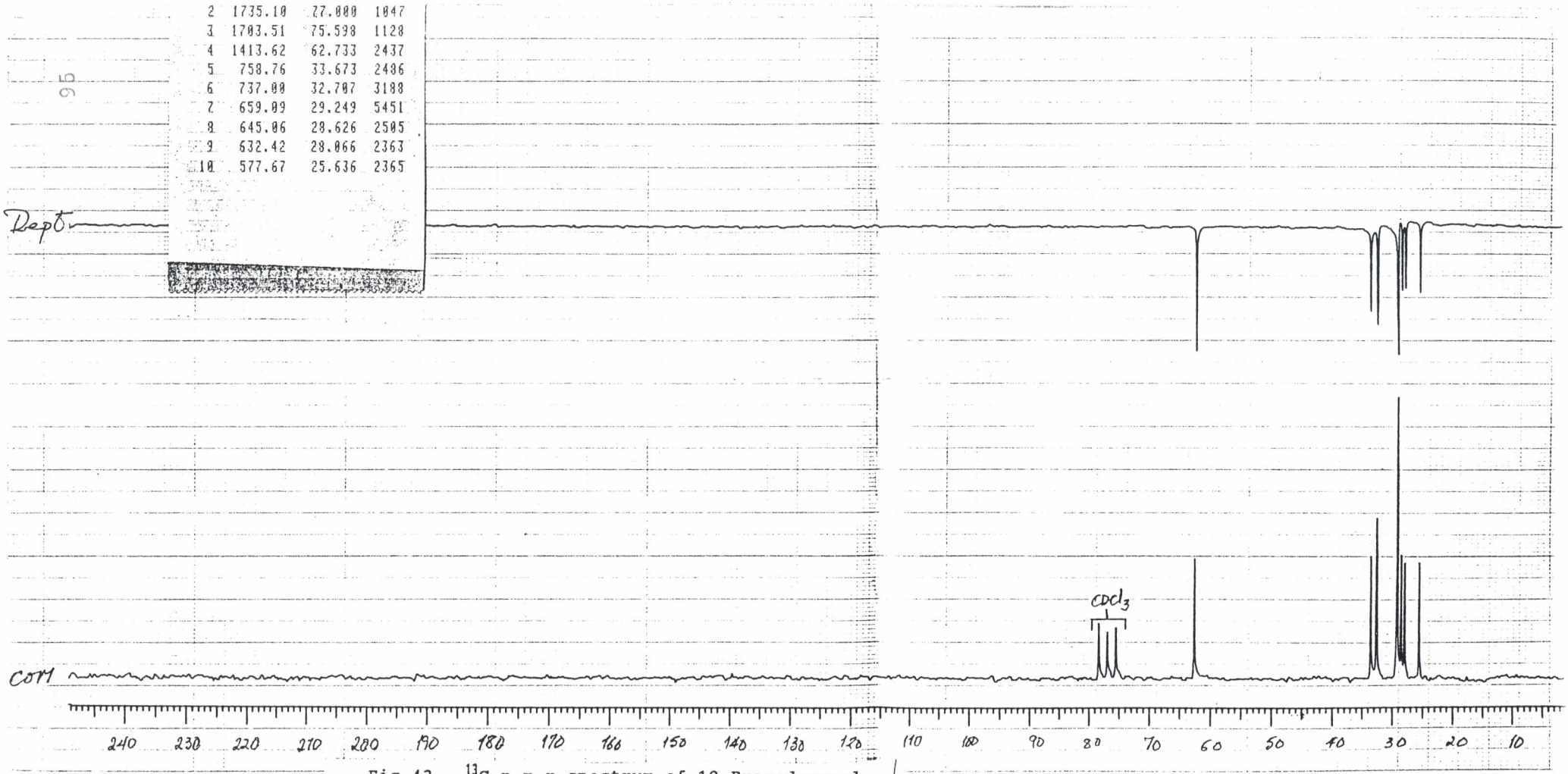


Fig 43. ^{13}C n.m.r spectrum of 10-Bromodecanol

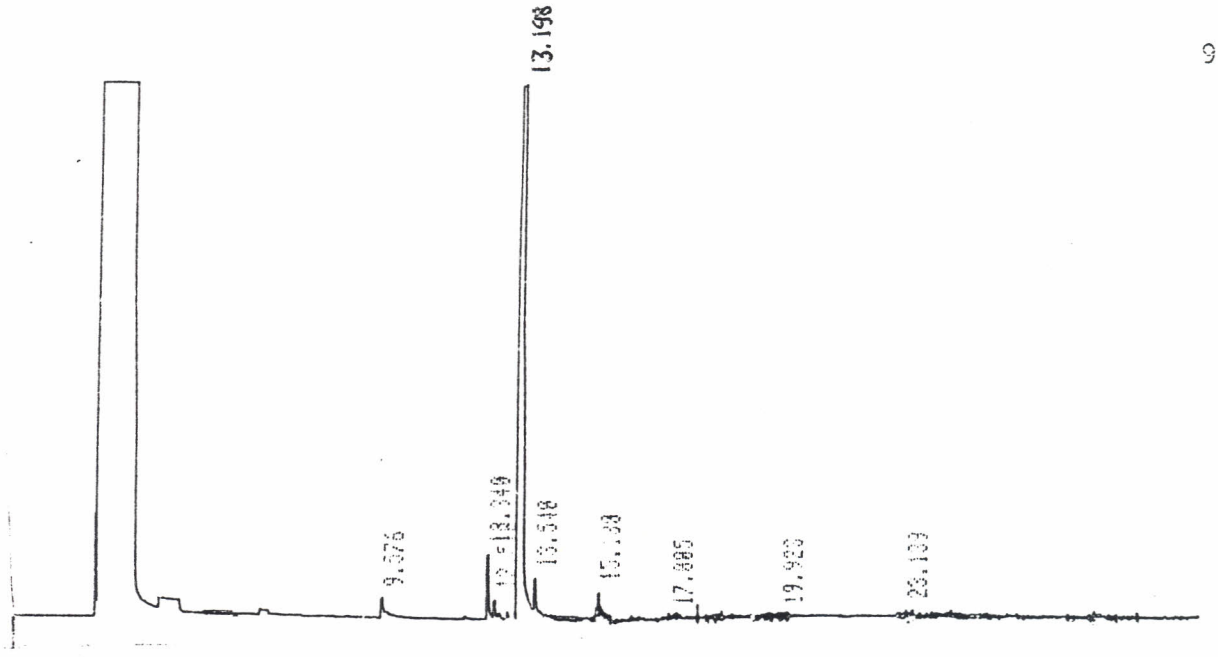


