CHARACTERIZATION OF THE ESSENTIAL OIL OF *THUJA OCCIDENTALIS L.* LEAVES AND BRANCHLETS

by

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ABSTRACT

The essential oil of *Thuja occidentalis* L. was isolated by steam distillation and chemically fractionated based on the presence or absence of ketone, aldehyde or carbonyl groups in the constituents. Leaf and branchlet samples of *Thuja* were collected from a site near Portland in Eastern Ontario. Monthly collections were made from October 1997 to September 1998.

Monthly variation in yield of oil and water content of the leaves and branchlets were measured. Yield of oil, based on fresh weight, ranged from a low of 0.59% (July 1998) to a high of 1.06% (Dec. 1997). In the summer months yield was approximately 30% lower than in the winter months. The oil was fractionated and analysed by gas chromatography and gas chromatography/mass spectroscopy to determine the chemical components.

The main ketone components of the oil were thujone and fenchone.
Unidentified minor malodorous constituents were concentrated in the aldehyde fraction.

The results suggest that biomass for oil extraction should be harvested during the winter months. Results also indicated that the Sukh-Dev fractionation method, based on the use of semicarbazide on silica gel, is effective in recovering both ketone and aldehyde components of the oil from the crude steam distillate.

Dedicated to the **Veras**

My mother and my mother-in-law

Acknowledgement

I want to thank John Balatinecz for his confidence in allowing me the opportunity to attempt an area of study with no background training. Dr. Balatinecz provided contacts, ideas and challenges to new possibilities to come up with this project. I had to work hard to establish the necessary foundations to continue on. In so doing I entered into the marvellous minute world of plant cells in full beauty. Now I see a tree not just for its simple aesthetic attractiveness but with extra images of tracheids, lignins, torus, pits, making this world so much more interesting. This project has also given me an understanding of chemistry, that strange world of complex molecules, ions and scribbled 'cartoon' symbols which I had no opportunity to study previously because of political upheavals in my youth. The new added knowledge of chemistry has stopped me from asking my husband "is this piece of paper any use or can I ditched it?" I can now share his interest in a more enjoyable way.

Thanks to my mother in-law Vera Slemon and to Mrs Betty Bond of Portland for their unfailing dependency in insuring that fresh cedar foliage was picked, packed and delivered to the bus parcel promptly to preserve the freshness. Thanks to Dr. Jan Oedenes, President of Torcan Chemical, for making it possible to work in his well equipped laboratory and sparing nothing to get my experiments done. A very special thanks to Bob Macel for spending his valuable time making repeat checks on the stability of the GC/MS machine before running my samples and in obliging me with constant requests of extra runs to test various hypotheses.

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Tarcisia (Tish) Khomasurya

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1.0 INTRODUCTION

Watching my brother and friend launch a new line of cosmetics (Larome) in Australia and Asia with perfume as the major product and helping in selection of new fragrances desirable for the Asian market introduced me to the world of perfumery. Coming from Indonesia, I grew up with eucalyptus oil, as a cure for various ailments and a recent visit to grasse perfumery in France demystified the complexity of the essential oil industry. In Ontario, cedar grows in wetland and way sides and in the little towns in the east of the province, the locals tell stories of the winter harvest. The old cedar chest for the storage of furs is true Canadiana.

Essential oil of cedar is a major oil of commerce in North America with a known content of 60% thujone. There is no mention in the literature searched to show that any attempt has been made to get higher content of thujone either by concentrating the components or by a different method of collection.

In this thesis, essential oil of *Thuja occidentalis* L., based on steam distillation of the leaves and branchlets, is **fractionated** into **ketonic**, **aldehydic,** non-ketonic and non-aldehydic fractions. A trace amount of an unidentified malodorous principal is

concentrated from the aldehydic fraction. The **monthly variation** in the yield of the oil is also examined.

It is hoped that the results of such studies will lead towards an optimised composition of the oil, perhaps with higher ketone assay and even better aroma and enhanced stability. Such improved oil may result in new and more intensive uses, necessary for further commercial exploration for isolation and recovery.

