DETERMINATION OF 1-TRIACONTANOL IN KENYAN BEESWAX (HONEYBEE, APPIS MELLIFERA L.) AND DEVELOPMENT OF A NEW APPROACH TO ITS SYNTHESIS.

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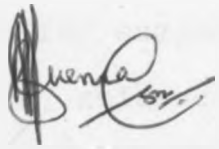
A thesis submitted in partial fulfilment for the degree of Master of Science in the University of Nairobi.

DECEMBER, 1987

This thesis is my original work and has not been presented for a degree in any other University.



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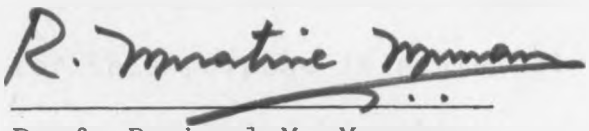

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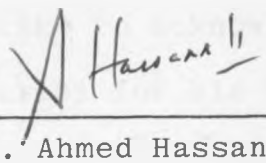

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CHAPTER I

INTRODUCTION

1.0 General

1-Triacontanol (1, hydroxytriacontane, $CH_3(CH_2)_{28}CH_2OH$) is a crystalline, naturally occuring straight chain primary alcohol present in a wide range of plant and animal waxes. Natural waxes are a mixture of high molecular weight acids, monohydroxy alcohols, esters and keto acids; the esters usually being predominant. Some of the other components of waxes are resins, lactones and sterols. 1-Triacontanol occurs either freely or as the alcohol part of the esters in natural waxes (Marsel, 1950; Warth, 1947).

Some natural waxes in which 1-triacontanol has been identified are shown in Table 1.

Plant waxes occur mainly as coatings on plant cuticles, stems, leaves and on certain berries and grasses, with some of the rare waxes appearing on flowers, roots and fruits. Animal and insect waxes are found in certain portions of the bodies of various animal and in the cellular fabrications of bees (beeswax) and other insects.

Beeswax consists mainly of simple esters formed from long, straight chain acids and alcohols, and are composed of even numbers of carbon atoms

Table 1: 1-Triacontanol content in selected natural waxes

Source	1-Triacontanol content (%)	Reference
Alfalfa (*Medicago sativa L.*)	13.30	Blair, H.E. *et al* (1953)
Ilex latifolia	0.32	Kariyone, T. *et al* (1953)
Sugar cane (*Saccharum officinarum*)	24.85	Kapil, Y.P. (1954)
Chamaecypanis obtusa	0.50	Kariyone, T. *et al* (1959)
Beeswax (honeybee, *Appis mellifera L.*)	9.61	Downing, D.T. *et al* (1961)
Sphaeranthus indicus	8.02	Tiwari, R.D. (1963)
Sucuriga suffructicosa	0.47	Mukhevjee, R. *et al* (1966)
Piper hookeri	2.82	Jagder, S. *et al* (1969)
Springwheat (*Triticum aestivum L.*)	3.36	Tulloch, A.P. *et al* (1973)
Baccharis cordifolia	5.66	Barbara, N.H. *et al* (1973)
Spilantes acmella	1.74	Krishnaswamy, N.R. *et al* (1975)
Tea (*Tea sinensis*)	0.08	Rao, J.M. *et al* (1987)

(from C_{14} to C_{36}). In beeswax, 1-triacontanol exists in the form of the ester, myricyl palmitate

($\underline{2}$, $C_{15}H_{31}COOC_{30}H_{61}$).

1-Triacontanol was first identified and characterised from beeswax by Gascard and Damoy (1923). Several workers (Holde and Bleyberg, 1930; Chibnall et al, 1934; Toyama and Hirai, 1951) thereafter also characterised the compound. Reports indicate that the compound constitutes an appreciable amount of the ester fraction in beeswax. Examination of Australian beeswax from the honeybee (Appis mellifera L.) by Downing and Kranz(1960) using spectroscopic and gas chromatographic techniques showed the wax to contain 9.61% 1-triacontanol. Analysis on the constitution of Czechoslovak beeswax (honeybee, Appis mellifera L.) has been undertaken (Stransky et al, 1972). The reported results indicate the compound to constitute 16% of the beeswax. Ouyang and co-workers (1983) conducted gas chromatographic studies on the chemical constituents of Chinese Hai-nan beeswax and observed the wax to contain a good amount of 1-triacontanol (27.6%).

In 1977, 1-triacontanol was discovered to be a powerful plant-growth regulator (Ries and Wert, 1977). This report has stimulated research in

synthesis, field tests on a wide variety of agricultural crops and mode of action studies to understand the exact role of 1-triacontanol in plant-growth control.

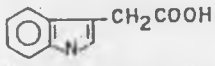
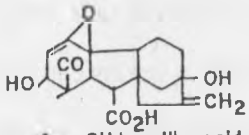
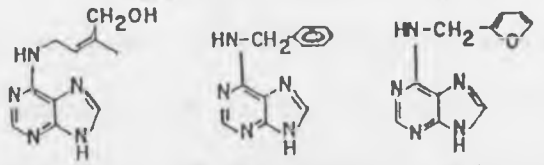
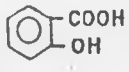
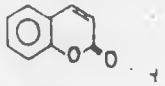
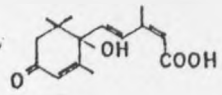
1.1 THE EFFECT OF 1-TRIACONTANOL ON PLANT-GROWTH

1.11 PLANT-GROWTH REGULATORS (Andus, 1972; Thimann, 1972).

Plant growth regulators consist of two types of chemical species. There are those which occur naturally in plants specifically called phytohormones and those which are synthetic or naturally occurring and resemble the hormones by mimicking their physiological and morphogenetic actions. There are five classes of phytohormones as shown in Table 2.

These hormones are synthesised in various parts of the plant from which they move either in basipetal, polar fashion (eg. the auxins) or in the vascular system (eg. cytokinins, gibberellins and the inhibitor, abscisic acid (10); Black and Osborne, 1965). These chemicals are active in minute amounts often at some distance from the cells which produce them. They act by exerting specific control of growth and development by influencing cell division, enlargement and differentiation (Whaley, 1961). The signifi-

Table 2: Plant Phytohormones (Leopold and Kriedmann, 1975).

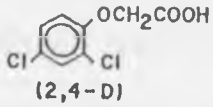
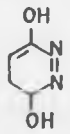
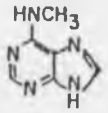
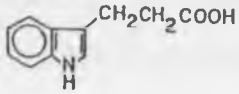
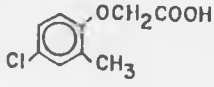
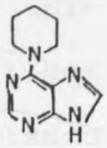
Phytohormone	Example
1. Auxins	 3, Indole acetic acid (IAA)
2. Gibberellins	 4, Gibberellic acid, GA_3
3. Cytokinins	 5, Zeatin; 6, Benzyladenine (BA); 7, Kinetin
4. Ethylene	$CH_2=CH_2$
5. Inhibitors	 8, Salicylic acid;  9, Coumarin
	 10, Abscisic acid

cantly affected processes are; stem growth, coleoptile growth, fruit growth, root growth, leaf growth, root initiation, bud formation, cambial division and delay of senescence. Hormonal control of growth however is not ascribed to a single hormone but depends on synergistic interaction between at least the five classes of phytohormones (Shantz and Steward, 1968).

The second type of growth regulators embraces a number of artificial mimics, naturally occuring or synthetically prepared, but which show the same physiological actions as the phytohormones.

Some examples are given in Table 3. Many of these substances are not so readily subject to enzymatic attack within the plant as the auxins. Due to this relative persistence they find usage in agriculture and horticulture example; to induce rooting or to control crop and fruit growth (Rains, 1983).

Table 3: Examples of synthetic plant-growth regulators.

11	 (2,4-D)	2,4 – Dichlorophenoxyacetic acid
12		Maleic acid hydrazide
1	$CH_3(CH_2)_{28}CH_2OH$	1 – Triacontanol
13		1- Methyl adenine
14		Indole – 3– propionic acid
15		4 – Chloro-2- methyl phenoxyacetic acid
16	$CH_3(CH_2)_7COOCH_3$	Nonanoic acid methyl ester
17		6 – Piperidino (1) purine

1.12 1-TRIACONTANOL AS A PLANT-GROWTH PROMOTER

With the discovery of plant-growth regulating activity of 1-triacontanol, its effect has been tested in quite a number of bioassay systems and its activity compared to that of the commonly known growth regulators (IAA 3, gibberellic acid 4 and kinetin 7).The compound has been shown to produce some growth responses including increase in water uptake or dry weight in these bioassay systems (Ries and Wert; 1984). In combination with other growth controlling substances, the compound has shown slight synergism. The rapid increase in water uptake or dry weight indicates that 1-triacontanol affects transpiration, but perhaps not directly. The increased dry weight accumulation of several species of plants with both foliar and root applications at low concentrations (2.3×10^{-8} M) of the compound suggests that this compound maybe involved in growth processes. It has thus been postulated that this lipoidal substance with a terminal polar group may have specific effects on membranes. Whether the growth is primarily associated with altered water uptake, with CO_2 fixation or with respiration has not been resolved.

The effect of 1-triacontanol on crop growth yield of several vegetable and field crops has been reported by Ries and co-workers (1978). Field studies on the compound's foliar spray of 5-500 mg/ha led to 10 to 40% increase in growth and yield of food plants such as corn, wheat, rice, soybeans, tomatoes, carrots etc. The plants were observed to apparently keep on growing even in the dark.

A detailed study of 1-triacontanol on seed germination, morphology and early growth of fifteen weed, crop and horticultural species was conducted by Hoagland (1980). He reported insignificant effect on germination of seeds of these species but noted inhibition in axis length of three species: lettuce (Lactosa sativa L.), sickle pod (Cassia obtusifolia) and cotton (Gossypium hirsulum). It was thus shown that at high concentrations, the compound can selectively inhibit growth.

1.12.1 Effect on Coffee (Coffea arabica):

Field trials covering a span of two years were carried out on arabica coffee sprayed with 1-triacontanol at a concentration of 50 ml/barrel at Chikmagalur, India. The data obtained showed good increase of average yields for both the fruit (berries) and clean coffee (Vasudeva, 1984).

1.12.2 Effect on Soybeans (Glycine max):

The application of 1-triacontanol on field grown soybeans has been reported by Nickey and Parker (1984). A spray solution was prepared containing 1-triacontanol, 0.0001-1.0 g/m^3 and calcium chloride, 75 g/m^3. The pH of the spray solution was adjusted to >10. Dual spray of 1-triacontanol at twenty minutes interval between the sprays showed significant increases in the soybean yields.

1.12.3 Effect on Mungbeans (Phaseolus aurea):

Kanaby et al (1983) examined the effects of 1-triacontanol on the growth and enzyme activity in mungbean seedlings. The procedure involved pretreating nine day old mungbean seedlings with four concentrations of the compound ($1 \times 10^{-4} - 10^{-7}$ mg/ml) prior to a fourteen day light exposure. Length measurements of leaf, stem and root tissue were carried out in addition to the assay, for ^{14}C-labelled auxin (IAA 3) destruction of the respective tissue homogenates. Their results indicated that all the four concentrations of 1-triacontanol employed promoted root growth in light exposed tissues and also some effect on auxin destruction in all the plant tissues studied.

1.12.4 Effect on corn (Zea mays L.):

A study involving foliar application of 1-triacontanol on field grown corn revealed that corn yields increased depending on the time of application (Oplinger and Basabe, 1981). In miniplot experiments, 1-triacontanol was found to be more effective when applied at eighteen days post-emergence. A formulation containing 1-triacontanol, acetone and a metal salt ($LaCl_3$) all dissolved in water was devised. When sprayed on corn, increases of 25-32% in weight and 25% in water uptake compared to untreated controls were noted.

1.12.5 Effect on rice (Oryza sativa L.):

A comparative study of the effect of the growth regulators (phytohormones) and 1-triacontanol on photosynthetic efficiency and translocation in rice was undertaken by Debata et al (1981). By foliar spraying the panicle of three cultivars with the plant growth regulators, it was found that these enhanced the ^{14}C photosynthesis and mobilised the photosynthates. The order of efficiency was reported as;
Kinetin (7)>1-triacontanol (1)>gibberellic acid (4)>-auxin (3)>control

whereas the order of mobilising the photosynthates was; 1-triacontanol (1), gibberellic acid (4)>auxin (3)>kinetin (7)>control.

The rating for increase in green leaf area and percent chlorophyll was also observed as follows; kinetin(7)>1-triacontanol(1)>gibberellic acid(4)> auxin(3)>control

In 1977, Ries and co-workers noted increase in dry weight and water uptake of rice one week after application of authentic 1-triacontanol. The application's optimum concentrations were found to lie between 0.01 and 0.1 mg/L of solution. The compound also increased both the percentage N and total N of rice seedlings within 6 hrs in the dark. Part of the data they obtained is shown in Table 4.

Table 4: Response of rice to authentic 1-triacontanol 1 week after application. Seedlings weighed 57 mg at the initiation of test

(Ries et al, 1977)

1-Triacontanol (mg/L.)	Rice	
	Water uptake (g/plant)	Dry weight (mg/plant)
0.000	32.7	81
0.001	37.0	103
0.010	38.8	107
0.100	39.0	106
1.000	33.4	91

1.12.6 Effect on sorghum (Sorghum vulgare):

The influence of 1-triacontanol has also been tested on growth, $^{86}Rb^{+}$ and $^{32}PO_4^{-}$ uptake and translocation in both drought resistant and susceptible sorghum varieties by Welebir (1981). It has been found that absorption and transportation of Rb^{+} and PO_4^{-} by sorghum seedlings, pretreated with different concentrations (10, 50 or 100µg/L) of the compound were enhanced though in general the compound caused differential effects on ion uptake in these two sorghum varieties.

1.12.7 Effect on wheat (Triticum durum):

A formulation consisting of 1-triacontanol (0.4 mg/gal), metal proteinates and phytohormones (50 ppm)

has been shown to synergistically stimulate plant growth by increasing the height, root length and nutrient uptake of wheat (Ashmead, 1978).

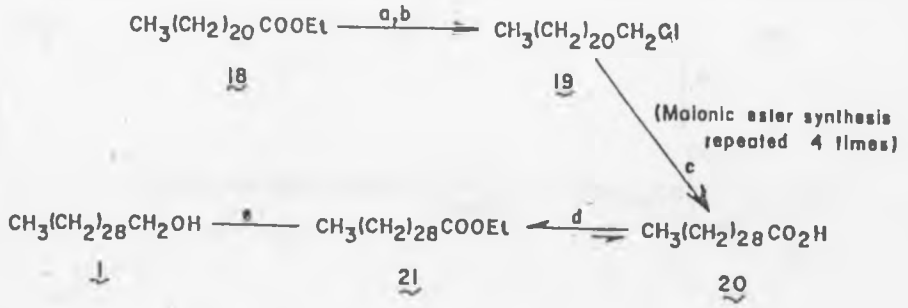
1.12.8 Effect on tomatoes (Lycopersion esculentum):

Growth regulating activity of 1-triacontanol has been noted in tomato seeds (Ries, 1977). The seeds were treated with 100µg 1-triacontanol/L for one hour. The shoot dry weight of 26 day old tomato seedlings were found to be significantly higher (135 mg) compared to those of controls (98 mg)

1.2 LITERATURE REVIEW ON THE SYNTHESIS OF 1-TRIACONTANOL

1-triacontanol was earlier prepared by Na-alcohol (ethanol/butanol) reduction of ethyl triacontanoate (21). Triacontanoic acid (20) was first synthesised by Bleyberg and Ulrich (1931) from ethyl behenate (18) by repeating the malonic ester synthesis sequence four times (scheme I).

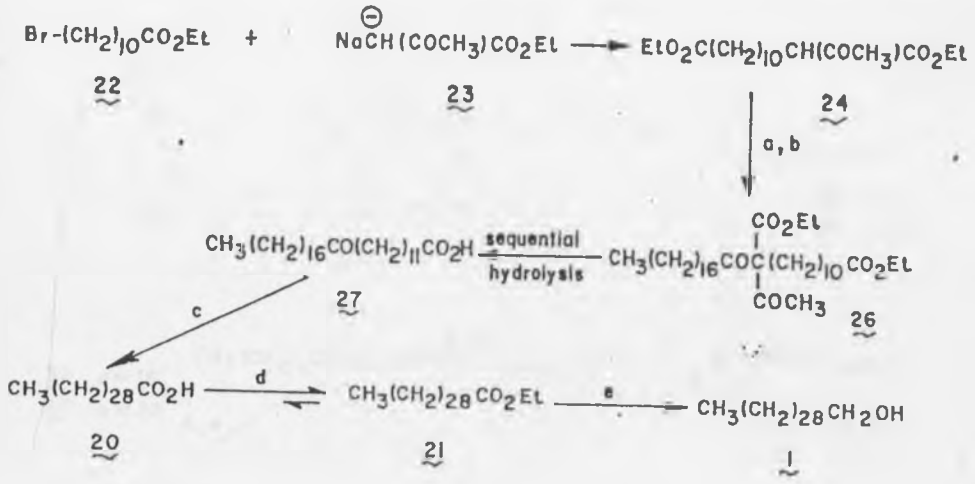
Scheme I



Reagents: (a) Na-BuOH; (b) $SOCl_2$; (c) $NaOEt/CH_2(CO_2Et)_2$; $H_3O^{\oplus}$, Δ, $-CO_2$; (d) $EtOH/H_2SO_4$ (e) Na-BuOH.

Triacontanoic acid (20) was also synthesised by Robinson (1934). Reaction of ethyl ω-bromoundecanoate (22) with ethylsodioacetate (23) gave ethyl-α-acetylbrassylate (24) whose sodio-derivative was condensed with stearoyl chloride (25) to generate ethyl-α-acetyl-α-stearoyl brassylate (26). Sequential hydrolysis of 26 afforded 13-keto-triacontanoic acid (27) which was subsequently reduced by Clemmensen's method to yield the titled compound (scheme II).