Fig 44. GC profile of 10-Bromodecanol

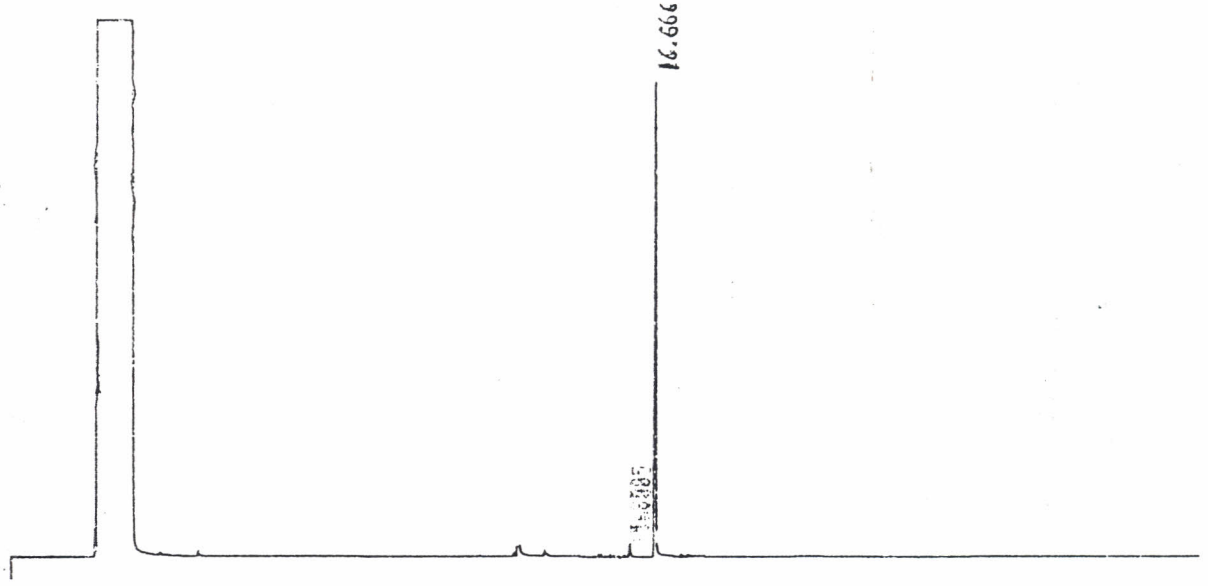


Fig 45. GC profile of $Br(CH_2)_{10}OTHP$