2.0 REVIEW OF THE LITERATURE OF ESSENTIAL OILS

2.1 Historical Overview

A simple, though incomplete definition of essential oil is the predominantly volatile material possessing odour and other characteristic properties of the plant which is isolated by some physical process. There are, however, several classes of structural product oils such as non-volatile or fixed oils, including fatty oils (e.g. corn or sesame seed oils) which are completely different in composition from the essential oils since they contain mainly glycerides (fatty acid esters of glycerol). The most important and well known oil type on earth is petroleum which is a product derived from prehistoric forests. Petroleum and its related products are composed of liquid and tarry-solid hydrocarbons.

The ethereal or volatile oils from vegetable materials were named essential oils by Paracelsus and other alchemists. They were thought to be the bases which include the total odour and flavour of each vegetable substance. Essential oils are composed of a wide variety of natural organic compounds and contain numerous functional groups and

molecular frameworks. The molecular weights of these components are mostly confined to the lower ranges because they are at least partially volatile.

Over 3000 oils have been identified from the vast number of plant species and several hundred have commercial uses (Olhmer, 1981). Some are extremely rare and produced in only kilogram quantities, e.g. violet oil, concretes (flower extracts), and angelica root oil. Several other botanical products are designated as oils, but do not completely follow the definition. The so-called concretes are extraction of flowers with a non-polar solvent. They contain the essential oil and some waxy or fatty material as well. By re-extraction with a polar solvent, the concrete is dewaxed or defatted giving an absolute oil that has improved solubility, higher odour and flavour intensity and often a better colour.

Other products related to essential oils are balsam, essences, extracts, fixatives, flower waters, gums, oleoresins, pomades, resinoids, resins, tinctures, infusions, and spices.

Long ago, essential oils were obtained by primitive methods from spices and gums. In the Indus valley, discovery of terra-cotta retorts together with fragrance pots (flacons) were reported to date from the 30th century BC (Rovesti 1977). The method of collection of the volatiles were no doubt very inefficient. The ancient Persians were aware of distillation but whether it was used in perfumery is not known (Guenther1947-1952). Paintings in Egyptian tombs show statues and bodies being anointed with fragrant oils. The Egyptians used not only cumin, marjoram, mint, rose, and myrtle which were secured locally but also cassia, cinnamon, and anise which could only have come from China.

The Egyptians pressed fragrant herbs with olive, castor and palm oils or macerated the herbs with hot oils to extract aroma into the oil. The Greeks and Romans imitated the Egyptians but up to that time no unadulterated essential oil was known to be produced.

Oil of turpentine seems to have been the first essential oil produced. It was mentioned first by Herodotus (450 BC) and also by Pliny and Dioscorides, but the production method was obscured. Pliny described ointment as 'body and juice'. The animal fats or bodies used in ointment were purified by repeated washing with sweet wine (water-ethyl alcohol) until the disagreeable odours were removed. Commercialisation of essential oils in this period is not clear though history recorded that the Romans chewed myrrh or mastic or gum, used mouthwash, and perfume pastilles. But it is the Arabs who were credited with the invention of distillation and condensation in the 9th and 10th centuries AD. Dry distillation of gums and herbs was practised earlier but until the 11th century the main fragrance products were fatty oils and pomades. This technology revolutionised the removing essences from natural products. Tradition, not documentation, credits Avicenna as the first to distil a flower water from rose petals. The Arabs were the first to distil alcohol which proved to be an excellent solvent for the quintessence of herbs and botanicals and for making tinctures for flavour, fragrance, and therapeutic application.

The first mention of authentic essential or volatile oils appears in the late 13th century by Arnold de Villanova who introduced therapeutic waters and perhaps several essential oils for medical uses. Therapeutic waters are apparently the aqueous phase and the water soluble portion of the steam distilled oil. In the early 16th century, a treatise was