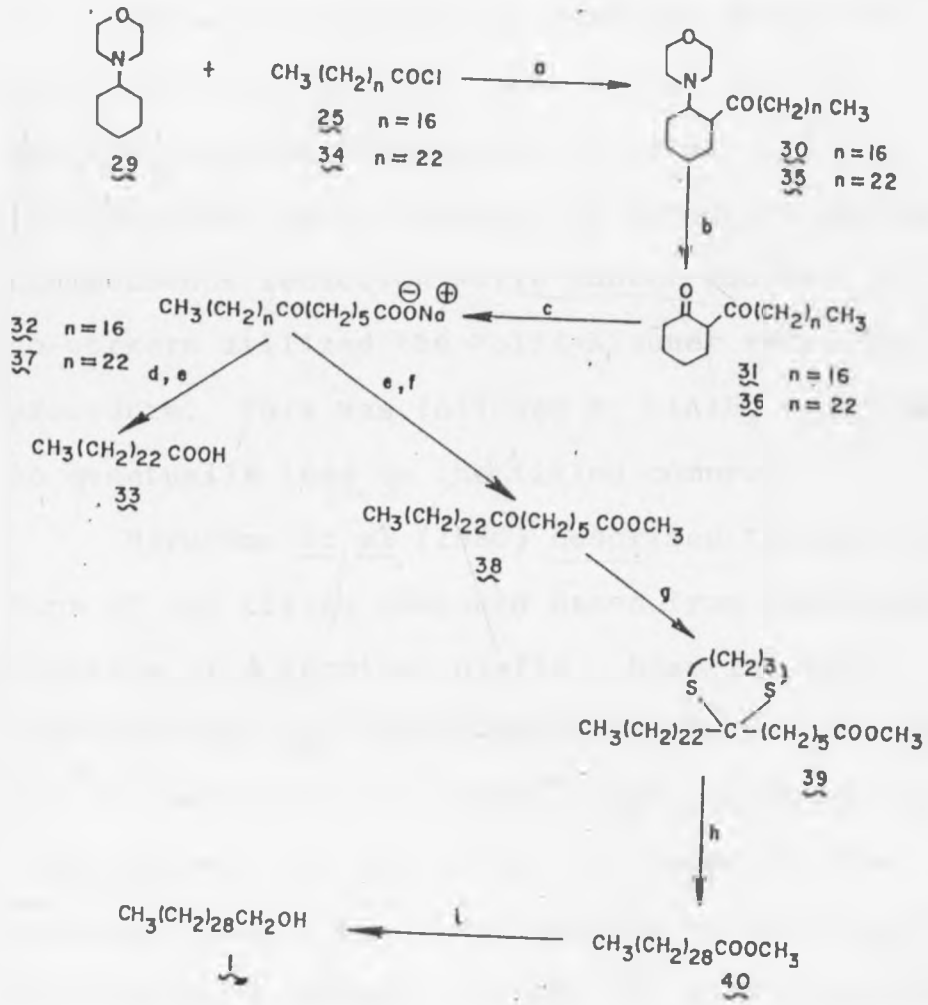
Scheme II



Reagents: (a) NaOEt; (b) $CH_3(CH_2)_{16}COCl$ (25); (c) Zn-Hg/HCl; (d) $EtOH/H_2SO_4$ (e) Na-BuOH.

Rao et al (1981) prepared 1-triacontanol from stearic acid (28) by making two successive additions of six carbon units through the 1-morpholino-1-cyclohexene (29) enamine intermediate. The overall process involved eleven steps and is represented in scheme III.

Scheme III

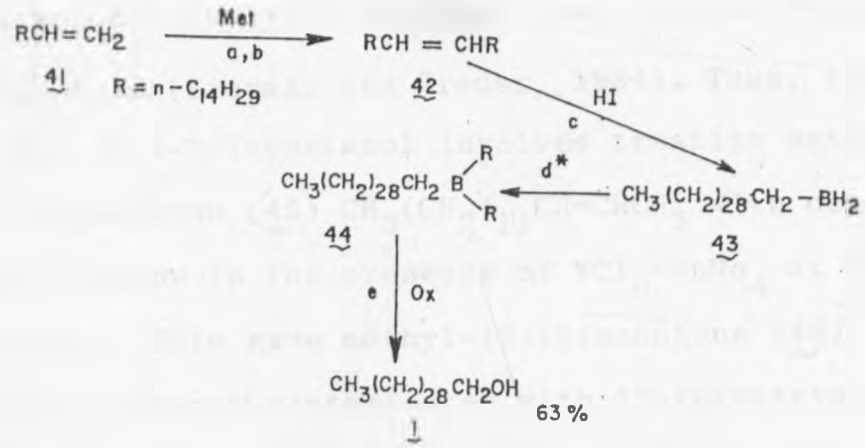


Reagents: (a) Et_3N; (b) HCl; (c) NaOH/EtOH; (d) KOH, N_2H_4; (e) HCl; (f) CH_2N_2,0^o; (g) HS SH/BF_3.OEt_2; (h) Raney Ni/H_2;(i) $LiAlH_4$/THF.

Yang et al (1980) and Hunter et al (1981) also similarly prepared the compound using the above outline synthetic route except for the reduction of the intermediate keto-acid, 7-oxo-triacontanoic acid. Yang et al directly employed Clemmensen's reduction while Hunter and his co-workers utilized the Wolff-Kishner reduction procedure. This was followed by $LiAlH_4$ reduction to eventually lead to the titled compound.

Maruyama et al (1980) described the preparation of the titled compound based from metathesis reaction of a terminal olefin. Starting with 1-hexadecene (41) he obtained 15-triacontene (42) through metathesis in 40-60% yield. Compound 42 then underwent hydroboration and isomerisation followed finally by oxidation with excess H_2O_2-NaOH. The procedure (scheme IV) gave 63% of 1-triacontanol contaminated with secondary triacontanol and triacontane.

Scheme IV



Met – Metathesis; HI – Hydrometalation – Isomerisation; Ox – Oxidation

Reagents: (a) WCl_6/CH_3CN; (b) $Bu_4Sn/CHCl_3$;
(c) BH_3-THF/diglyme; (d) $2RCH = CH_2$;
(e) 160^{O}, 12h/H_2O_2-NaOH

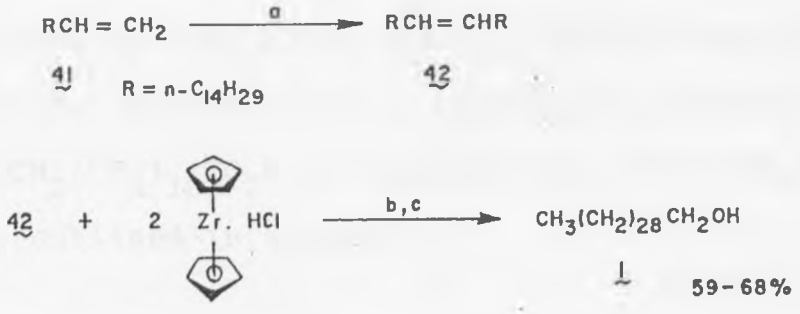
*Addition of 1-hexadecene (41) at monohydroboration stage was noted to improve yields of 1-triacontanol.

Co-metathesis of functionalised olefins, $R^1CH=CHR^4$ (R^1=H,n-alkyl; R^4=terminal functionalised alkyl) and $R^2CH=CHR^3$ (R^2.R^3=H, n-alkyl) with a soluble transition metal compound as catalyst has also been reported to yield long chained primary alcohols (Bierman and Preuss, 1984). Thus, the preparation of 1-triacontanol involved treating methyl-1-teradecene (45) $CH_3(CH_2)_{11}CH=CHCH_3$ with octadecene in toluene in the presence of WCl_6-$SnMe_4$ at 8° under argon. This gave methyl-13-triacontene (46) as a major product contaminated with 17-tetratriacontene. Compound 46 was hydrogenated over Cu-Zn to give 87.5% 1-triacontanol.

Gibson (1982) and Qian et al (1984) both exploited the observation that hydrozirconation of internal olefins followed by oxidation with anhydrous t-butyl hydroperoxide, $(CH_3)_3COOH$ lead to long chain primary alcohols to synthesise 1-triacontanol in high yields (scheme V). Their report first described the preparation of the olefin intermediate, 15-triacontene (42) by metathesis reaction of 1-hexadecene (41). The obtained intermediate, 42 underwent hydrozirconation with two equivalents of CpZr.HCl (Cp = η^5-cyclopentadienyl) in tetrahydrofuran at 40° for 96h. Subsequent oxidation

with anhydrous t-butylhydro peroxide in 1,2-dichloroethane at room temperature gave 1-triacontanol (59-68%) contaminated with a mixture of olefins.

Scheme V

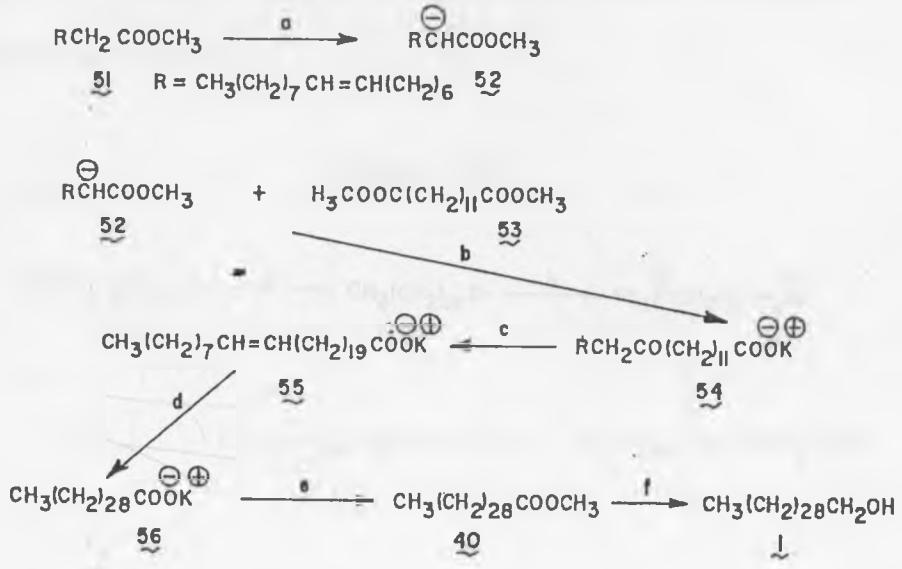


Reagents: (a) WCl_6, $Bu_4Sn/CH_3CO_2C_2H_5$; (b) Reflux, 96h, 40°; (c) t-BuOOH/$ClCH_2CH_2Cl$.

Starting from 1,10-decanedicarboxylic acid, (47, $HO_2C(CH_2)_{10}CO_2H$), Qian et al (1981) perfomed acid derivatisation to $EtO_2C(CH_2)_{10}COCl$ (48). Intermediate 48, was alkylated with octadecanyl-chlorozincate, (49, $CH_3(CH_2)_{17}ZnHCl$) and hydrolysed to 12-oxotriacontanoic acid (50, $CH_3(CH_2)_{17}$-$CO(CH_2)_{10}CO_2H$). Huang-Minlon reduction followed by esterification with ethanol and subsequent $LiAlH_4$ reduction gave 1-triacontanol in 23% overall yield .

Krause (1982) carried out the synthesis of the titled compound via Claisen condensation of fatty alkyl ester with a di-methyl ester of a higher saturated dicarboxylic acid. This was achieved in six steps starting from methyl oleate (51) and di-methyl brassylate (53) via the intermediate, unsaturated keto salts; (54, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COCH_2(CH_2)_{10}CO_2K$ and (55, $CH_3(CH_2)_7CH{=}CH(CH_2)_{19}CO_2K$ as outlined in scheme VI.

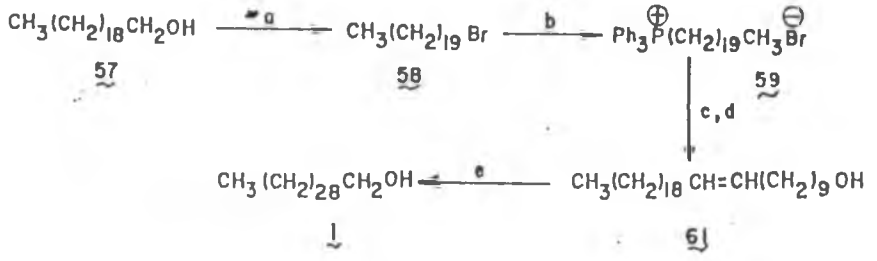
Scheme VI



Reagents: (a) KOEt; (b) $H_3O^{\oplus}$, Δ, $-CO_2$; (c) Zn-Hg/HCl; (d) Raney Ni/H_2; (e) CH_2N_2; (f) $LiAlH_4$/THF.

A novel synthesis of the titled compound was reported by Penninger (1984) utilising the Wittig reaction (scheme VII). The work involved the treatment of 1-eicosanol (57, $CH_3(CH_2)_{18}CH_2OH$) at 130° with hydrobromic acid for 2.5h giving 90% eicosanylbromide (58). Compound 58 was refluxed with triphenylphosphine in acetonitrile for 40h to give 95% of the phosphonium salt, $CH_3(CH_2)_{19}P^{\oplus}Ph_3Br^{\ominus}$ (59). Wittig reaction of 59 with sodium methoxide and 10-hydroxydecaldehyde (60, $HO(CH_2)_9CHO$) in t-butylmethylether gave 80% of $CH_3(CH_2)_{18}CH=CH(CH_2)_9OH$ (61) which on hydrogenation afforded 1-triacontanol.

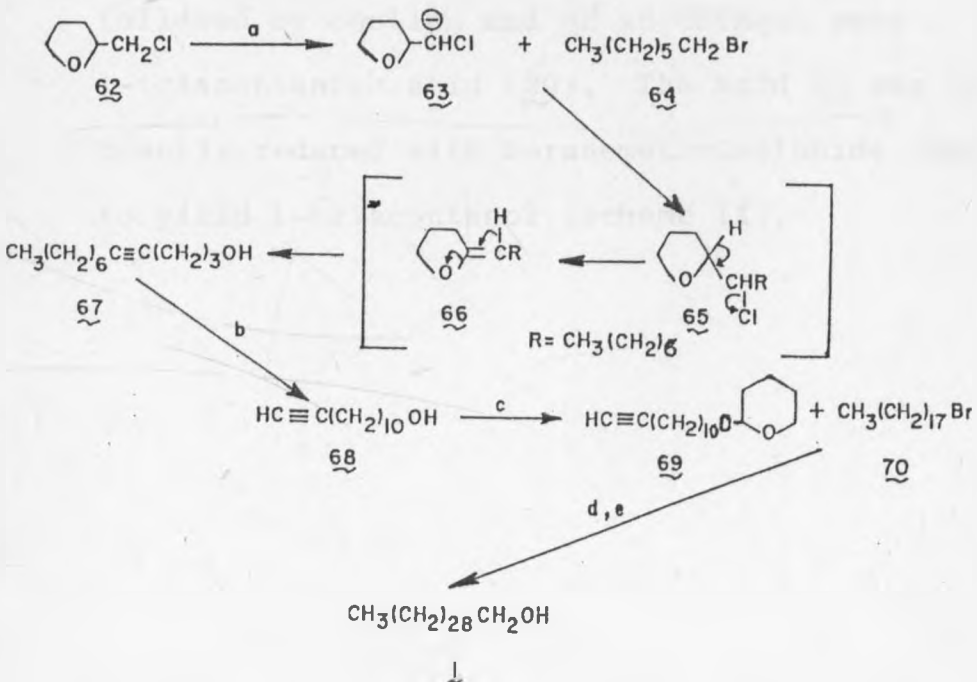
Scheme VII



Reagents: (a) HBr, 130°/2.5h; (b) Ph_3P/CH_3CN;
(c) $NaOCH_3/t\text{-}BuOCH_3$; (d) $HO(CH_2)_9CHO$ (60);
(e) H_2/Pt.

Rao et al (1984) again reported a new synthetic route to the titled compound from 4-dodecyne-1-ol, (67, $CH_3(CH_2)_6C{\equiv}C(CH_2)_3OH$) intermediate (scheme VIII). Compound 67 was prepared by treating tetrahydrofurfuryl chloride (62) with lithium amide in ammonia and heptanyl bromide (64). The obtained intermediate (67) underwent a smooth re-arrangement reaction with sodium amide in 1,3-diaminopropane to afford 11-dodecyne-1-ol (68) whose hydroxyl group was protected by dihydropyrane. The ether 69 formed was coupled with 1-bromooctadecane (70) and hydrogenated. Subsequent acid work-up gave 1-triacontanol.

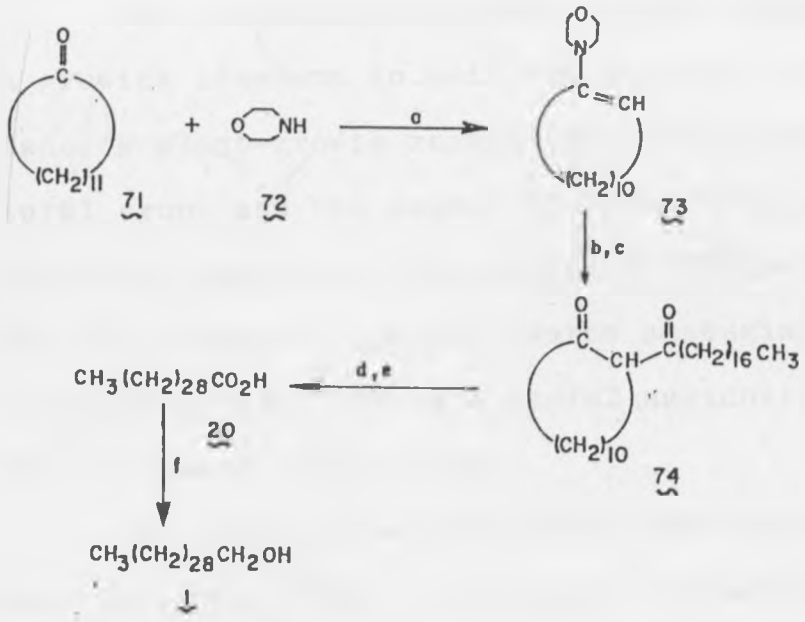
Scheme VIII



Reagents: (a) $LiNH_2/NH_3$; (b) $H_2N(CH_2)_3NH_2/NaNH_2$; (c)  , $H^{\oplus}/H_2O$; (d) H_2/Pd; (e) $H_3O^{\oplus}$.

In 1984, Nickey et al also developed a synthetic route to 1-triacontanol starting from cyclododecanone (71). Compound 71 was treated with morpholine (72) in the presence of p-toluene sulphonic acid in toluene and the formed 1-morpholino-1-cyclododecane (73) coupled with stearoyl chloride (25) in the presence of triethylamine at 0-10°C. The resulting intermediate, 2-n-hexadecyl cyclotetradecanedione (74) was hydrolysed under acidic condition, then treated with potassium hydroxide in diethylene glycol and refluxed with hydrazine hydrate distilling at 190-210°. Continued reflux of the mixture followed by cooling and pH adjustment gave 1-triacontanoic acid (20). The acid 20 was subsequently reduced with boranemethylsulphide complex to yield 1-triacontanol (scheme IX).