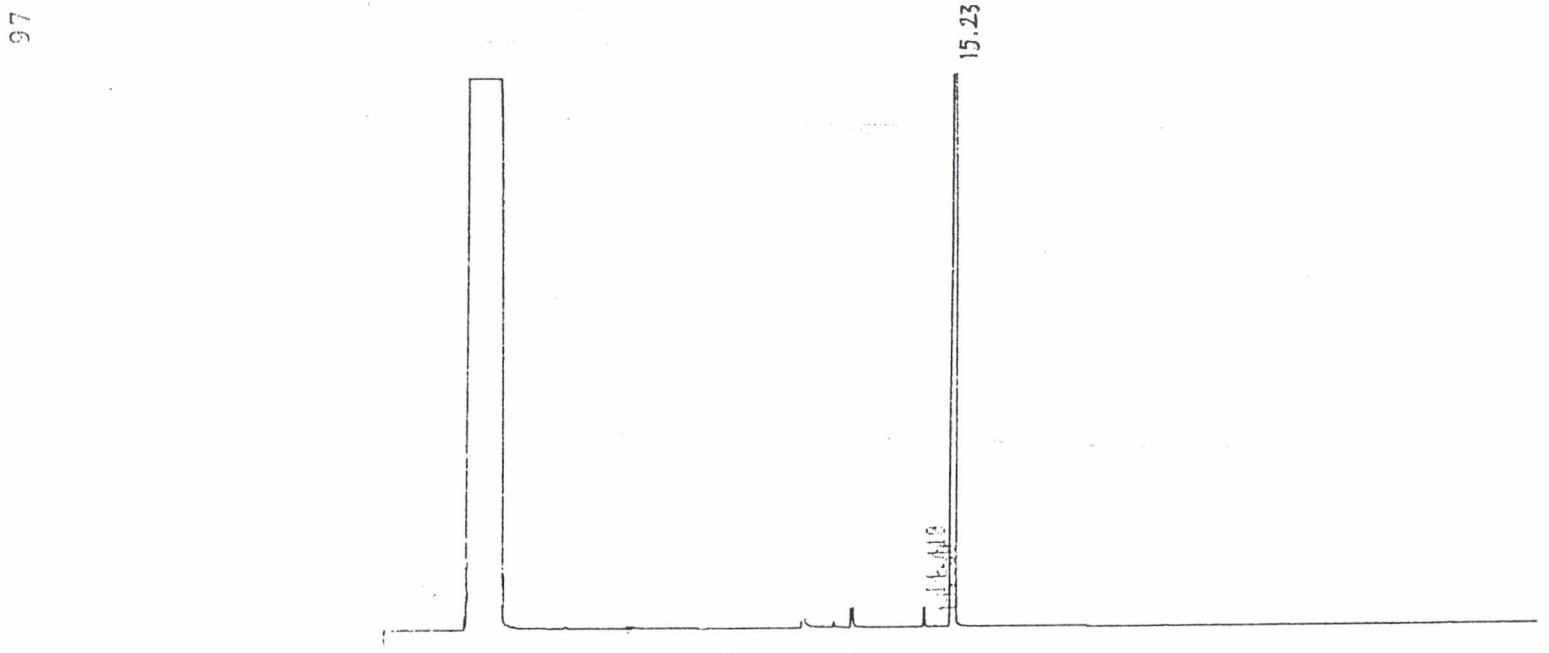


Fig 46. GC profile of $C_4H_9C{\equiv}C(CH_2)_{10}OTHP$

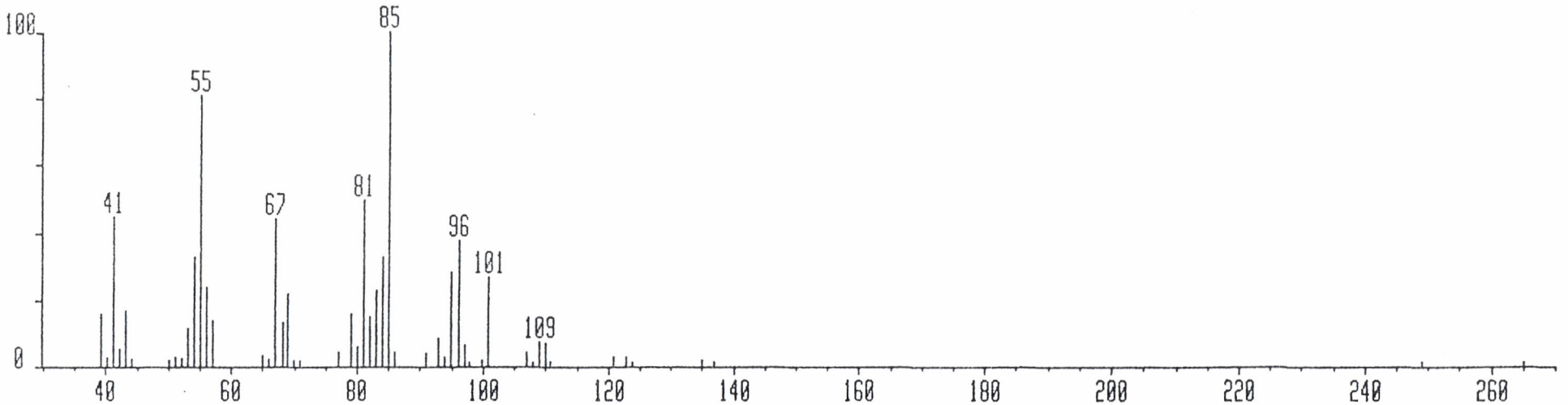