published in Strasbourg describing distillation in general and the production of oil from Juniperus communis L. ; turpentine, rosemary, spike and various resins. In 1551, a compendium of methodologies called the Krauterbuch was published. This date is regarded as a turning point in the understanding of the nature and importance of essential oils. Up until the 18th century, pharmacists manufactured and dispensed essential oils because since ancient time greatest emphasis had been on the therapeutic effects of these spices and herbs derived products. Even today drug stores are major outlets for perfumes and cosmetics. The technology of essential oils has progressed to today's advanced methods, with the application of modern science. At first and even in recent times, essential oils were usually manufactured close to the growing area, in undeveloped places and shipped to technologically advanced countries for isolation of the oil. More often than not, this resulted in poor quality, because essential oils are easily adulterated and this was often irresistible temptation to local producers. Nowadays, quite a few oils are still produced under very primitive conditions. To keep pace with modern agricultural methods, hybrid plants are grown and new essential oils are isolated and evaluated every year though not many are fully commercialised. New growing areas for specific oils are opened up all the time which can offer economic or quality advantages such as soil conditions, irrigation, and the availability of labour. Today essential-oil crops are grown in areas which may still be remote from the processors' location.

2.2 Origin of essential oils in plants

The popularity of spiced food has led in recent years to increase in consumption of spices and essential oils. Black pepper, ginger and cloves for example are grown in Indonesia.

More and more temperate zone spices are being grown in the United States, such as basil, parsley, thyme, mustard seed, tarragon and marjoram. Most of these are processed for dry spices, only dill is converted to an essential oil. Essential oils are isolated from various parts of the plant, such as leaves (patchouli, pines, cedar), fruits (mandarin), bark (cinnamon), root (ginger), grass (citronella), gum (myrrh & balsam oils), berries (pimenta), seed (caraway), flowers (rose) twigs (clove stem), buds (cloves), wood (amyris), heartwood(cedar),and saw dust(cedar oil). These plants are processed to yield their quintessence or essential oils by separation from cellulose, glycerides, starches, sugars, tannins, salts, and minerals in the botanicals. The most widespread physical method for isolating essential oils from the botanical is co-distillation with steam. The details of the technology will be expanded upon in a later section.

A small group of products are exception to the simple definition of an essential oil. Garlic oil, mustard oil, sweet birch oil and the like require enzymatic release of the volatile components before they can be freed from the residual biomass by steam distillation. There are other flower oils or resinoids obtained by extraction which contain only a small portion of volatile oil, but nonetheless are called essential oils. There are also several oils coming via dry-distillation which also contain only a limited amount of volatiles, but which nonetheless fall within the designation of essential oil, e.g. oil *Labdanum*, oil balsam Peru.

Getting the odorous principal from the botanical is called 'expressing' the essential oil. In the case of the citrus oils, this in fact does involve 'pressing' as implied in the verb

itself. Formerly produced by tedious hand-pressing or, sponge-pressing, they are now produced by modern high speed, multifunctional machines. Many flower oils are extracted with a purified petroleum solvent. Enfleurage, an old process in which delicate flower petals were physically stuck onto a purified fat, is no longer in common use. Maceration as a process is used frequently today.

The yield of essential oils from botanicals varies widely. Nutmegs yield 10-12 wt % of oil, whereas onions yield less the 0.1% after enzymatic treatment. Thuja wood oil yield is typically between 0.6 -1.0% wet material or 0.9-1.3% dry material (Collin 1993). Essential oils are typically liquid at room temperature. Semi-solid *Mentha arvensis* (Brazilian mint) and oil of guaiac wood are examples of solids under ambient conditions.

2.3 Purification of essential oils

Rectified oils are re-distilled essential oils, processed to improve a particular property or characteristic. Thus, an objectionable flavour or fragrance note may be removed, e.g. natural oil of peppermint is rectified (~2-3% forerun taken) to remove dimethyl sulfide, a green weedy note with high impact, important to chewing gum and mouthwash but objectionable in creme de menthe liqueurs. Colour may be removed e.g. cassia by vacuum steam distillation, leaving a residue of coloured high boiling components. A desirable component my be increased by rectification e.g. eucalyptus oil, which contain ~85% cineole. The oil is rectified by removal of forerun and residue to produce a eucalyptus oil consisting of 95% cineole. Concentrated or folded oils are processed by various physical methods to remove wholly or partly undesirable or non-flavour

components such as terpenes or sesquiterpenes, which have poor solubility, very low flavour value and poor stability. The processing methods include fractional distillation, solvent extraction, counter-current extraction, thin-film evaporation and molecular distillation. In some cases both distillation and solvent extraction are needed for complete removal of terpenes. When half of the volatile constituents of the oil are removed, their removal is said to double the concentration and the oil is then called twofold. In the past, distinction was made between terpeneless and sesquiterpeneless oils, but this distinction has now been abandoned since it is practically only fractional distillation that can remove mono-terpenes without removing the sesquiterpenes at the same time.