Scheme IX



Reagents: (a) $p\text{-}CH_3C_6H_4SO_3H/CH_3C_6H_5$;

(b) $CH_3(CH_2)_{16}COCl$ (25)/Et_3N; (c) $H_3O^{\oplus}$;

(d) $KOH/O(CH_2CH_2OH)_2$; (e) $N_2H_4.H_2O, H_3O^{\oplus}$;

(f) $(CH_3)_sS.BH_3$.

1.3 Aims and Objectives of the present study.

The foregoing literature review clearly shows a growing interest in both the study of 1-triacontanol's plant-growth regulating activity on agricultural crops and the search for expedient natural or synthetic sources of the compound. These studies are all primarily geared towards assessing the compound's viability as a useful agricultural input for increased crop yields.

The aims and objectives of the present study were two-fold: First, to conduct chromatographic analysis of local beeswax sample in order to determine it's constitution and also ascertain 1-triacontanol content. Second, to develop a new synthetic approach to the compound from tetracosanoic acid ($\underset{\sim}{33}$, $CH_3(CH_2)_{22}CO_2H$) a principal component of the oil from the readily available Cassia grandis seeds.

CHAPTER TWO

RESULTS AND DISCUSSION

2.10 GENERAL CHROMATOGRAPHIC INVESTIGATION OF KENYAN BEESWAX.

The crude sample of beeswax was purified by the method of Khalique et al (1967) to give a pale, cream-coloured wax. The physico-chemical properties of the purified wax were determined using standard analytical procedures (Lewkowitsch, 1934). The analysis included determination of physical constants (colour, melting point and density) and chemical constants (saponification value, acid value, iodine value and acetyl value). Saponification value is defined as the number of milligrams of potassium hydroxide required for complete saponification of 1.0g of wax and is indicative of the average molecular mass of the wax. Acid value is a measure of the quantity of free acids in wax and is defined as the number of milligrams of potassium hydroxide required to neutralise the free fatty acids in 1.0g of wax. Iodine value indicates the average degree of unsaturation and is defined as the number of grams of iodine absorbed by 100g of wax. Indication of the quantity of free hydroxy-acids in the wax is

given by the acetyl value. This is defined as the number of milligrams of potassium hydroxide required to neutralise the acetic acid produced by hydrolysis of 1.0g of acetylated wax.

The results of these analyses are summarised in Table 5. The determined properties of the Kenyan beeswax sample were found to show close similarity to those of beeswax from the honeybee, Appis mellifera from various parts of the world except for the sample's degree of unsaturation (iodine value) which was found to be relatively lower. This low iodine value is probably attributed to the initial permanganate oxidation process during the purification of the crude beeswax sample.

Separation of the beeswax into constituent classes was carried out using the scheme set out in Figure 1. Saponification of the wax was accomplished by dissolving the wax in petroleum ether (80-100°) and refluxing in the presence of ethanolic potassium hydroxide solution (0.5N). Addition of brine solution to the resulting reaction mixture resolved the mixture into organic and aqueous phases. The organic phase was separated off and the aqueous phase extracted with petroleum ether (80-100°). Evaporation

Table 5 Properties of the purified beeswax

Property	
Colour	Pale cream
Melting point (^{o}C)	63-67
Density (at 23^{o}C)	0.96
Saponification value	91.4
Acid value	17.6
Iodine value	7.3
Acetyl value	15.8

of the organic phase and combined petroleum ether extracts afforded the unsaponifiable fraction as a white solid (62.4%). The residual alkaline phase was neutralised with dilute sulphuric acid (3N), extracted with petroleum ether (80-100^{o}) and evaporated to afford the acid fraction as a white solid (37.6%).

The constituent components of the saponified wax were determined by TLC analysis of both the unsaponifiable and the acid fractions obtained. A mixture of synthetic compounds; tetracosane, docosanol, 1,23-tetracosanediol, eicosanoic acid and 16-hydroxyhexadecanoic acid was employed as a reference system. Small amounts of the two fractions and the standard mixture were dissolved in chloroform (1-2% solutions) and spotted on TLC

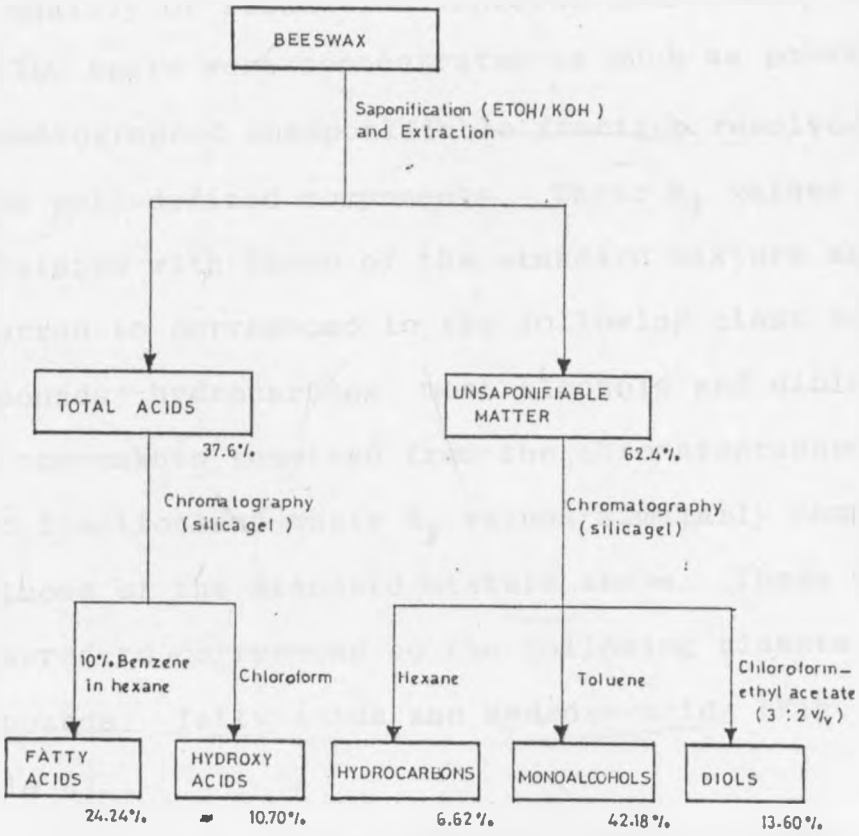


Figure 1.: Fractionation of beeswax components into classes. Fractions were identified by thin-layer chromatography and infra-red spectroscopy.

(silica gel) plates. A solvent system consisting of benzene, dichloromethane, and ethyl acetate (7:3:3 v/v) gave the most satisfactory resolution. The quality of resolution improved appreciably when the TLC spots were concentrated as much as possible. The chromatographed unsaponifiable fraction resolved into three well-defined components. Their R_F values were correlated with those of the standard mixture and inferred to correspond to the following class of compounds: hydrocarbons, mono alcohols and diols. Two components resolved from the chromatographed acid fraction and their R_F values similarly compared to those of the standard mixture above. These were inferred to correspond to the following classes of compounds: fatty acids and hydroxy acids (Fig. 2; Table 6).

Thin-layer chromatography was thus found to be a suitable technique in the study of long chain aliphatic constituents in beeswax. The saponified Kenyan beeswax was successfully resolved by the system designed. Moreover, the TLC data obtained correlated well with those published by Holloway et al (1966). This method is therefore recommended for routine qualitative analysis of beeswax. The technique developed was also found useful in

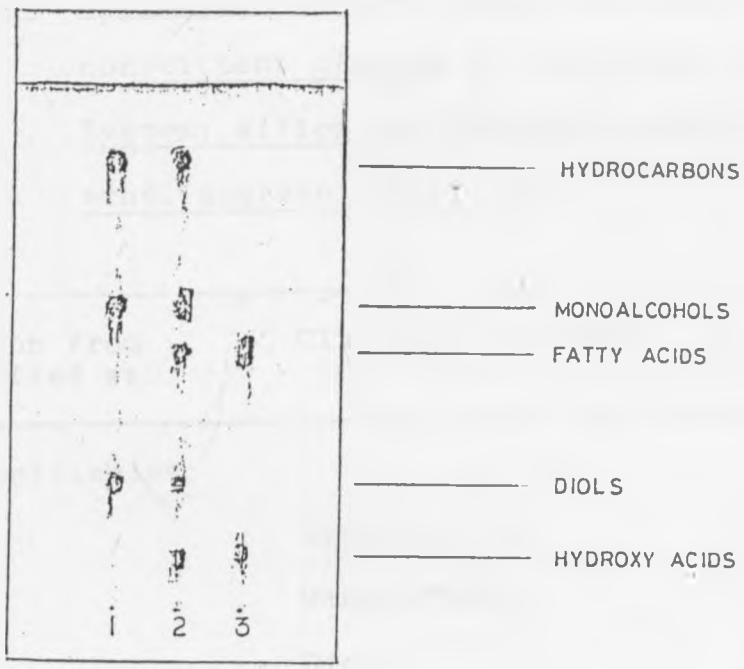


Figure 2 TLC OF SAPONIFIED BEESWAX

1. Unsaponifiable fraction of beeswax

2. Reference mixture of: tetracosane, docosanol, eicosanoic acid, 1,23-tetracosanediol, 16-hydroxyhexadecanoic acid

3. Acid fraction of beeswax

Solvent system: Benzene-dichloromethane-ethyl acetate (7:3:3 v/v).

monitoring fractions collected from column chromatography.

Table 6: R_F values obtained with the various constituent classes of saponified beeswax. System; silica gel/benzene-dichloromethane-ethyl acetate (7:3:3 v/v).

Fraction from saponified wax	Class of compounds	R_F value
Unsaponifiables:		
	Hydrocarbons	0.86
	Monoalcohols	0.56
	Diols	0.23
Total acids:	Fatty acids	0.48
	Hydroxy acids	0.10

We addressed ourselves next to the problem of isolating the pre-determined (TLC) composite constituents of the saponified wax by column chromatography. A portion of the unsaponifiable fraction (10g) was first subjected to a column chromatographic (silica gel) separation. Hexane was employed as the initial eluant and afforded a

chromatographically pure fraction appearing as white waxy-like crystalline plates, mp 58-60^{o}. IR examination showed peaks at 2855(s), 1470(m) and 720(m) cm^{-1} association with long chain alkanes and the fraction was inferred to consist of hydrocarbons (1.06g, 10.6%). Co-spotting with an authentic hydrocarbon sample, tetracosane on TLC using chloroform-benzene (7:3 v/v) as eluant gave no resolution of the constituent, confirming that the isolated fraction consisted of hydrocarbons.

Warm toluene was employed next and eluted a well-defined second fraction in the form of fine white crystals, mp 76-80^{o}. IR analysis exhibited sharp peaks at 3340 cm^{-1} attributable to the hydroxyl group and a characteristic carbonyl group, C-O (str.) at 1050 cm^{-1} suggesting that the fraction consisted of aliphatic monoalcohols (6.76g, 67.6%). Further R_F comparison on TLC with an authentic primary alcohol sample, docosanol using chloroform-ethylacetate (1:1 v/v) as the solvent system confirmed that the fraction was composed entirely of mono alcohols.

The last fraction of the unsaponifiable fraction was strongly adsorbed on the column and was only eluted by a warm chloroform-ethyl acetate (3:2 v/v)

solvent mixture. The fraction in the form of a white powder, mp 92-94^{o}, gave a yield of 2.18g (21.8%). Inspection of the IR spectra showed a strong band at 3350-3400 cm^{-1} and medium intense overlapping bands in the region, 1120-1055 cm^{-1} depicting the presence of hydroxyl groups. In conformity to this inference, R_F comparisons on TLC with an authentic diol sample, 1,23-tetracosane diol also confirmed that the fraction consisted of diols.

Isolation of the constituent components of the acid fraction obtained was undertaken by applying a portion of the acid fraction (5.0g) onto a silica gel column. Elution with 10% benzene in hexane gave a chromatographically pure white crystalline product, m.p. 81-86^{o}. IR analysis gave a broad strong band at 3375 cm^{-1} and a medium intense peak at 1730 cm^{-1} attributable to carboxyl group providing strong evidence that the fraction consisted of fatty acids (3.24g, 64.8%). These observations from IR were further confirmed by TLC employing chloroform-ethyl acetate (1:1 v/v) as the eluant. The R_F's of the fraction and that of an authentic fatty acid sample, eicosanoic acid were compared and found to be superimposable. Further elution using chloroform

gave a white solid fraction m.p. 94-98^{o} from the chromatographic column. The IR spectra of the fraction showed a broad strong peak at 3600 cm^{-1} and a sharp peak of medium intensity at 1718 cm^{-1} attributable to the carboxyl group. Peaks of medium intensity were observed in the region, 1050-1100 cm^{-1} suggesting the presence of the hydroxyl group. The IR spectra data thus suggested that the fraction was likely to consist of hydroxy acids (1.43g, 28.6%). Comparison of the fraction's TLC behaviour with an authentic hydroxy acid sample, 16-hydroxyhexadecanoic acid using chloroform-ethylacetate (1:1 v/v) solvent mixture gave identical R_F values, providing further evidence that the fraction was composed of hydroxyacids. Final elution from the column using chloroform yielded a brown gummy solid (0.31g, 6.2%) which showed no resolution on TLC using the above described conditions. This fraction, which was not further investigated, probably consists of artefacts from pigments and propolis of honeycomb frames from which the wax was originally isolated. Table 7 summarises the percent composition of the chief constituents of saponified beeswax.

Table 7: Composition (%)* of saponified beeswax

Component	%
Hydrocarbons	6.62
Mono alcohols	42.18
Diols	13.60
Fatty acids	24.24
Hydroxy acids	10.70
Unidentified (pigments,propolis,etc.)	2.32

*Calculated from the weights of components obtained by column chromatography (silica gel).

2.11 GAS CHROMATOGRAPHIC ANALYSIS OF 1-TRIACONTANOL IN ALCOHOL FRACTION OF SAPONIFIED BEESWAX.

The GC analysis of the alcohol fraction obtained was conducted in order to determine the content of 1-triacontanol along with identifying and estimating the amount of other components present. The underivatised alcohol fraction was analysed initially but resolution of the various components was poor with appreciable tailing. The fraction showed ten asymmetric peaks which were attributed to a homologous series of primary alcohols. This was demonstrated by comparison of the fraction's retention times with a reference mixture consisting of eicosanol, docosanol and 1-triacontanol. The series was found to extend from C_{18} to C_{36} with a maximum at C_{30} due to 1-triacontanol. Better GC resolution of the alcohol fraction was obtained by conversion to the correspoding acetylated derivative. The acetylated alcohol fraction gave sharp peaks and resolved all the ten components satisfactorily (Fig. 3). The components of the acetylated fraction exhibited longer retention times than those of the free alcohol fraction and the same emergence temperatures were observed when co-injected with the acetylated reference mixture.

The percentage composition of the resolved components were determined (Table 8) and 1-triacontanol

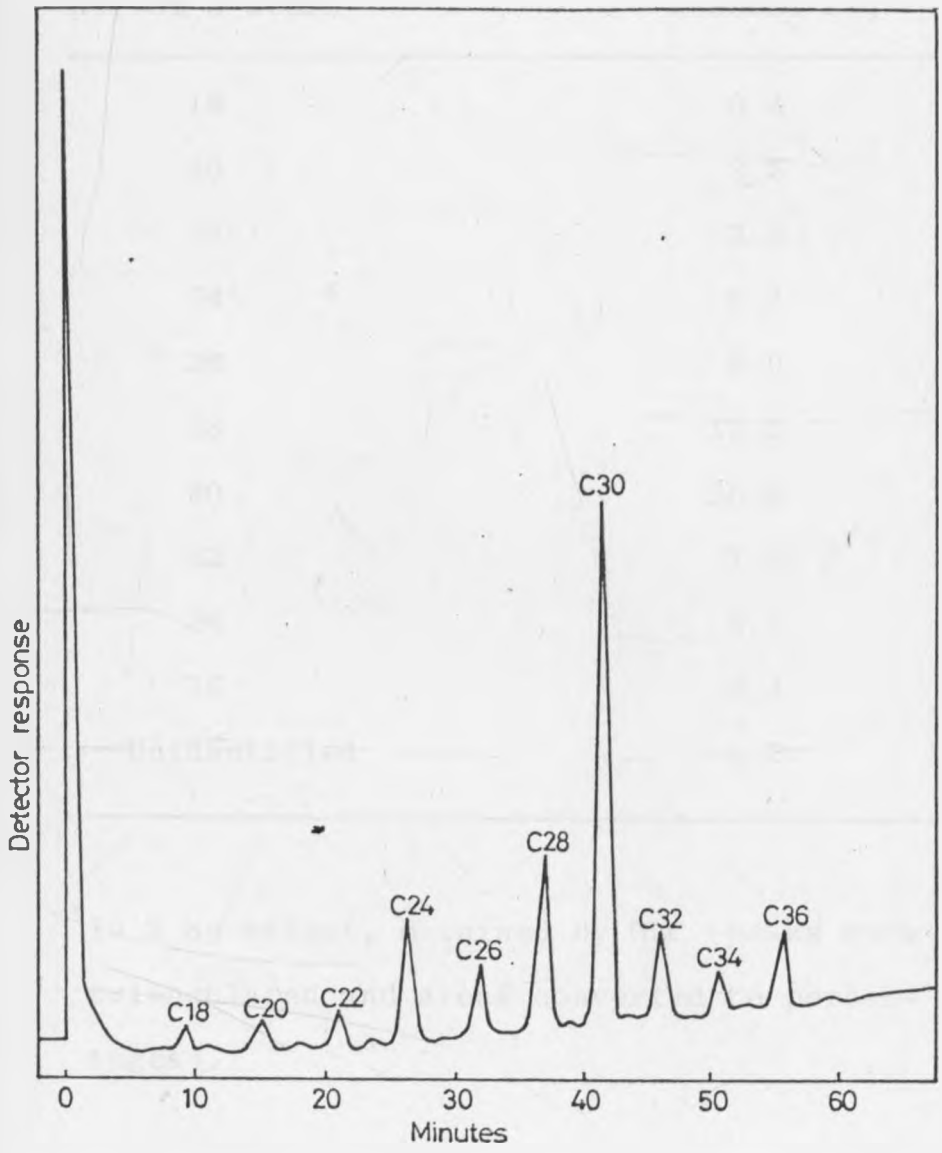


Fig. 3. GLC Separation of the acetylated alcohol fraction.

Table 8: Composition* of alcohol fraction from saponified beeswax.

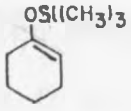
No. of C atoms	Alcohol (%)
18	0.4
20	2.5
22	2.3
24	9.1
26	5.0
28	13.9
30	50.8
32	7.3
34	3.1
36	4.4
Unidentified	1.2

*In % by weight, obtained by GLC (peaks were triangulated and areas converted to percentages).

was found to constitute 50.8% of the alcohol fraction. This represents 21.4% of the whole beeswax sample obtained from Kitui district, Kenya.