Fig 47. Mass spectrum of $C_4H_9C{\equiv}C(CH_2)_{10}OTHP$

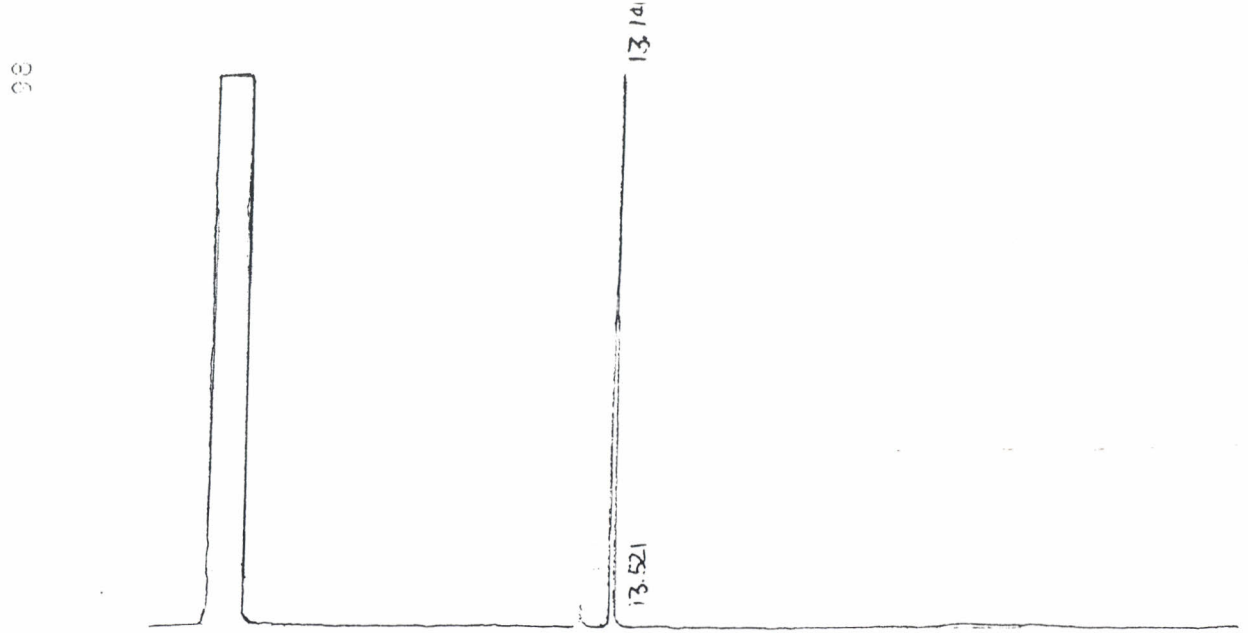