Aroma chemicals are isolates or chemically treated oils or components of oils. Some components are removed physically, and others chemically. Several hundred essential oils have commercial importance. Forty two specific oils are on the US Department of Commerce import list. Among the 295 plant families, 87 produce essential oils and only 17 of these, like *Thuja* oil, grow in temperate climates (McNair 1922).

2.4 Uses of essential oils

Essential oils are used for flavour and fragrances. Essential oils are concentrated, rectified, extracted, or chemically treated. This is to further isolate vital components, or to purify, or to adjust the properties, or to increase the concentration for significant flavour or fragrance components. The oil of *Thuja*, is used for decongestant, cough suppressant, miticide and fragrance.

2.5 The role and location of essential oil

The function of essential oil in the plant is not fully understood. Microscopic examination of plant parts that contain the oil sacs readily shows their presence as in *Thuja* when the protruding sac on the foliage is pricked, the aroma appears. The odour or aroma of the flowers are said to act as attractants for insects involved in pollination, and thus aid in preservation of species and natural selection. Essential oils are almost always bacteriostatic and often bactericidal. Many components of essential oils are chemically active. They are sources of plant metabolic energy if present in large quantities although some chemists have labelled them as waste products of plant metabolism. Exudates such as balsam and resins, all of which contain essential oils act as protective seals against disease or parasite, prevent loss of sap and are formed readily when the tree trunks are damaged.

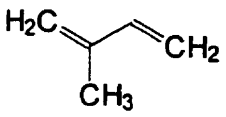
2.6 Composition of essential oils

The volatile components of essential oils usually contain fifteen carbon atoms or less. Some small droplets of non-volatile liquids maybe swept into the receiver during the distillation. Essential oils are made up of three elements almost exclusively carbon, hydrogen, and oxygen. By far the most common component class is the terpenes. The structure of terpenes can be rationalised as the joining together of identical branched units each of which comprises of five carbon atoms (see fig. 1).

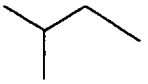
Terpenes can form building blocks by joining together in a "head-to-tail" configuration to form monoterpene, sesquiterpene, diterpene and larger sequences. (see fig. 2). Besides chain structures, nature produces structures with rings by creating additional bonds but in most cases the basic monoterpenes, sesquiterpenes or diterpenes sequence can still be detected in the complex structure. In Thuja, the most prominent constituents of the oil are thujone-isothujone, fenchone and camphor (see fig. 3).

It is not uncommon for an essential oil to contain over two hundred components and often the trace substances (in ppm) are essential to the odour and flavour. The absence of even one component may change the aroma. The same species of botanical grown in different parts of the world, usually has the same components, but the percentages may be different. Age, climatic and topographical conditions affect plants and can alter the essential oil both quantitatively and qualitatively. Flavour and fragrance industry expends considerable effort to determine the principal components of essential oils.