2.20 SYNTHESIS OF 1-TRIACONTANOL

The reviewed published synthetic routes to 1-triacontanol suffer generally from low yields with some requiring vigorous reaction conditions. This led us to seek a synthetic approach which would attempt to overcome these constraints. In our approach we envisaged the addition of a six-carbon unit to tetracosanoic acid (33) by coupling the silyl enol ether of cyclohexanone, 76 to tetracosanoyl chloride (34). The resulting β-diketone 36 would then be cleaved with alkali to the keto-acid salt 37 which would then be reduced to the desired compound.



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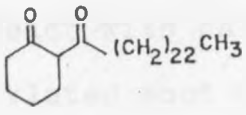

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In designing our approach to l-triacontanol, we set as our first target the preparation of the silyl enol ether intermediate 76. The use of silyl enol ethers in recent years as synthetic intermediates in organic synthesis is well documented (Rasmussen, 1977; House, 1972; Baukov et al,1970; Stork et al, 1968). The ability of silyl enol ethers to generate stabilised enolate anions with high regiospecific nucleophilicity, have made them suitable intermediates for subsequent condensation (albeit often catalysed) transformations and have thus afforded useful new synthetic routes.

Cyclohexanone (75) was thus converted to silyl enol ether 76 by the procedure of House and co-workers (1968). Compound 75 was treated with a mixture of excess chlorotrimethylsilane and trimethylamine in refluxing dimethyl formamide. The role of the amine was to react with the ketone 75 to form an enolate anion which would then react with chlorotrimethyl-silane to form the O-silylated enol ether. Compound 76 was readily isolated, after work-up, by fractional distillation as a colourless oil (bp. 74-75^{o}/20 mm) in 72% yield. IR spectra gave a characteristic peak at 1670 cm^{-1} associated with the cyclohexenyl olefinic bond and other peaks at 2940 (s), 1250

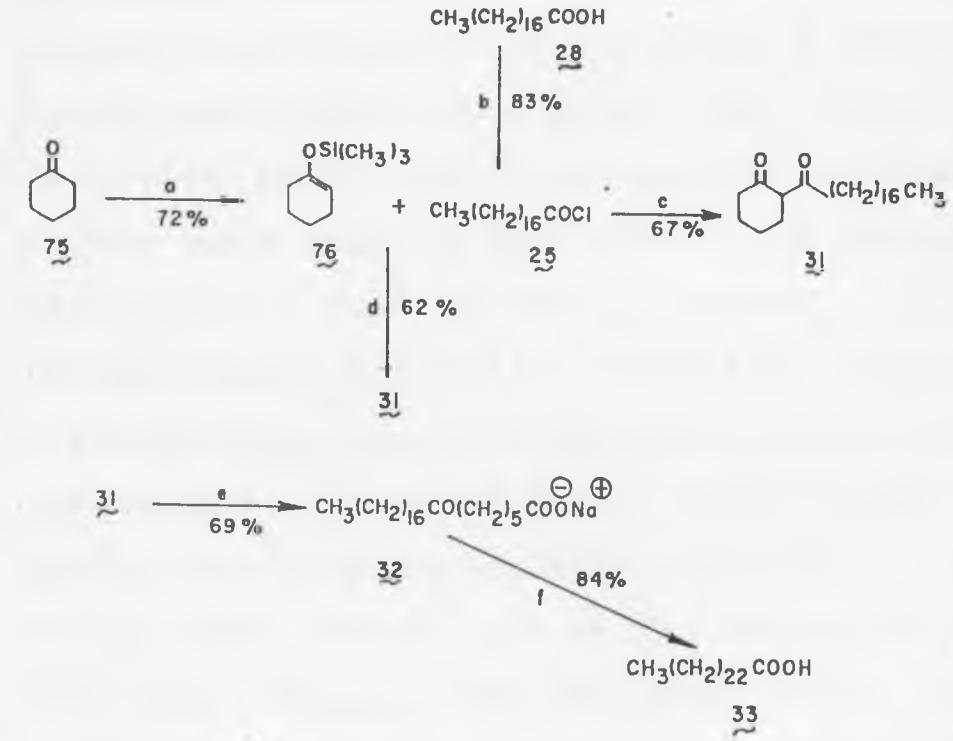
(s) cm^{-1} due to $Si-CH_3$ bond. Proton NMR spectrum gave the diagnostic vinyl proton signal as a multiplet centred at δ4.73.

We next explored the possibility of making the β-diketone 36 which would constitute a key intermediate in the construction of the carbon skeleton of 1-triacontanol. In a model study, we chose to prepare the β-diketone, 2-stearoylcyclohexanone (31) from the readily available stearoylchloride (25) Scheme X .

The acid 28 was transformed to compound 25 using excess thionylchloride in dichloromethane. Vacuum evaporation of solvent and excess thionyl-chloride followed by vacuum distillation afforded stearoyl chloride (25) (bp $185^{o}/2.5$ mm) as a clear liquid in 83% yield. IR spectra showed the presence of C-Cl bond at 950(m) cm^{-1} and the C=O signal at 1820(s) cm^{-1} typical of acid chlorides. ^{1}H-NMR gave resonance peaks at δ0.49 (t), δ1.15-1.25(m) and δ2.3(t) assigned to terminal CH_3, methylenes and $COCH_2$ groups respectively.

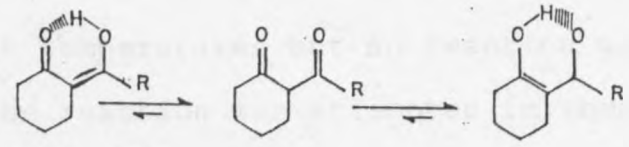
Generation of the β-diketone 31 was achieved by the C-acylation coupling reaction between the acid chloride 25 and the silyl enol ether 76 using two different synthetic methods. The first method was

Scheme X



Reagents: (a) $ClSi(CH_3)_3$, Et_3N/DMF; (b) $SOCl_2$, CH_2Cl_2;
(c) $CH_2Cl_2/ZnBr_2$; (d) Et_3N/THF, HCl(2M);
(e) NaOH/EtOH; (f) KOH/N_2H_4, HCl(2M).

adapted from established procedures on the acylation reactions of silyl enol ethers (Brown, 1974; Fleming and Peterson, 1979; Ladjama et al, 1979). This proved attractive in that, it involved mild reaction conditions and was essentially a one-pot reaction which gave a good yield of the product. It involved mixing the two reactants in dichloromethane under anhydrous conditions in the presence of a catalytic amount of zinc(II) bromide. Chromatographic purification followed by recrystallisation from hexane afforded crystalline 2-stearoyl cyclohexanone (31) in 67% yield. ^{1}H-NMR of the product gave resonance signals at δ0.8 (t), δ1.25-1.55(m), δ2.25(t) and δ4.1(t) assigned to CH_3, methylenes $-(CH_2)_{15}$, $COCH_2$ and COCHCO groups respectively. IR spectra exhibited peaks at 3600 (w, br) cm^{-1} due to OH group and at 1710(m), 1610(m) cm^{-1} due to C=O group consistent with the existence of keto-enol tautomeric equilibria:

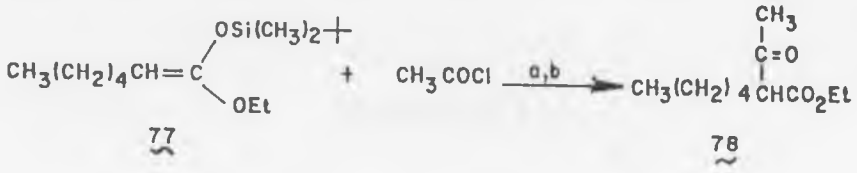


31

The mass spectrum gave a weak parent ion peak at z/e 364 thus confirming the identity of the compound.

Our second method was based on that of Rathke and Sullivan (1973) who showed that O-silyl ketene acetals and acid chlorides could be readily coupled with the help of triethylamine under relatively mild conditions to give quantitative yields of β-ketoesters (Scheme XI).

Scheme XI

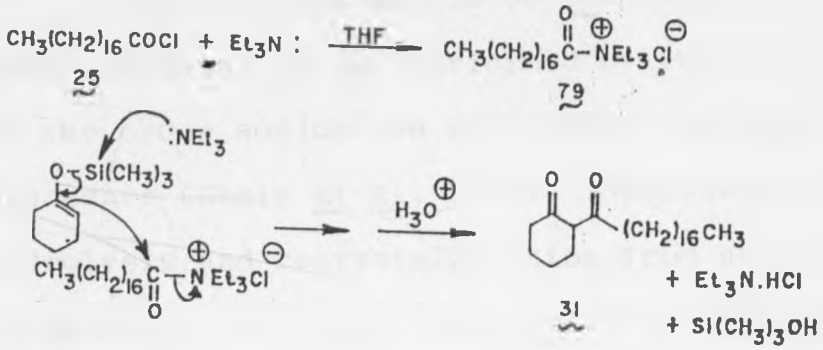


Reagents: (a) Et_3N/THF, 25°; (b) HCl, H_2O.

Condensation of the silyl enol ether 76 with stearoyl chloride 25 to 31 was first attempted in dichloromethane and hexane as reaction solvents at ambient temperatures but no reaction was evident. When the reaction was attempted in these solvents at elevated temperatures, complex uncharacterisable reaction products were formed. The use of tetrahydrofuran was however more rewarding, with

the reaction cleanly occuring at 25° to give product 31 in 62% yield. The IR, ^{1}H-NMR and MS data observed for the product were identical to the material obtained by the previously described zinc(II) bromide catalysed synthetic method. The fact that the reaction proceeded readily in the more polar solvent, tetrahydrofuran, but poorly in dichloromethane and hexane suggests that activation of the acyl C=O through the formation of the polar zwitterionic acyl ammonium salt 79 is crucial for the reaction to occur smoothly.(Scheme XII).

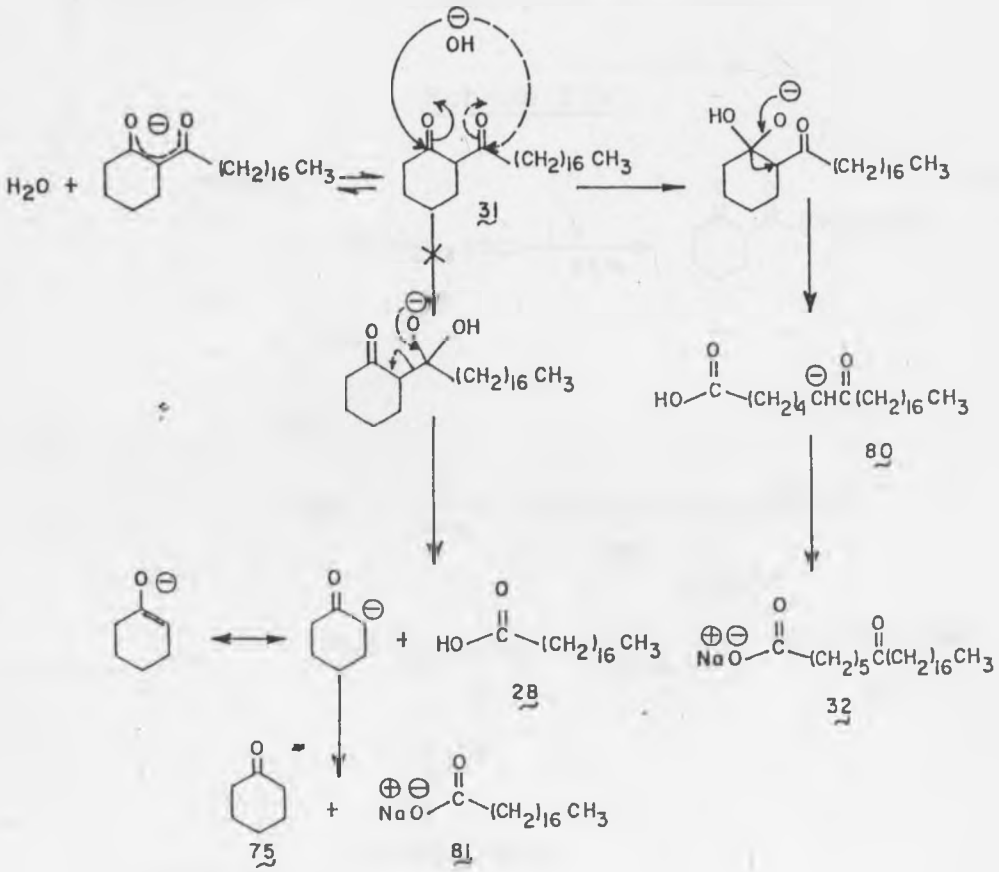
Scheme XII



Completion of the synthesis was finally achieved by a sequence of reactions (see scheme X). where 31 was first allowed to undergo an hydroxide initiated cleavage in refluxing ethanol to sodium oxo-tetracosanoate (32). Examination of the product's IR spectra showed the characteristic carboxylate salt peak at 1680 cm^{-1} and 1600 cm^{-1} due to $C(\overline{\quad\cdots\cdots}O)_2^{\ominus}$ stretching band. Two possible mechanisms for the cleavage of 31 are shown in scheme XIII. The fact that the attack by the hydroxide ion on cyclic C=O is more favoured than that on the open chain C=O probably suggests a reduced steric hindrance on this carbon as a result of the folding of the cyclic methylene groups.

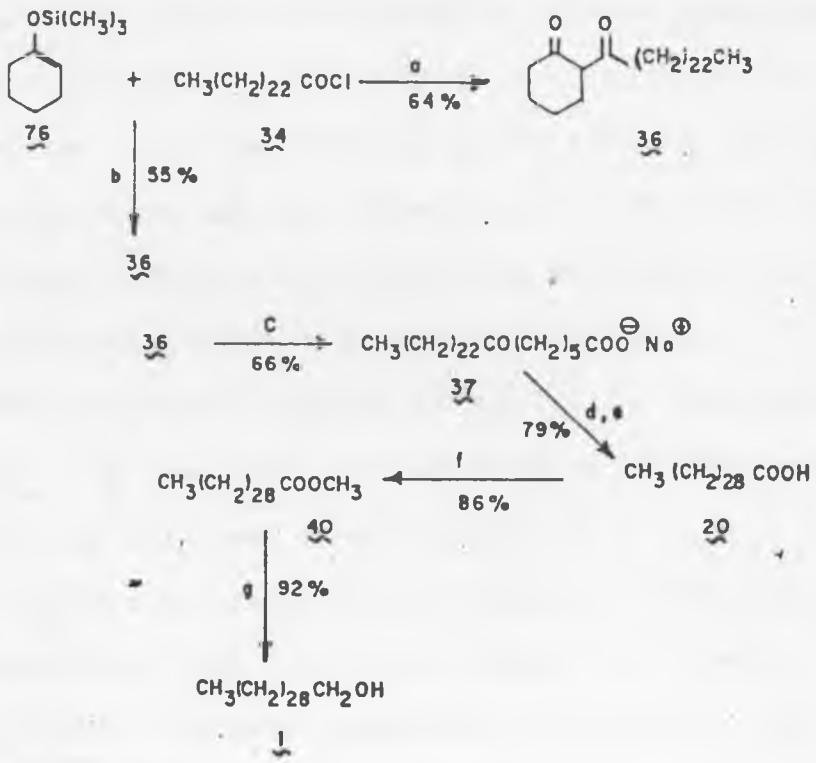
Final transformation of 32 to the targeted model compound 33 was performed by the keto-reduction of the crude sodium-oxo salt using the Huang-Minlon procedure (Hunig et al, 1961). Subsequent acid hydrolysis and recrystallisation from acetic acid afforded white crystalline plates of tetracosanoic acid 33 (mp 86-87^{o}) in 32.2% overall yield. The sample prepared was shown to have essentially identical characteristics (mmp, TLC, IR and ^{1}H-NMR) with those of an authentic sample of tetracosanoic acid.

Scheme XIII



Having demonstrated the preparation of tetracosanoic acid (33) with encouraging results in our model synthesis, we next extended this methodology to the synthesis of our titled compound, 1-triacontanol as summarised in scheme XIV.

Scheme XIV

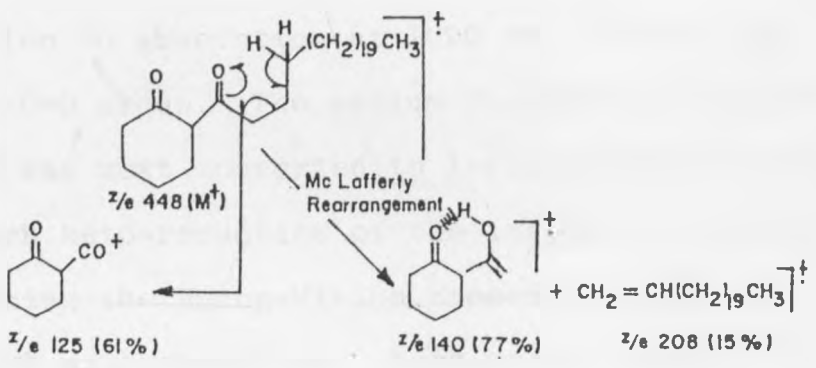


Reagents: (a) $CH_2Cl_2/ZnBr_2$; (b) Et_3N/THF, HCl (2M); (c) NaOH/EtOH; (d) KOH, N_2H_4, Triethyleneglycol; (e) HCl (6M); (f) $MeOH/H^{\oplus}$ (g) $LiAlH_4$/THF.

Our synthesis began with the conversion of tetracosanoic acid (33) to its acid chloride 34 where the acid was treated with excess thionyl chloride in refluxing dichloromethane. Subsequent work-up provided a 91% crude yield of the corresponding tetracosanoyl chloride (34), mp 116-118°. TLC showed a single spot with only trace impurities. IR spectrum showed the presence of C-Cl bond at 940 (m) cm^{-1} and the C=O signal at 1785 (s) cm^{-1}. ^{1}H-NMR spectrum showed a downfield shift at δ2.8(t) associated with the acid chloride methylene protons - CH_2COCl with respect to the acid protons, $-CH_2COOH$ resonance signals at δ2.1 (t). The acid chloride, 34 was used as such without further purification in the next transformation.

Conversion of 34 to the desired, 2-tetracosanoyl cyclohexanone (36) was accomplished by either of the two synthetic methods discussed previously. The first method which utilised zinc(II) bromide as the catalyst in the condensation of 34 with silyl enol ether 76 afforded after work-up and purification, a 64% yield of crystalline 36 mp 50-52° (Lit. 48-50°, Ries and Wert, 1977). IR spectra gave a broad signal at 3540 cm^{-1} due to OH group besides the two ketonic, C=O signals at 1780 cm^{-1} and 1720 cm^{-1} respectively,

characteristic of the expected keto-enol tautomeric equilibria. Further structural evidence was provided by the mass spectrum where a weak molecular ion peak was observed at z/e 448 together with moderately intense fragmentation ions at z/e 208, 140 and 125 among others, ascribed to the following postulated fragmentation pathway.