Fig 48. GC profile of $C_4H_9C{\equiv}C(CH_2)_{10}OH$

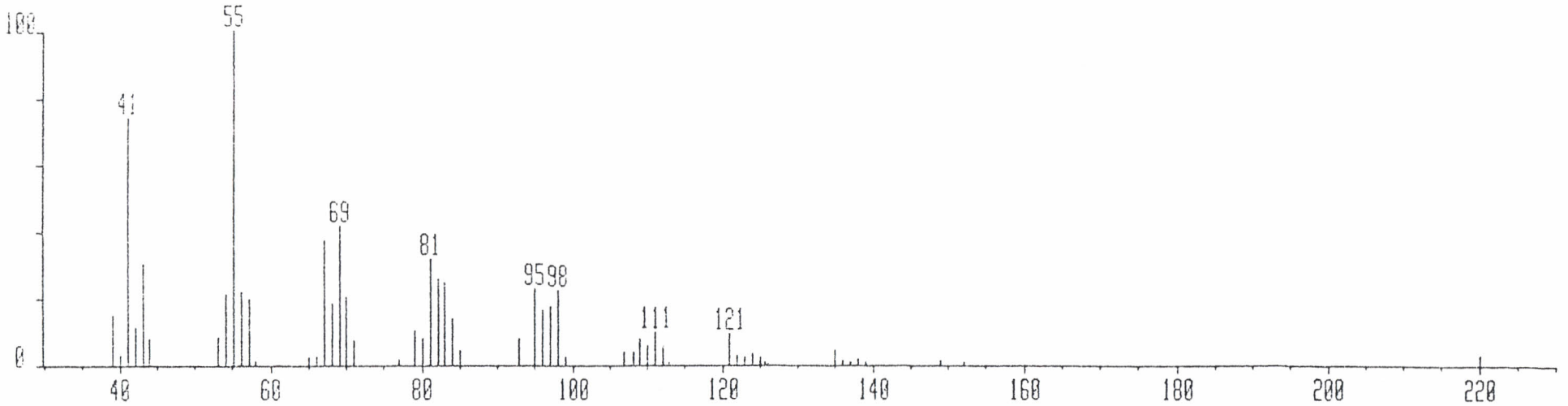
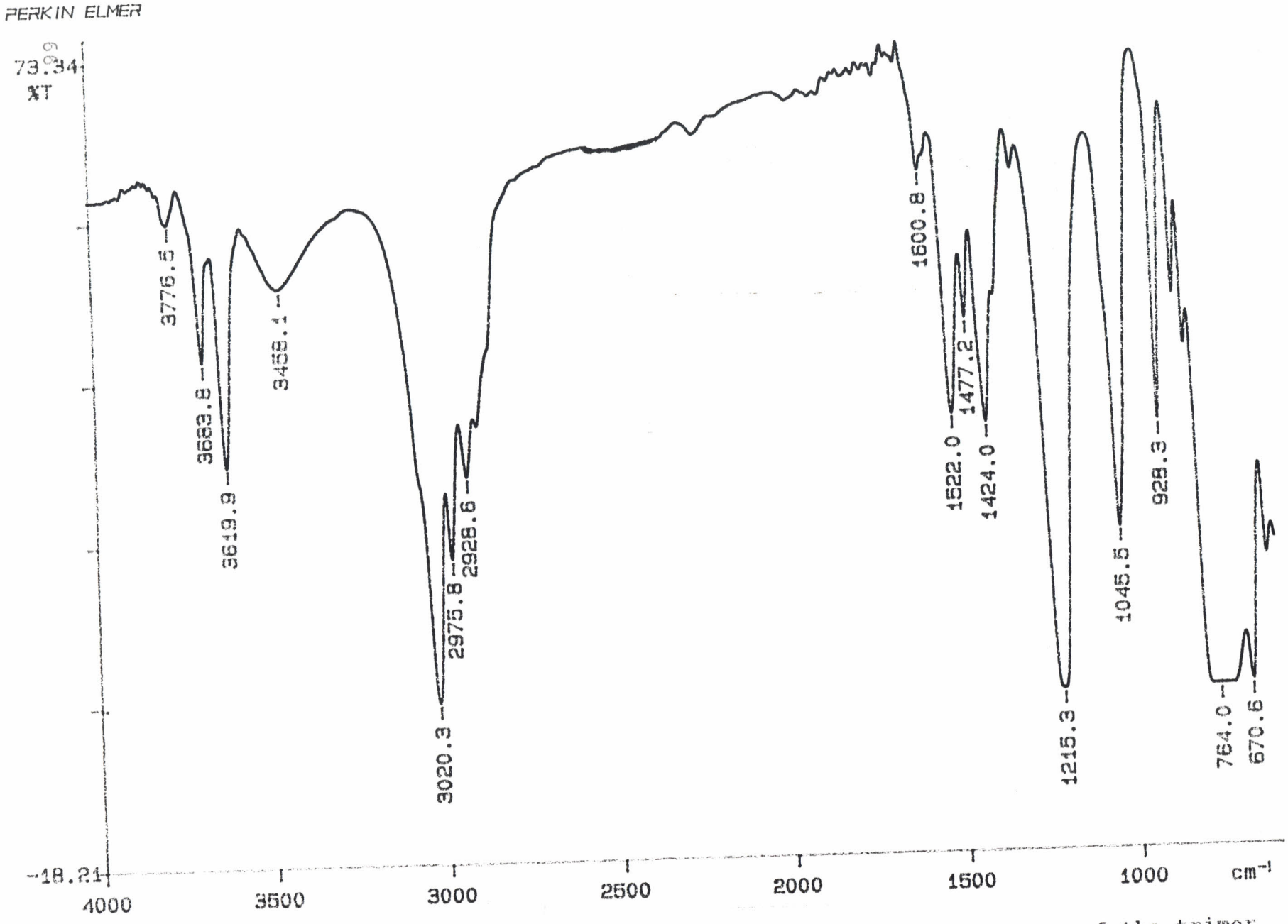