Figure 1

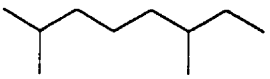


Isoprene

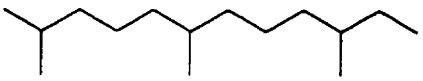


Isoprene unit
the basic building block of terpenes

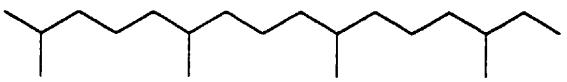
Figure 2



2 Units = Monoterpene

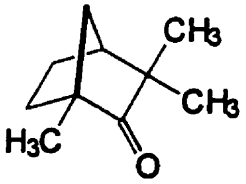


3 Units = Sesquiterpene

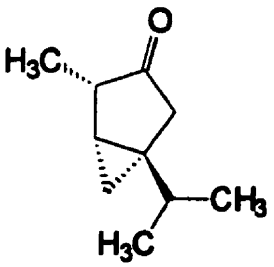


4 Units = Diterpene

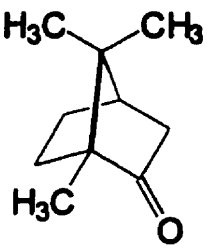
Figure 3



Fenchone



Thujone



Camphor

2.7 Methods of isolation of essential oils

Methods to isolate essential oils are practised differently in different parts of the world depending on local knowledge, accessibility to chemicals and final usage. The methods can be grouped into steam distillation, solvent extraction and enfleurage.

2.7.1. Steam distillation

Steam distillation is the most common method for producing an essential oil. It is the method used to remove the cedar leaf oil from the plant samples in the present work. All steam distillations are true distillations. The product fraction has passed from the still pot over into the condenser in the gaseous stage. It underwent transformation from liquid to vapour and returned to liquid. Materials which cannot significantly vaporise at the distillation temperature are effectively removed from the essential oils. The only exception to this is the small amounts of non-volatile materials which may get trapped in mists or aerosols and are swept over with the steam.

Organic compounds containing just carbon hydrogen and oxygen and having ten to fifteen carbons are typically sufficiently volatile to be distilled without water but with the application of vacuum. Normal atmospheric pressure is 760 mm of mercury. Compounds of the type above could be expected to be distilled at 20 mm of mercury or less with the possibility of reducing the pressure as low as 0.15 mm even in industrial large scale apparatus. The reasons direct water free distillation is not performed and would not be effective are three:

- The biomass samples from which the oil is to be extracted are bulky solids and the heat required to produce vaporisation of the oil in the sample cannot be efficiently transmitted from the walls of the container to the bulky contents. If this is attempted there is charring of the biomass particularly at the point of contact to the reactor.

- The biomass already contains water. If one wanted to try to distill in the absence of water, the biomass has to be first dried (our cedar leaf collection characteristically contain an average of 59% of water by weight).

- The higher temperatures which would be required for dry distillation would destroy some of the natural components and create unnatural artifacts. For materials whose significant properties is their aroma, these unnatural artifacts are particularly damaging.

Steam distillation employs either water, wet steam or dry steam. The plant can be immersed in water which is heated to boiling. The oil and water vapour are passed into a condenser. The oil is separated automatically from the water phase. Water distillation is a mild and slow process but it yields a superior product. In what is called wet steam distillation, the plant is placed on a grid in the still, there may be water below the grid or it may accumulate during the process. Steam is introduced from an outside source into the still. Initially sufficient water condenses in the cool charge to wet it slightly. External heat may be applied to limit the build-up of water and wetting of the charge.

Steam distillation is a reasonably rapid method and can be used for most oils with the exception of those containing high concentrations of low volatility components. In dry steam distillation, the plant is placed on a grid in the still. Direct steam is applied and outside heat, supplied by a steam jacket is maintained at a temperature sufficient to prevent any water condensation. This method is used when the plant contains a higher percentage of sesquiterpenes than most essential oil such as sandalwood, ginger and celery. Care must be exercised to prevent charring (creation of hot spot in the jacket) and channelling (result of hole in the charge which prevent contact of steam with the entire charge).

A process related to steam distillation, but which in fact is not distillation is called in the literature hydrodiffusion (Simon and Beliveau 1987). Hydrodiffusion in fact is a gravity fed extraction of the biomass with a mixture of steam and hot water. Since all the oil components which are recovered have not necessarily passed through the gaseous stage it cannot be properly called distillation of any type.

2.7.2 Solvent extraction.

If steam distillation is ruled out (for example essential oils containing some key components that are sensitive to heat or which contain a major non-volatile constituent) then these oils are extracted with solvent (e.g. piperine in black pepper). A proper solvent has low boiling point, is free of odour and impurities and does not react with the extract. For food product, the solvent must be approved by the FDA (Food and Drug Administration). Pentane or hexane are preferred for flower oils, toluene for aromatic components, alcohol or acetone for phenolic components and chlorinated solvents for

extracts containing amides. Mixed solvents are sometimes used. The plant is placed in an extractor with a removable bottom and a filter bed, usually burlap. The solvent is percolated either with or without heat for a certain period of time. The extract is drained and the solvent recovered by distillation and recycled. In general three or four extractions are sufficient.