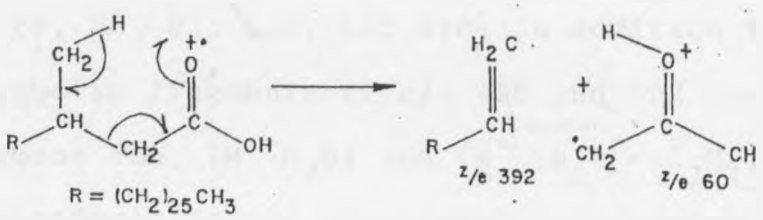
Compound 36 was also prepared using the second synthetic method where a mixture of 34 and 76 in tetrahydrofuran was treated with triethylamine at room temperature for 24 hr. Work-up of the reaction mixture gave a crude product which on the basis of TLC showed no apparent contamination of products due to the competitive O-acylation

reaction. Recrystallisation from hexane provided a 55% yield of 36 as crystalline plates which was found by melting point and spectral comparison to be identical with the sample prepared through zinc(II) bromide catalysis.

The reaction of 36 with sodium hydroxide in refluxing ethanol proceeded smoothly to afford the desired cleavage product 37 in 66% isolated crude yield. IR spectrum showed characteristic bands of the carboxylate, $C(\overset{\ominus}{\text{----}}O)_2$ at 1670 cm^{-1} and 1570 cm^{-1} in addition to absorption at 1690 cm^{-1} due to the ketonic, C=O group. The sodium 7-oxotriacontanoate salt, 37 was next converted to 1-triacontanoic acid 20 through keto-reduction of the internal carbonyl centre using the Huang-Minlon procedure with subsequent acid quenching. Work-up followed by chromatographic purification and recrystallisation from diethyl ether afforded a 79% yield of tetracosanoic acid 20 as plate-like crystals mp 87-89°. The IR spectrum of the product showed characteristic carboxylic acid absorptions at 3350-2500, 1670 and 1280 cm^{-1}. The mass spectrum consisted of two series of peaks resulting from α C-C bond cleavages with retention charge either on the oxygen-containing fragments at z/e 45, 59, 73 etc. or on the alkyl

fragments at z/e 43, 57, 71 etc. These ion clusters of medium intensity at intervals of 14 mass units are typical of the fragmentation pattern of long chain monocarboxylic acids. A characteristic peak for straight chain monocarboxylic acids was also observed at z/e 60 due to β-bond cleavage accompanying McLafferty rearrangement.

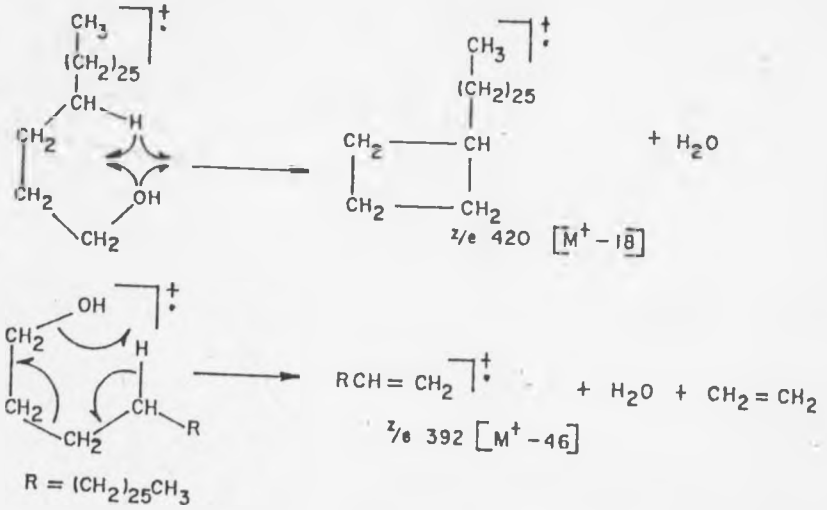
β-Cleavage with McLafferty rearrangement



The acid 20 was then esterified in excess methanol in the presence of a catalytic amount of concentrated sulphuric acid to the corresponding methyl ester 40 in 86% yield. Subsequent work-up and recrystallisation from ethanol afforded methyl-1-triacontanoate (40) in crystalline form. The IR spectral data showed strong bands at 1740, 1240 and 1050 cm^{-1} expected of an ester whereas ^{1}H-NMR spectra revealed the presence of methoxy protons, OCH_3 as a singlet

centred at δ4.1.

The methyl ester 40 was finally reduced to the desired target alcohol using lithium aluminium hydride in refluxing tetrahydrofuran. Work-up with aqueous sodium hydroxide and recrystallisation from hexane afforded 1-triacontanol mp 85-87° (Lit. 87-88°, Steicher et al, 1968) in 92% yield. The IR spectrum of 1 exhibited characteristic aliphatic alcohol absorptions at 3450-3100 cm^{-1} due to OH group and at 1450, 1060 cm^{-1} due to C-O group. Mass spectrum depicted characteristic alkyl fragments at z/e 69, 83, 97, 111, 125 etc. in addition to the diagnostic fragments at z/e 420 and 392 due to fragment ion, $[M^+-H_2O]$ and $[M^+-(H_2O + C_2H_4)]$ respectively formed as a result of the following fragmentation:



The titled compound obtained was also found to be identical to an authentic sample in all respects (IR, ^{1}H-NMR, MS, mixed mp and thin-layer mobility in dichloromethane). Thus the synthesis of 1-triacontanol was accomplished in 6 steps in 24% overall yield from tetracosanoic acid 34.

CHAPTER THREE

EXPERIMENTAL

3.0 MATERIALS AND GENERAL METHODS

All organic reagents were of the highest grade commercially available and were used as obtained. Analar grade or distilled reagent-grade solvents were used for all reactions and chromatographic separations. Where required, they were purified following standard procedures (Keese and Müller, 1982) and used immediately as such or stored over molecular sieves (type 4A^{O}) before use. All inorganic materials were commercial products and were used as such. Gravity column chromatography and flash column chromatography were carried out on E. Merck 0.063-0.200 - mm and 0.040-0.063 - mm silica gel respectively. Qualitative analysis of fractions collected from column chromatography and monitoring of all reactions was done by thin-layer chromatography (TLC) performed on either silica gel (Merck H, 0.25mm thick) pre-coated plastic sheets or commercial pre-coated glass plates (size, 20x20 cm) cut to appropriate sizes. Melting points were measured on an Electrothermal capillary melting point apparatus and are reported uncorrected. Infrared spectra were

recorded on a Pye Unicam, SP3-300 infrared spectrophotometer calibrated against the 1601-cm^{-1} band of polystyrene. Mulls were films in potassium bromide pellets and liquids were films between sodium chloride plates. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined in either carbon tetrachloride or deuterochloroform on either a Perkin Elmer, R12 (60 MHz) or a Varian, XL-300 (200 MHz). All chemical shifts are reported in ppm(δ) downfield from tetramethylsilane used as the internal standard where s, d, t and m designate singlet, doublet, triplet and multiplet respectively. GC data were obtained on a Pye Unicam - GCD chromatograph equipped with a 1.5m silicone SE30 glass column and a flame ionisation detector. Low-resolution mass spectra were obtained on a VG 12-250 quadrupole mass spectrometer coupled to an HP 5790A gas chromatograph operated at 70eV using electron-impact (EI) mode, and data are reported as z/e (percent base).

The sample of beeswax from the honeybee, Appis mellifera L. was kindly donated by the livestock production division (Beekeeping research section-Lang'ata, Nairobi) of the ministry of livestock development on October, 1985. The wax which originated from Kitui district was light yellow in colour and showed no traces of honey or pollen.

3.1 CHROMATOGRAPHIC INVESTIGATION OF KENYAN BEESWAX AND 1-TRIACONTANOL CONTENT.

Purification of crude beeswax (Khalique and Nuran, 1967)

The crude wax (100g) was melted and treated with 50ml of 0.1% aqueous potassium permanganate solution. The mixture was boiled with stirring for 2.5hr at 70^{o} and allowed to stand overnight. The wax was washed with water (2x150ml), remelted and treated with 20% aqueous sodium bisulphite (50 ml) for 30 min. It was finally air-dried to yield a pale cream sample (97.6g).

Determination of the saponification value (Whiteley and Heibron (eds), 1949).

The wax (2.06g) was weighed into a 250-ml quick-fit conical flask equipped with a stirring bar and a condenser. Ethanolic potassium hydroxide (25ml, 0.5N) was added and the contents refluxed with stirring in a boiling-water bath (30 min.). A second identical flask containing only alcoholic alkali (25ml) was similarly treated at the same period of time. The flasks were removed and 20 drops of 1% ethanolic solution of phenolphthalein added to each. The contents were then titrated with 0.5N hydrochloric acid, while hot.

The saponification value was obtained by multiplying the difference between the two titrations (6.7ml) by 28.05 and dividing by the weight in grams of wax (2.06) taken.

Determination of the acid value.

2.5g of wax were placed into a 250-ml quick-fit conical flask equipped with a stirring bar and a condenser. 50ml of neutralised 2-propanol and toluene, 5:4 at 70^{o} was added onto the contents followed by five drops of phenolphthalein test solution. The whole was brought to reflux with stirring and the contents titrated, while still hot, with 0.1N potassium hydroxide solution to a permanent pink colour.

The acid value was obtained by multiplying the titer (7.8ml) by 5.61 and dividing by the weight of wax (2.5g) used.

Determination of iodine value (Furman, 1939)

A mixture of wax (0.5g), chloroform (10ml) and iodine solution (30ml) was placed in a 250ml conical flask fitted with a well-ground stopper. (The iodine solution was prepared by dissolving iodine (35.5g) and bromine 2.0g in glacial acetic acid and

the solution made up to 250ml). The whole was shaken to dissolve and allowed to stand for 30 min. Aqueous potassium iodide (10%; 10ml) was then added to the contents, followed by water (100ml). The contents were shaken and the excess iodine of this resulting solution titrated with 0.1N sodium thiosulphate solution. A blank determination under the same conditions without the wax was also conducted at the same time.

The iodine value was obtained by multiplying the difference between the blank and test titer (2.8ml) by 1.269 and dividing by the weight of wax (0.5g) taken.

Determination of the acetyl value.

In a 100-ml round-bottomed flask equipped with a condenser, was added wax (15.0g) and acetic anhydride (20ml). The mixture was refluxed for 2hr until all the wax dissolved. The hot solution was poured onto a 1-litre beaker containing hot water (500ml) and the resulting mixture boiled for 30min. with stirring and then allowed to cool to room temperature where the aqueous layer was separated off. The process of separating off the aqueous layer was repeated thrice and the acetylated wax

pressed as dry as possible and left to dry in a dessicator. A portion of the obtained acetylated wax (5.0g) was saponified with 0.5N ethanolic potassium hydroxide (50ml) and the alcohol distilled off. The obtained soap was dissolved in water (200ml) and 0.5N sulphuric acid (50ml) added with gentle warming until a waxy layer (fatty acids) separated out completely. The layer was filtered off and washed with hot water. The filtrate and the washings were then titrated against 0.1N ethanolic potassium hydroxide while still hot. At the same time, 5.0g of the original beeswax was treated in the same manner described above, starting from saponification with 0.5N ethanolic potassium hydroxide (50ml).

The acetyl value of the beeswax was obtained by multiplying the difference between the two titrations (14.1ml) for the acetylated and non-acetylated waxes by 5.61 and dividing by the weight of the two waxes (5.0g) used.

Saponification and partitioning of wax into acid (saponifiable) and unsaponifiable fractions.

Beeswax (5.0g), petroleum ether, 80-100^{o} (50ml) and 0.5N ethanolic potassium hydroxide solution (100ml) were introduced into a 250-ml round-bottomed

flask equipped with a stirring bar and a reflux condenser. The mixture was then refluxed gently with stirring for 4hrs. The reaction mixture was then transferred while hot into a 500-ml separatory funnel and a saturated sodium chloride solution (30ml) added. The resulting mixture separated out into an aqueous and an organic layer on vigorous shaking. The organic layer was extracted with hot petroleum ether, 80-100^{o} (3x20ml). The combined petroleum ether fractions were concentrated in *vacuo* to yield on air-drying a white unsaponifiable solid (3.12g, 62.4%).

The residual alkaline aqueous alcoholic solution was concentrated under vacuum to a small volume; and to this solution, dilute sulphuric acid (3N) was added drop-wise until the solution became neutral. Monitoring of the solution's pH was done using litmus paper. The solution was poured into a 250-ml separatory funnel and extracted with hot petroleum ether, 80-100^{o} (3x20ml). The petroleum ether fractions were combined and concentrated in *vacuo* to yield on air-drying a white acidic solid (1.87g, 37.6%).

TLC analysis of the unsaponifiable and acid fractions from saponified wax.

Small amounts of the two fractions and a standard mixture of tetracosane, docosanol, 1,23-tetracosanediol, eicosanoic acid, and 16-hydroxyhexadecanoic acid were dissolved in chloroform (1-2%) and spotted on TLC plates using glass capillary tubes. The loaded plates were developed at room temperature (23-25^{o}) in glass-jars lined with filter papers by the ascending solvent technique. The eluant employed was a solvent mixture of benzene-dichloromethane-ethyl acetate (7:3:3 v/v). Detection of spots was done by charring the developed plates with 5% methanol in concentrated sulphuric acid. This was effected by fine spraying of the chromogenic reagent onto the plates using an aerosol spray gun and heating at oven temperature of 120^{o} until black spots appeared. The chromatographed unsaponifiable fraction resolved into three well defined components whose R_F values were correlated with the R_F's of the corresponding components in the standard mixture and inferred to consist of hydrocarbons, monoalcohols and diols. The chromatographed acid fraction was observed to resolve into two components which correlated with the R_F values

of the acidic and hydroxy acidic components of the standard mixture (Fig. 2; Table 6).

Chromatographic separation of the unsaponifiable fraction.

Silica gel (350g) was packed onto a glass column (74.0x4.0 cm o.d.) using hexane. A portion of the unsaponifiable fraction (10.0g) was dissolved at 40° in chloroform (40ml). The resulting solution was mixed with activated silica gel (30g) and the solvent removed in vacuo. The dried mixture of silica gel and the adsorbed sample was then introduced onto the packed column. Hexane was employed as the first eluant and fractions 1-12 (50ml each) were collected. Their compositions were monitored by TLC on silica gel using chloroform-benzene (7:3 v/v) as the eluant. Fractions 1-9 showed R_f values (0.89) consistent with the hydrocarbon class of compounds. These fractions were combined and the solvent distilled off to afford white, flaky, wax-like crystals (1.06g; 10.6%) m.p. 58-60°. IR(CCl_4) spectra showed bands at 2855(s), 1470(m), 720(m) characteristic of long aliphatic chains.

Fractions 10 to 12 showed no components on TLC and were discarded.

The second eluant employed was warm toluene. Fractions 13-30 (100ml each) were collected and their compositions also monitored by TLC on silica gel using chloroform-ethyl acetate (1:1 v/v) solvent mixture. Fractions 13-26 showed one component on TLC (R_f, 0.52) consistent with the monoalcohol class of compounds. However, fractions 27-30 showed two components on TLC (R_f, 0.52 and 0.18). These unseparated fractions were rechromatographed and the obtained fractions with R_f, 0.52 were combined with the corresponding fractions from the principal chromatography. These were pooled, concentrated in vacuo and air-dried to yield fine white crystals (6.76g; 67.6%) mp. 76-80°. IR(CCl_4) spectra exhibited absorption bands at 3340(s), and 1050(m) cm^{-1} attributable to hydroxyl group.

Final elution was effected using warm chloroform-ethyl acetate (3:2 v/v) solvent system and fractions 31-38 (100ml each) were collected. Their compositions were then analysed by TLC using chloroform-ethyl acetate (1:1 v/v) as the solvent system. Fractions 31-35 showed one component R_f, 0.18 consistent with the diol class of compounds. These fractions were combined with the toluene

fractions 27-30 which had the same R_f values. Fractions 36-38 gave no spots and were discarded. The combined fractions were evaporated to dryness to yield a white powder (2.18g; 21.8%) mp. 92-94^{o} IR(CCl_4) spectra gave absorption peaks at 3400-3350 (br,s) cm^{-1} and overlapping bands at 1120-1055(m) cm^{-1} due to hydroxyl group.

Chromatographic separation of the acid fraction.

A portion of the obtained acid fraction (5.0g) was dissolved in a minimum amount of chloroform and applied on a glass column (65.0x3.0cm o.d.), packed with silica gel (160g) using hexane. Fractions 1-11 (50ml each) were collected using 10% benzene in hexane as the eluting solvent system. Final elution was performed using chloroform and fractions 12-23 (50ml each) collected. Analytical TLC using hexane-ethyl acetate (1:3 v/v) as eluant gave R_f of 0.57 for fractions 1-8 and these were inferred to belong to fatty acids. Fractions 9 to 11 showed no product and were discarded. Fractions 1-8 were then combined and concentrated under reduced pressure to yield a white crystalline solid (3.24g; 64.8%) mp. 81-86^{o}. IR (CCl_4) spectra showed a strong, broad absorption band at 3375 cm^{-1} and a medium intense peak at 1720 cm^{-1}

due to carboxyl group.

Fractions 12-19 gave R_f 0.26 on TLC using the above solvent system and were inferred to consist of hydroxy acids. These fractions were combined and concentrated in vacuo to yield a white crystalline solid (1.43g; 28.6%) mp. 94-98°. IR (CCl_4) analysis indicated a broad strong peak at 3600 cm^{-1} and an intense peak at 1718 cm^{-1} attributed to the carboxyl group. Other broad peaks of medium intensity ranging from 1050-1100 cm^{-1} due to hydroxyl group were also observed.

Fraction 20-23 showed spots that were not eluted on TLC using the above conditions. These were combined and concentrated under reduced pressure to yield a brown gummy solid (0.31g; 6.2%) which was not characterised further.

A summary of column separations of acid and unsaponifiable fractions of the beeswax is presented in figure 1.

Gas chromatographic analysis of 1-triacontanol in alcohol fraction of saponified beeswax.

GC analysis of the alcohol fraction was carried out using a Pye Unicam - GCD chromatograph under the following conditions: a 4-mm i.d. glass column, 1.5m

long, packed with chromosorb W(80-100 mesh) coated with 5% silicone SE 30; injection temperature 250°; detector temperature 270°. The column temperature was programmed from 100 to 250° at a rate of 2 deg/min, with nitrogen as the carrier gas (flow rate, 30ml/min). A flame ionisation detector (FID) was used.