Fig 49. Mass spectrum of $C_4H_9C{\equiv}C(CH_2)_{10}OH$



94/05/09 19:22 Beniam K.
Z: 4 scans, 4.0cm-1, apod weak, diff, smooth

Fig 50. IR spectrum of the trimer.

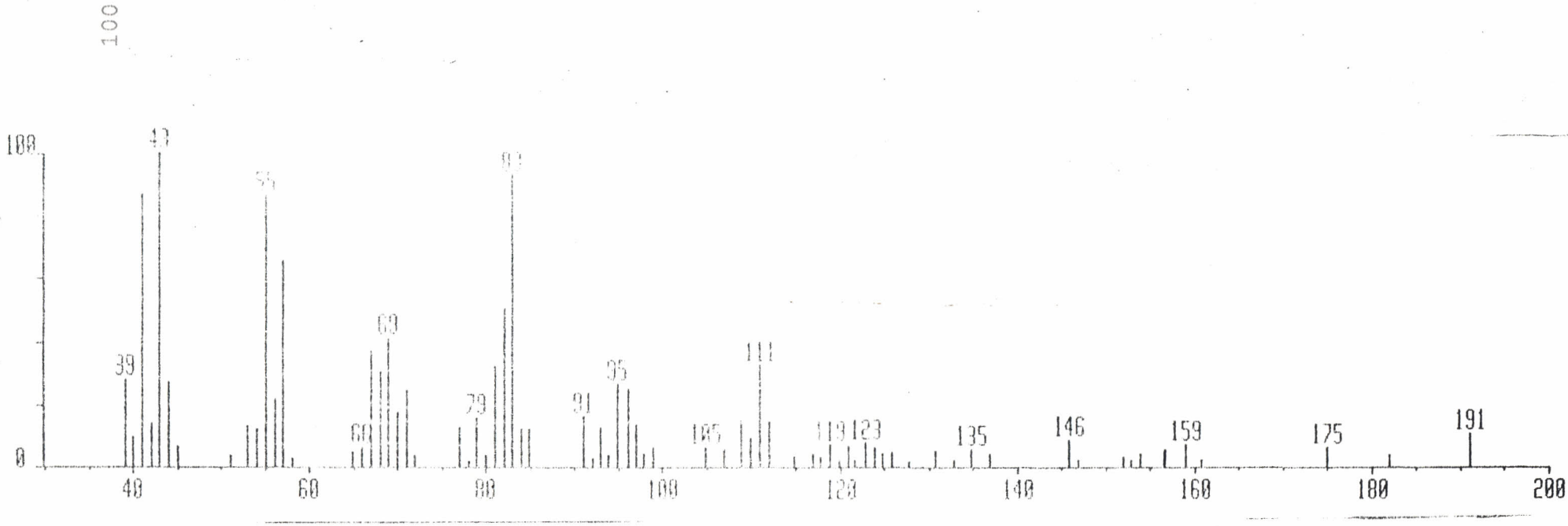


Fig 51. Mass spectrum of 8-bromooctanyl formate.

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