A standard mixture of eicosanol, docosanol, and 1-triacontanol was injected (1-4% solution in chloroform) to estimate the amounts of compounds in the alcohol fraction.

The alcohol fraction and the standard mixture were converted to their corresponding acetylated derivatives by the procedure of Purdy and Truter (1963) in order to compare the GC resolution efficiency with the underivatised ones. The derivatives prepared on a milligram scale where 0.2ml redistilled acetylchloride was added to 2mg of either the alcohol fraction or the standard mixture containing 0.067mg each of the three alcohols, and heated for 2hrs at 60°. The excess acetylchloride was evaporated off and the reaction mixtures chromatographed directly.

Qualitative evaluation and identification was done by comparing the retention volumes of the

alcohol fraction with those in the standard mixture (Fig. 3). Quantitative evaluation of the chromatograms was done by comparing the products of retention times and the corresponding peak heights without using correction factors. The areas of the ten resolved peaks (triangulation method) were first calculated and the percentage composition for each peak was then computed from their total areas (Table 8).

3.2 SYNTHESIS OF 1-TRIACONTANOL

Stearoylchloride (25)

Freshly distilled thionylchloride (2.56ml., 35.2mmol) was placed in a 3-necked 100 ml round-bottomed flask equipped with a stirring bar, a condenser and fitted with gas exit tube packed with fused calcium chloride. Using a dropping funnel, a solution of stearic acid (28) (5.0g; 17.6mmol) in 40 ml of dry dichloromethane was added dropwise at room temperature with stirring. The reaction mixture was stirred at room temperature until the evolution of hydrogen chloride gas ceased (ca. 20min.). The mixture was then refluxed gently for 3hr on an oil-bath maintained at a temperature of 35-40°. The solvent and excess thionyl chloride were completely removed by means of a water pump aspirator and the crude brown liquid product distilled under vacuo (bp 185°/2.5mm) to obtain pure stearoyl chloride (25) as a colourless liquid (5.6g, 83%).

IR (film) C=O 1820(s), C-Cl 950(m) cm^{-1}

^{1}H-NMR(CCl_4) δ0.95(t,3H,CH_3), 1.15-1.25(m, 30H,methylenes), 2.3(t,2H,$COCH_2$)

1-Cyclohexenyloxytrimethylsilane (76)

In a 100 ml, 3-necked round-bottomed flask fitted with a condenser, a nitrogen gas inlet and a magnetic stirrer, cyclohexanone (5.0g; 50.9mmol) was added as a rapid drip with stirring onto a solution of chlorotrimethylsilane (6.0g; 55.2mmol) and dry triethylamine (10.82g; 107mmol) in 20ml of dry dimethylformamide. The resulting mixture was refluxed with stirring under dry nitrogen for 4 hr. Some pale yellow solid (presumably triethylamine hydrochloride) was observed to separate out immediately and more separated out during the reaction process. The reaction mixture was then cooled to room temperature, diluted with 40ml of hexane and extracted thrice with 40ml portions of cold aqueous 5% sodium bicarbonate solution. The organic layer was combined with the hexane extracts from the aqueous washings and washed rapidly in succession with two, 20 ml portions of cold aqueous 1.5M hydrochloric acid and cold aqueous 5% sodium bicarbonate. The resulting hexane solution was dried over anhydrous magnesium sulphate and concentrated to leave a light yellow oil. This was vacuum distilled through a short vigreux column to give a forerun of the starting ketone bp 24-25^{o} (20mm) and the titled

product 76, bp 74-75°(20mm) as a clear viscous liquid (6.06g, 72%).

IR(film) S-CH_3 2940(s),1250(s), C=C 1670 cm^{-1}

^{1}H-NMR(CCl_4) δ0.13-0.25(s,9H,$(CH_3)_3SiO$),4.73 (m,1H, vinyl CH).

2-Stearoylcyclohexanone (31). Acylation of 76 with 25 under zinc(II) bromide catalysis.

A dry 100 ml round-bottomed flask equipped with a stirring bar was flushed with dry nitrogen gas and immersed in an ice-water bath. A solution of stearoylchloride (3.3ml; 10mmol) in 30ml of dry dichloromethane was introduced into the flask followed by 76 (1.68g; 10mmol) and anhydrous zinc(II) bromide (0.05g). The ice-water bath was removed and the reaction mixture stirred for 5 hrs at room temperature. The colour of the mixture changed from clear to dark brown. The progress of the reaction was monitored by TLC (hexane-chloroform; 5:1) until no spots attributable to the starting reagents were observed. The reaction mixture was extracted with water (4 x 10ml) and the organic layer dried over anhydrous magnesium sulphate and concentrated in vacuo to a small volume. This concentrate was subjected to flash chromatography on silica gel (120g) using hexane-

-chloroform (3:2) as eluant. Eluted first was a light yellow semi-solid which was decolourised with activated charcoal and recrystallised from hexane to afford white plate-like crystals of compound 31 (2.54g, 67%), mp 44-45^{o} (Lit. 46^{o}, Rao et al, 1980).

IR(KBr) broad O-H 3600(w), C=O 1710(m), 1610(m) cm^{-1}.

MS,z/e (relative intensity) 364 (M^{+},2)

Eluted next was a white solid which gave after recrystallisation from hexane white crystals (0.22g, 6%) mp 70-71^{o} with characteristics (TLC, IR, mmp) identical with that of the starting authentic stearic acid.

Alternative preparation of 31 by condensation of 76 with 25 in the presence of triethylamine in tetrahydrofuran.

To a stirred cold (0^{o}) solution of stearoyl chloride (5.0ml; 15mmol) and 76 (2.5g; 15mmol) in dry tetrahydrofuran (40 ml) placed in a 100 ml round-bottomed flask immersed in an ice-water bath and purged with nitrogen, was added anhydrous triethylamine (2.1ml; 15mmol) dropwise. After 1.5-2hr the mixture was allowed to warm to room temperature and the reaction left to stir overnight. The reaction

was monitored by TLC with dichloromethane - hexane (1:4) as the eluant, where the spot at R_f 0.7 due to 76 was gradually replaced by a new spot at R_f 0.47. The reaction mixture was then extracted with water (3x10 ml) and the organic layer dried over anhydrous magnesium sulphate. Evaporation of the solvent under reduced pressure gave 4.92g of a white solid product which was then treated with 15ml of dilute hydrochloric acid (2M) at room temperature with vigorous stirring for 1hr and the crude product extracted with dichloromethane (3x10 ml). The combined extracts were washed with aqueous 10% sodium bicarbonate (2x10 ml), dried over anhydrous magnesium sulphate and filtered off. Removal of the solvent, afforded a white solid weighing 4.78g. Chromatography of the crude product on a silica gel column (150g) eluted with dichloromethane - hexane (1:4) under medium pressure gave product 31 after pooling fractions of R_f 0.5. Recrystallisation of 31 from hexane provided white crystalline plates (4.16g, 62%) mp 45-47°. Compound, 31 thus prepared was shown by spectral (IR, ^{1}H-NMR), TLC and mixed mp comparisons to be identical to material obtained by the previously described zinc(II) bromide catalysis method.

Rearrangement of 2-stearoylcyclohexanone (31) to sodium 7-oxotetracosanoate (32).

In a 100ml round-bottomed flask equipped with a stirring bar and a condenser, 2-stearoylcyclohexanone (3.64g, 10mmol) was added to sodium hydroxide (0.8g, 20mmol) in absolute ethanol (60ml, 92.6%). The reaction mixture was refluxed with stirring until the sodium salt, was observed to precipitate out (2hr). The reaction contents were allowed to cool to room temperature and the precipitated sodium salt was collected on a 5-cm Büchner funnel and pressed as dry as possible. The moist salt was suspended in absolute ethanol (30ml) with stirring for 30 minutes, collected on the Büchner funnel as before and washed with acetone followed by water. Final vacuum-drying yielded the sodium salt 32 (2.80g; 69%) mp 121-123°.

IR(KBr) C=O 1680(m), $C(\overset{\ominus}{---}O)_2^{\ominus}$ 1600 cm^{-1}.

Preparation of tetracosanoic acid (33).

Powdered potassium hydroxide (0.56g; 10mmol) in ethylene glycol (10ml) was placed in a 50 ml round-bottomed flask fitted with a condenser and equipped with a magnetic bar. The mixture was refluxed until it dissolved (40 min.) and the resulting solution cooled to 80-100°. The sodium salt 32

(2.02g; 5mmol) and hydrazine hydrate (6ml; 99%) were then introduced to this solution. The contents were warmed gently (the reaction being strongly exothermic) and then refluxed for 2hr. The water formed during the reaction and excess hydrazine hydrate were distilled off; refluxing of the mixture was continued for another 2hr at 190-200^{o} at which considerable foaming that occurred was contained by occasional addition of a few drops of aqueous silicone anti-foaming agent. The reaction mixture was cooled to 100-110^{o}, poured into water (20ml), acidified with hydrochloric acid (6M) to Congo red and filtered off. The white solid residue obtained was air-dried and crystallised from acetic acid to afford white crystalline plates of tetracosanoic acid (33) (1.54g, 84%) mp 86-87^{o} (Lit. mp 87-88^{o}, Steicher *et al*, 1968) which was found to have characteristics (TLC, IR, ^{1}H-NMR, mmp) identical to that of an authentic sample (Aldrich Chemical Co.)

IR(KBr) broad OH 3400(m), C=O 1710(m) cm^{-1}

^{1}H-NMR (CCl_4) δ0.8(t,3H,CH_3), 1.25(m,42H, methylenes) 2.2(t,2H,$COCH_2$),12.2(s,1H,OH)

Conversion of tetracosanoic acid (33) to tetracosanoyl chloride (34).

The acid chloride 34 was prepared in a manner analogous to the procedure outlined above for the preparation of stearoyl chloride. In a 3-necked 100ml round-bottomed flask bearing a magnetic stirrer and reflux condenser, was placed freshly distilled thionylchloride (2.8ml; 37.8mmol) and a gas exit tube packed with fused calcium chloride fitted onto the condenser. By use of a dropping funnel, a solution of tetracosanoic acid (7.0g; 18.9mmol) in 50ml of dry dichloromethane was added dropwise at room temperature with stirring. Stirring was continued until the evolution of hydrogen chloride gas ceased (ca. 30 min.). The mixture was then gently refluxed on an oil-bath for 5hr, maintaining a temperature of 35-40°. The solvent and excess thionyl chloride were completely removed in vacuo to leave a crude white solid of tetracosanoyl chloride (6.35g, 91%), mp 116-118°. TLC showed only trace impurities and compound 34 was used as such without further purification in the subsequent transformation.

TLC R_f, 0.44 (hexane-chloroform; 4:1)

IR(CCl_4) C=O 1785(s), C-Cl 940(m) cm^{-1}

^{1}H-NMR(CCl_4) δ0.8-0.9 (t,3H,CH_3),
1.1-1.3(m,42H,methylenes),2.2(t,2H,$COCH_2$)

Preparation of 2-tetracosanoylcyclohexanone (36) by acylation of 76 with 34 under zinc(II) bromide catalysis.

In a dry 100ml round-bottomed flask fitted with a mechanical stirrer and immersed in an ice-water bath was placed tetracosanoyl chloride (5.0g; 12.9mmol) in 50ml of dry chloroform and 76 (2.2g; 12.9mmol). Anhydrous zinc(II) bromide (0.05g) was then added. The reaction mixture was allowed to warm to room temperature with stirring under nitrogen atmosphere where it was observed to change colour from clear to dark brown. Stirring was continued at this temperature for 8hr. Examination of the reaction by TLC (hexane-chloroform, 4:1) after 8hr indicated the absence of starting materials and the presence of a new product, R_f 0.52. The reaction mixture was washed with water (4x10ml) and the resulting organic layer dried over anhydrous magnesium sulphate and taken to dryness in vacuo. The brown oily residue obtained was subjected to chromatography on a column of silica gel (120g)

and eluted with hexane - chloroform (5:1). Appropriate fractions were combined and the solvent removed in vacuo to yield a white solid product. Recrystallisation from hexane followed by rapid cooling in an ice-water bath and scratching the sides of the flask with a glass rod gave 36 as white crystalline plates (3.71g; mp 50-52°C (Lit. 48-50°C, Ries and Wert, 1977).

Silica gel TLC R_f, 0.52 in hexane-chloroform (4:1);

IR(KBr) broad O-H 3540(w), C=O 1780(m), 1720(m) cm^{-1};

Ms, z/e (relative intensity) 448 [M^+], 431(4), 208(15), 140(77), 125(61), 43(100).

Alternative preparation of 36 by condensation of 76 with 34 in the presence of triethylamine in tetrahydrofuran.

An oven-dried, 50 ml round-bottomed flask equipped with a stirring bar was cooled in an ice-water bath under a slow stream of nitrogen. The flask was charged with a solution of tetracosanoyl chloride (2.0g; 5.2mmol) in 20 ml of dry tetrahydrofuran followed by 76 (0.88g; 5.2mmol). Anhydrous triethylamine (0.70ml; 5.2mmol) was then added drop-wise onto the reaction mixture. A white

precipitate was observed to form during this addition. The ice-bath was removed and the resulting mixture stirred for 24hr at room temperature at the end of which it was extracted with 10ml of water. The organic layer was dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure to give 1.48g of a white solid product. This was subsequently treated with 5ml of dilute hydrochloric acid (2M) with vigorous stirring at room temperature for 1hr and the crude product extracted with dichloromethane (2x10ml). The combined organic extract was washed with 10ml of 10% aqueous sodium bicarbonate and dried over anhydrous magnesium sulphate. Filtration of the mixture followed by removal of the solvent under reduced pressure gave 1.42g of crude product 36. Chromatography of the crude material on silica gel (50g) and elution with hexane - chloroform (5:1) gave traces of a pale brown tarry solid as the first fraction. Further elution yielded a second fraction containing product 36 which was recrystallised from hexane to give white crystalline plates (1.27g; 55%), mp 50-52^{o}. Product 36 was shown by TLC behaviour, mmp, ^{1}H-NMR and IR spectra to be identical to material previously prepared using zinc(II) bromide catalysis.

Generation of sodium 7-oxotriacontanoate(37) by rearrangement of 36 with sodium hydroxide in absolute ethanol.

A mixture of sodium hydroxide (0.45g; 11.16mmol) and 20ml of absolute ethanol was refluxed with mechanical stirring in a 50ml round-bottomed flask until all the sodium hydroxide dissolved (ca.10 min .). The solution was cooled to room temperature and a warm solution of 36 (2.5g; 5.58mmol) in 10ml of ethanol added. The mixture was brought to reflux on an oil-bath and heating continued for 2hr during which a white precipitate of the sodium salt was observed to separate out. The reaction mixture, now a thick white mush, was cooled to room temperature and the salt collected on a Büchner funnel and pressed as dry as possible. The moist salt was then suspended in 10ml of absolute ethanol with mechanical stirring, collected on the Büchner funnel as before and finally washed with a mixture of acetone and water to give on vacuum-drying the sodium salt 37 (1.87g, 66%), mp 149-151°.

IR(KBr) C=O 1670(m), $C(\overset{\ominus}{=\!=}O)_2^{\ominus}$ 1570(s) cm^{-1}.

Preparation of 1-triacontanoic acid (20) by keto-reduction of 37

A 100ml round-bottomed flask equipped with a reflux condenser and a magnetic stirrer was charged with powdered potassium hydroxide (0.34g; 6.14mmol) and 10ml of triethylene glycol. The reaction mixture was heated under reflux with stirring until it dissolved (ca 10 min.) and the solution cooled to 80-100°. Hydrazine hydrate (4.0ml, 99%) and the sodium salt 37 (1.5g; 3.07mmol) in 20ml of triethylene glycol were then introduced cautiously but rapidly to the stirred reaction mixture and refluxing continued for 2hrs. The water formed during the reaction and excess hydrazine hydrate were distilled off and the temperature allowed to rise rapidly to 200-210° at which further refluxing was continued for additional 4hrs. Foaming occuring during the reaction was suppressed by occasional addition of a few drops of silicone antifoaming agent. The reaction mixture was cooled to about 100°, diluted with 30ml of hot water and acidified to a pH of 2-3 with hydrochloric acid (6M). The acid 20 was extracted with diethyl ether (3x10ml), dried over anhydrous sodium sulphate and evaporated in vacuo.

The crude acid obtained was then chromatographed over silica gel (50g) using a mixture of hexane - chloroform (1:3) to give the product as a white solid which was recrystallised from hexane - diethyl ether to yield white crystalline plates (1.10g; 79%) of 20, mp 87-89^{o} (Lit. 91-93^{o}, Heibron, 1965).

IR(KBr) broad O-H 3350-2500(m), C=O 1670(m), C-O 1280(m) cm^{-1}

^{1}H-NMR(CCl_4) δ0.8(t,3H,CH_3), 1.25(m,54H,methylenes), 2.2(t,2H,$COCH_2$), 12.5(s,1H,OH),

MS, z/e(relative intensity), 452(M^+,26), 129(22), 73(48), 43(100).

Methylation of 1-triacontanoic acid.

In a 50ml round-bottomed flask equipped with a reflux condenser and a mechanical stirrer was placed 1-triacontanoic acid (0.8g; 1.77mmol) and 20ml of dry methanol. The contents were then heated gently on an oil-bath with stirring until all the acid dissolved. A drop of concentrated sulphuric acid (0.1ml) was added cautiously and the whole was refluxed for 2hr . The mixture was cooled to room temperature, 10ml of water added and the organic phase extracted with diethyl ether (2x10ml). The

combined ethereal extract was washed successively with water (10ml), 5% aqueous sodium bicarbonate (10ml) and saturated brime (5ml). The extract was then dried (anhydrous sodium sulphate), filtered and concentrated under reduced pressure to leave a white solid which was recrystallised from absolute ethanol to give 40 (0.68g; 86%) as a white crystalline solid, mp 69-71°.

IR(KBr) C=O 1740(s), C-O 1240(s), 1050(m) cm^{-1}

^{1}H-NMR(CCl_4) δ0.8-0.9(t,3H,CH_3), 1.0-1.7(m, 54H,methylenes), 2.3(t,2H,$COCH_2$), 4.1(s,3H,OCH_3).

Preparation of 1-triacontanol (1) by reduction of methyl triacontanoate (40) with lithium aluminium hydride.

A suspension of lithium aluminium hydride (0.02g; 0.55mmol, BDH chemical Co.) in 15ml of dry tetrahydrofuran was placed in a 50ml three-necked round-bottomed flask equipped with a reflux condenser, dropping funnel, a magnetic bar and protected from moisture until completion of the reaction by fused calcium chloride tubes attached to the openings. The whole was kept at -10° by immersing the flask in salted ice-bath. To the stirred suspension, a

solution of methyltriacontanoate (40) in 10ml of dry tetrahydrofuran was added dropwise through a dropping funnel. The temperature was then allowed to rise to room temperature over a period of 1hr and the mixture left stirring at this temperature for 4hr . The excess hydride was decomposed by successive drop-wise addition of water (1.0ml), 15% aqueous sodium hydroxide (1.0ml) and finally water (3ml). After vigorous stirring for about 15 minutes, the mixture was filtered under suction and the fine precipitate taken up in tetrahydrofuran (10ml). The tetrahydrofuran solution was dried (anhydrous sodium sulphate), filtered and the solvent removed in *vacuo* to leave a white solid residue which on crystallisation from hexane, furnished white crystalline plates of 1-triacontanol (0.46g; 92%), mp 85-87^{o} (Lit. 87-88^{o}, Steicher *et al*, 1968)

IR(KBr) broad O-H 3450-3100(w), C-O 1450(w), 1060(w) cm^{-1}

^{1}H-NMR(CCl_4) δ0.9(t,3H,CH_3), 1.25(m,56H, methylenes), 3.65(m,2H,CH_2O).

MS, z/e (relative intensity), 420($M^{+}-H_2O$), 125(9), 97(41), 57(100), 43(91).

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A P P E N D I X

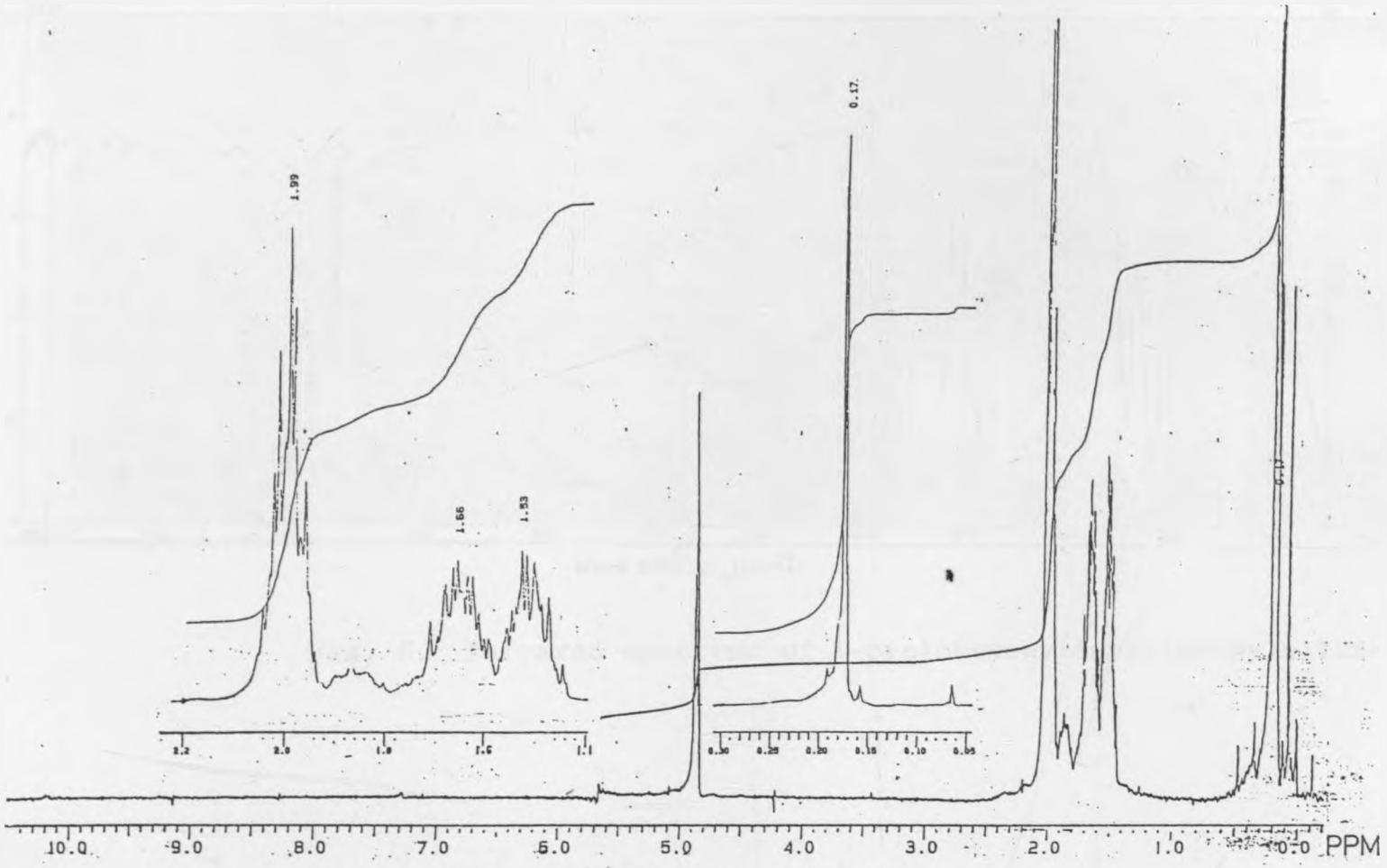

Fig. 4. 'H-nmr spectrum of 1-cyclohexenyloxytrimethylsilane

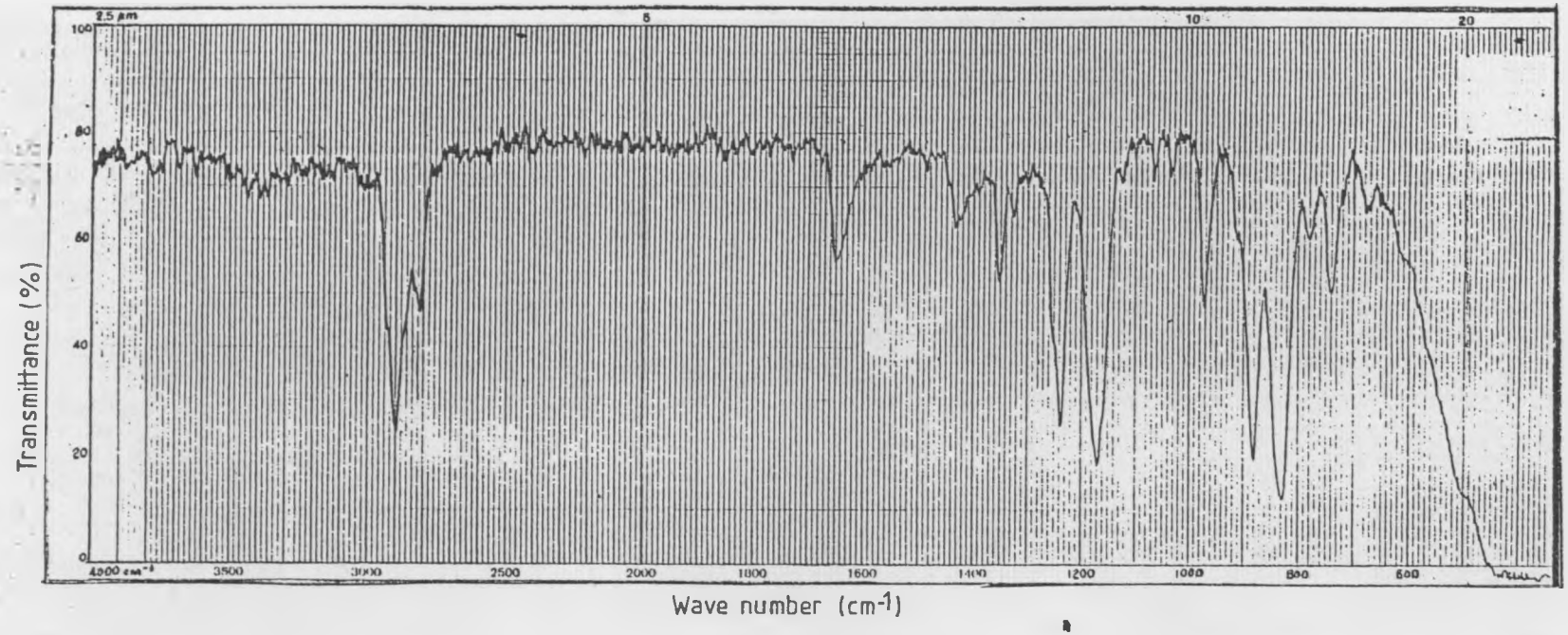

Fig. 5. Infrared spectrum of 1-cyclohexenyloxytrimethylsilane

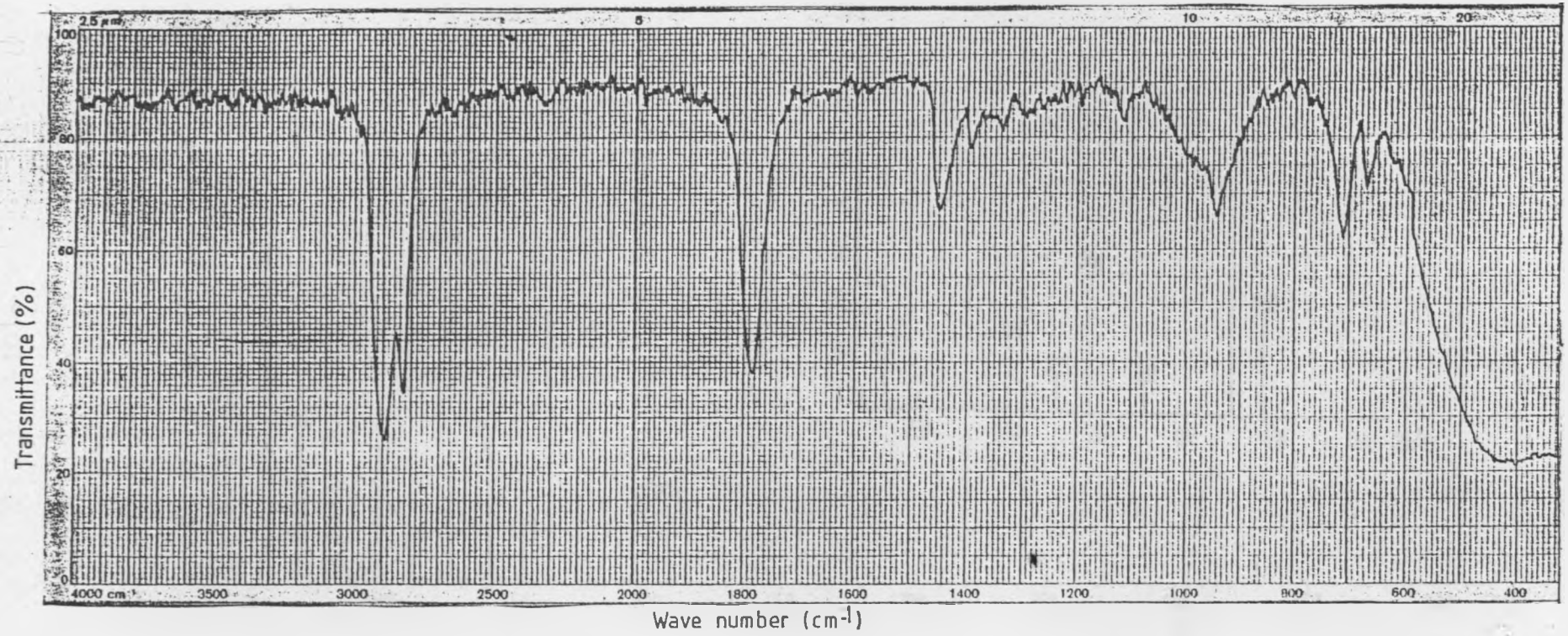

Fig. 6. Infrared spectrum of tetracosanoyl chloride

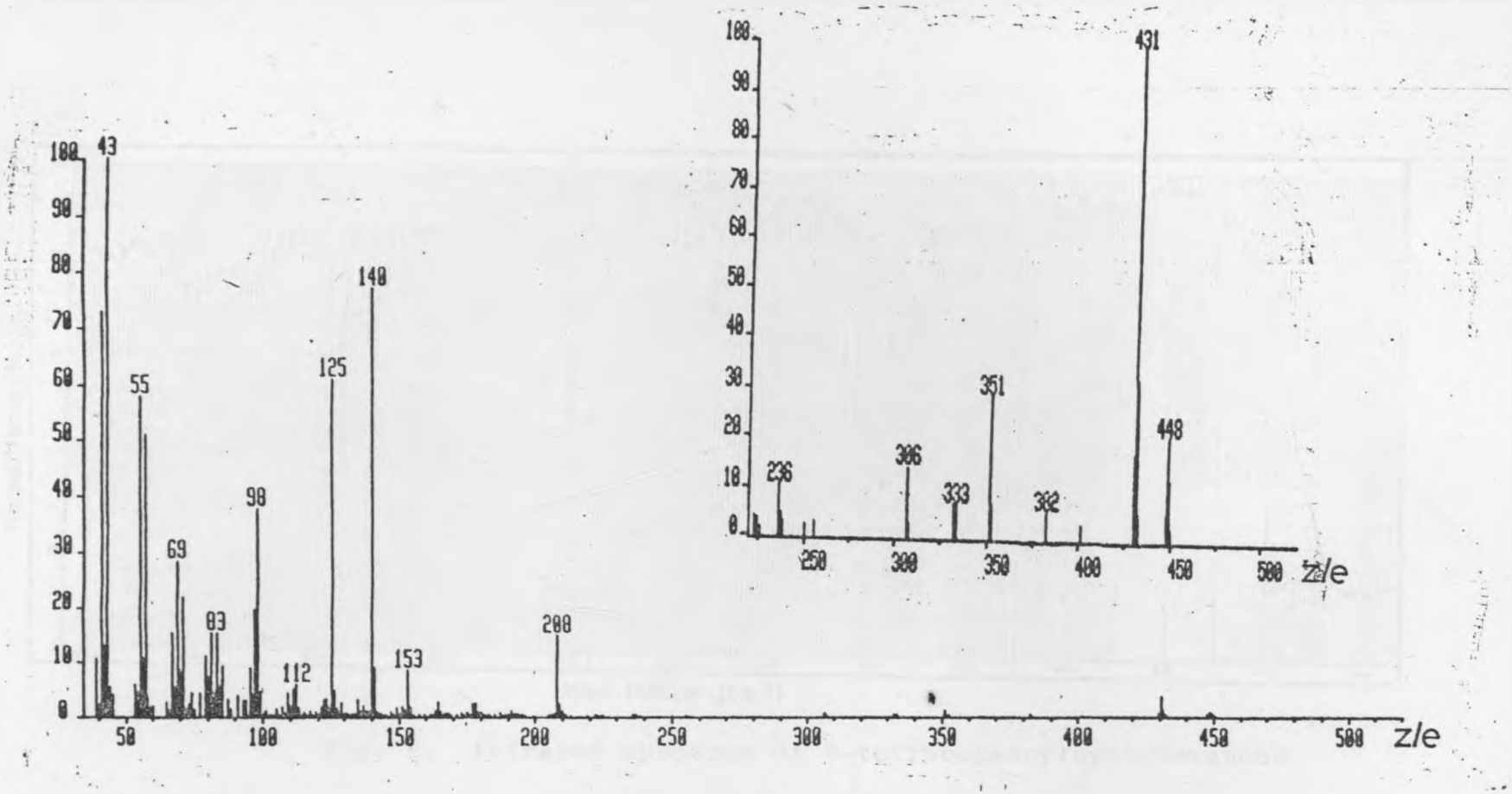

Fig. 7. Mass spectrum of 2-tetracosanoylcyclohexanone

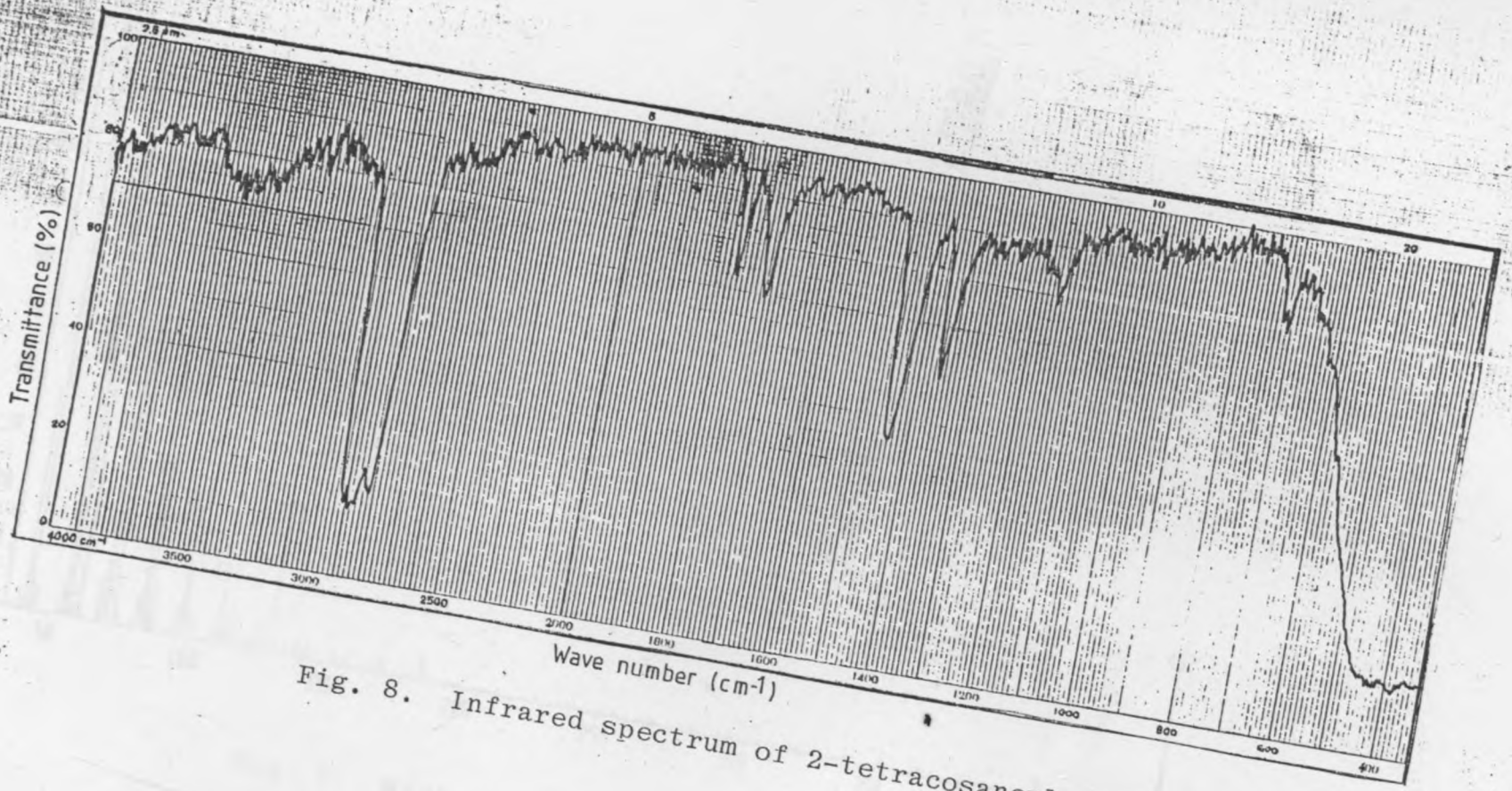

Fig. 8. Infrared spectrum of 2-tetracosanoylcyclohexanone

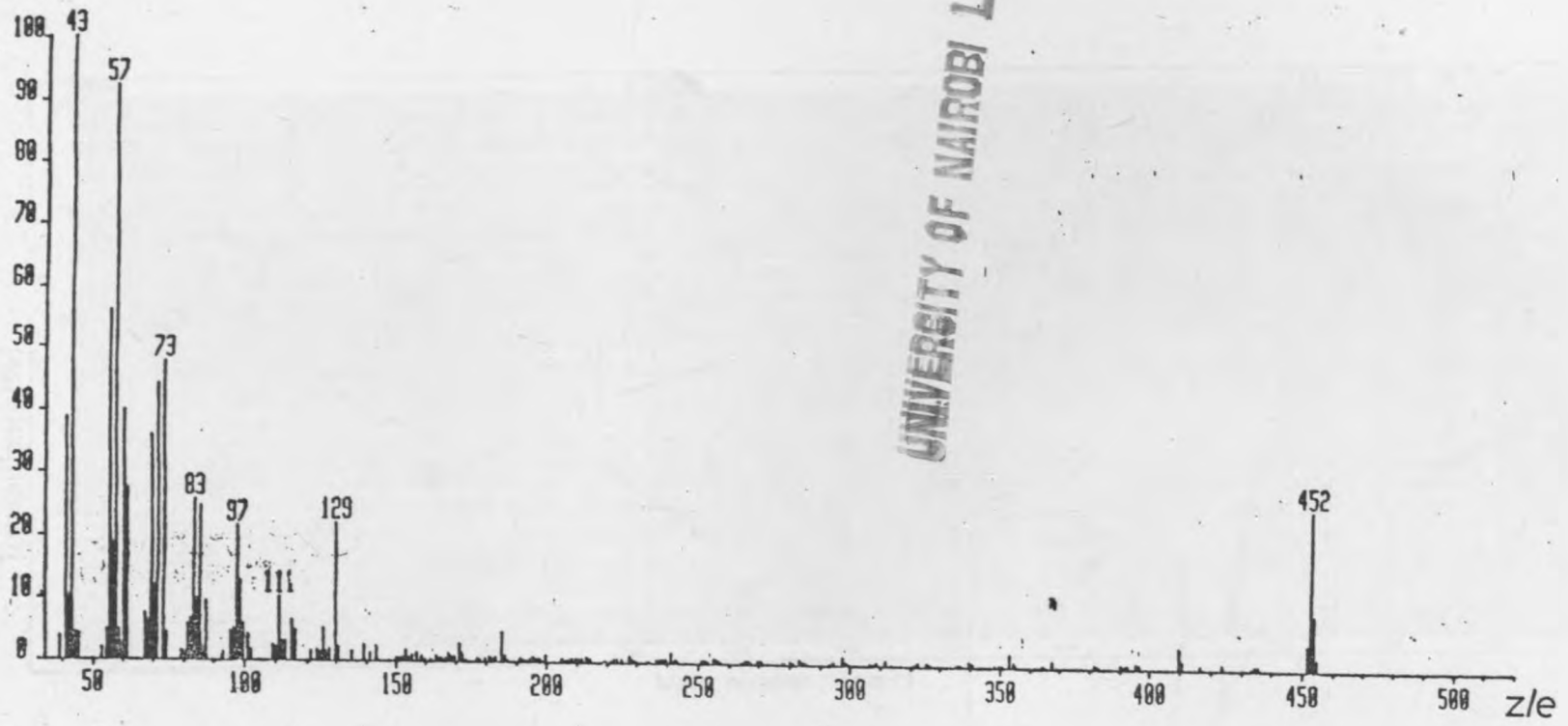

Fig. 9. Mass spectrum of 1-triacontanoic acid

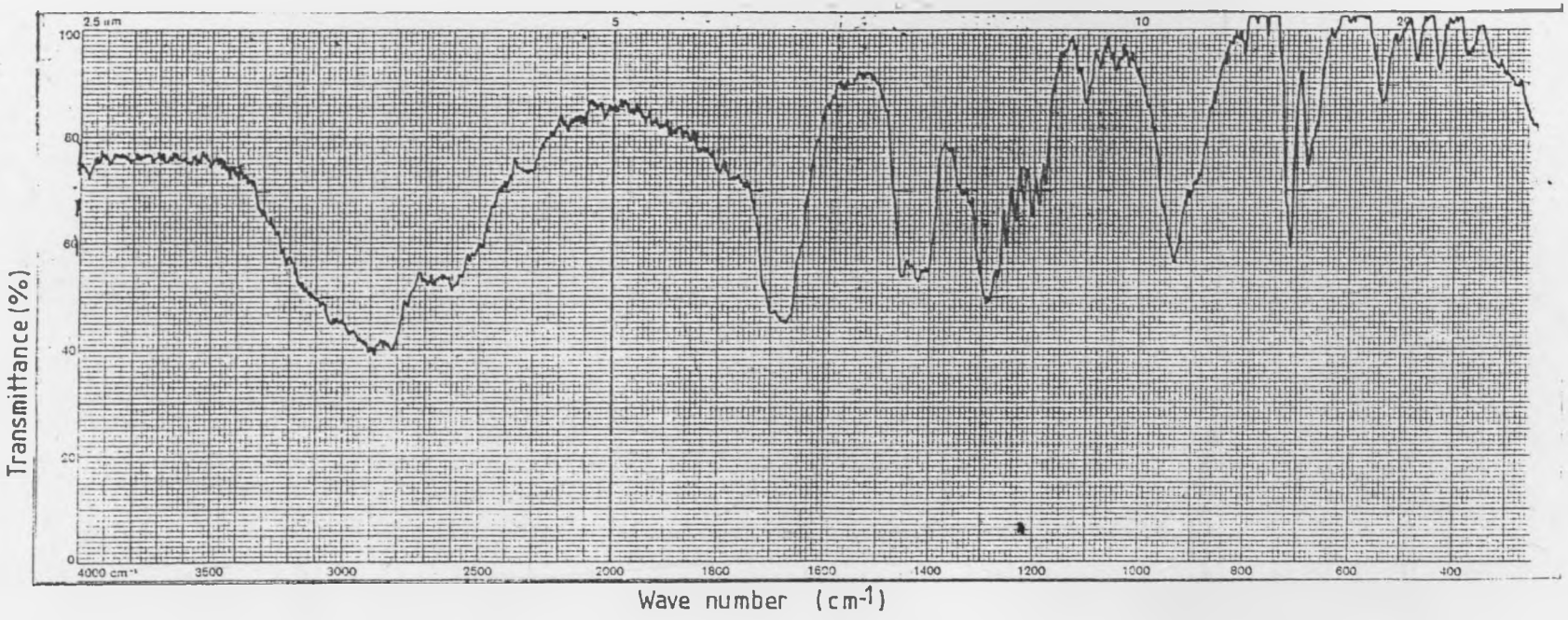

Fig. 10. Infrared spectrum of 1-triacontanoic acid

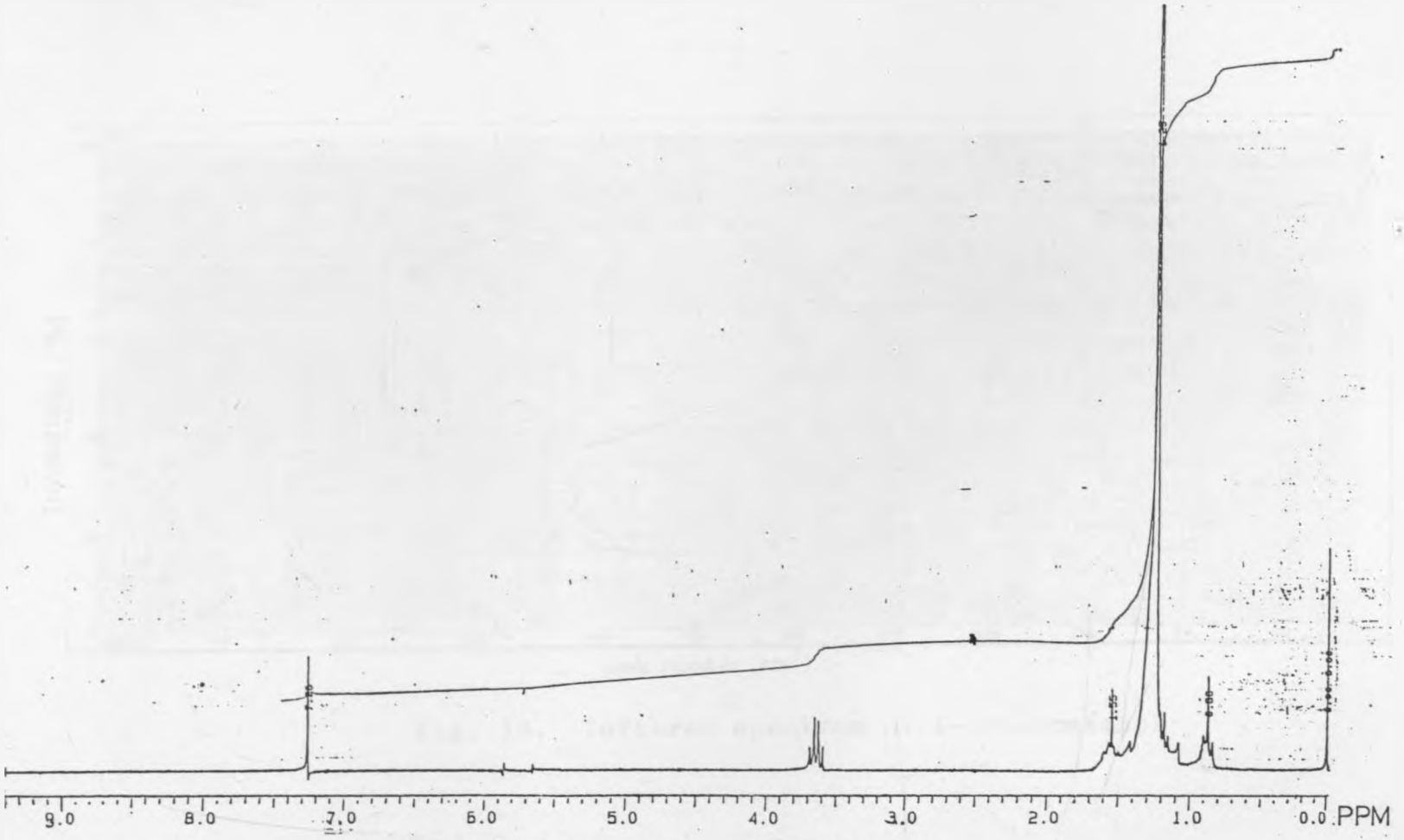

Fig. 11. 'H-nmr spectrum of 1-triacontanol

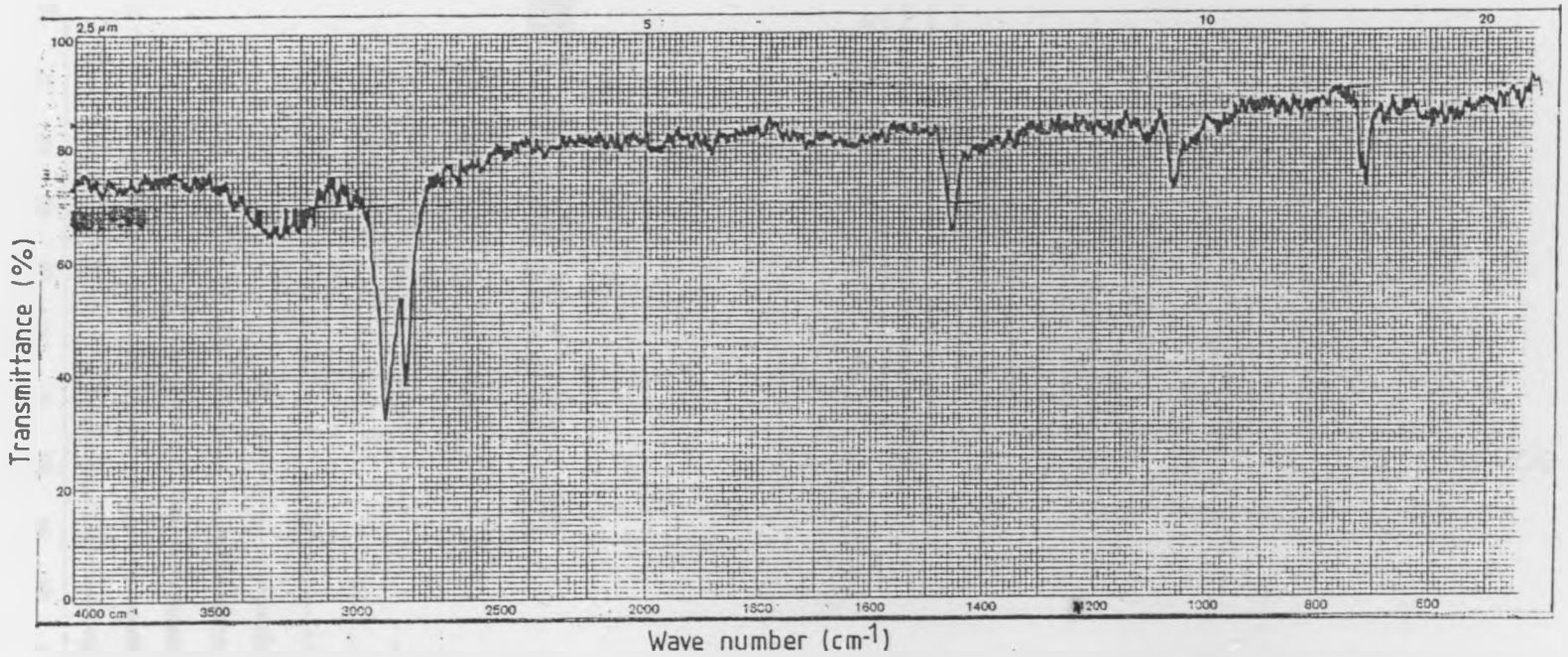

Fig. 12. Infrared spectrum of 1-triacontanol

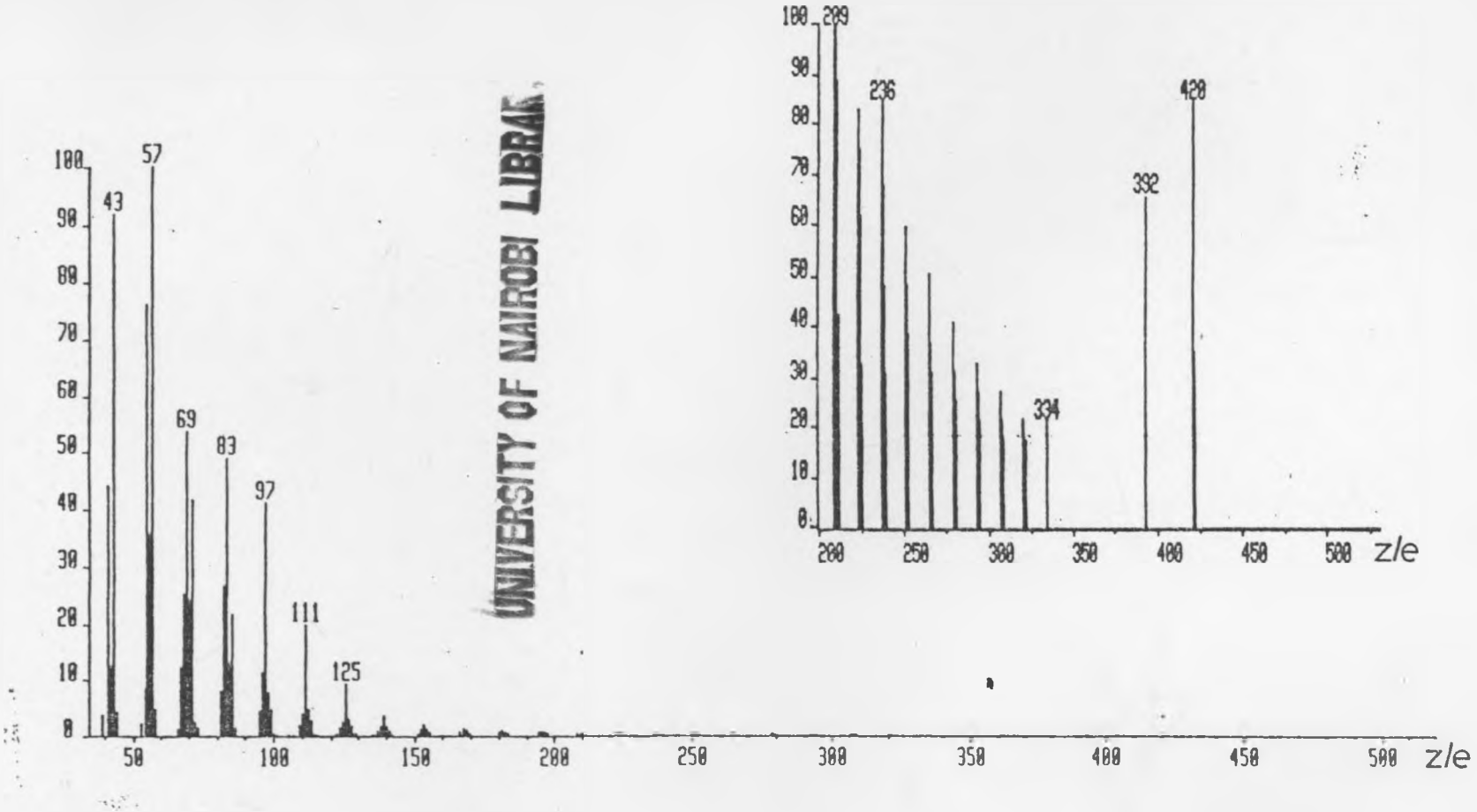

Fig. 13. Mass spectrum of 1-triacontanol