2.7.3. Enfleurage.

It is a method used for delicate flower petals which is the absorption of oil on purified fat on a special pressing equipment. The process is repeated many times until the fat is saturated. The fat is removed and extracted with alcohol and recycled. In some modifications, the flower petal is pressed in hot fat at 40-60^{0}C for a shorter period of time in an attempt to reduce the time and cost of the enfleurage extraction step.

2.8 Characterization of essential oils

Once the chemical composition of a particular oil has been established, the analysis of a similar oil is rapid and very convenient since only milligram amounts of the oil to be compared are required. These methods of analysis can be used for questions relating to plant physiology, morphology and taxonomy. Two observations which may affect the analysis by GLC must be pointed out. On some columns the retardation of components can be outside of the error of measuring retention time due to the large amount present. With higher temperature, peak height of the same amount of material can be larger but the trace components are more superior. At higher temperature, larger samples can be used before a loss in the degree of separation is noticed so that detection of trace

components is easier. Comparison of retention data (obtained on columns having different polarities) facilitates the identification of individual components, such identifications should not be accepted without correlation with at least one other reliable technique. Isolation of individual components in more than milligram amounts is difficult, unless the separation factor is relatively large. Pre-fractionation can overcome this difficulty only in part. Such small amounts of terpenes seldom allow chemical identification and physical methods have to be relied upon. Positive identification may fail when two components are so closely related that differences in physical properties are too small to be detected.

2.9 Fractionation of essential oils

Fractionation is the separation of a mixture into its ingredients or into portions, each portion having a distinctly different properties, such as the alcohol fraction, which has distinct hydroxyl or (OH) in the molecular structure.

Since essential oil mixtures contain compounds that can be grouped as carbonyl, non-carbonyl, ketonic, non-ketonic, aldehydic, and non-aldehydic groups, it is desirable that separation or fractionation of the compounds can be made. A variety of methods of fractionation exist. The acids and bases can usually be extracted from mixtures of natural products by using aqueous base or acid. Carbonyl (aldehydes and ketones) compounds, important constituents of essential oils occur in mixture with hydrocarbons, carbinols, and esters.. To recover the carbonyl components of such mixtures, the aldehydes and ketones are converted into water soluble derivatives. To achieve this, separation or

fractionation must be undertaken and a number of reagents to be used for the reaction have to be developed to efficiently isolate the groups. One well known pair of reagents for separating the carbonyl functional group are the Girard reagents.

Girard reagents T and P are quaternary ammonium hydrazides. These reagents work because the hydrazine end of the molecule reacts with aldehydes and ketones affording hydrazones which are soluble in water by virtue of the quaternary ammonium functional end of the molecules.

Girard-T reagent (trimethylammonium acethydrazide chloride)

$$(CH_3)_3\overset{+}{N}CH_2CONHNH_2\,Cl^-$$

Girard-P reagent (pyridinium acethydrazide chloride)

Girard T and P were first prepared by Andre Girard and George Sandulesco (Girard and Sandulesco, 1936). The most famous application of the reagents was by Reichstein (1936) in isolation of many steroidal adrenocortical hormones from beef adrenal glands. Aldehydes and ketones react on heating with the Girard reagents in methanol in the presence of acetic acid to form the hydrazones ($R_3NCH_2CONHN{=}CR_2\,Cl$). The

unreacted non-carbonyl containing compounds are usually removed by partially neutralising the acetic acid, by diluting with water and extracting with ether. The carbonyls are recovered from the aqueous layer by ether extraction after the solution is heated with dilute mineral acid to hydrolyze the hydrazones.

The Girard-T and P reagents have been widely used to separate ketones from essential oils such as vetiver and lavender essences. The *alpha* and *beta*-ventivones can be separated using the fact that the alpha isomer was regenerated more readily from its hydrozone than the conjugated beta-isomer. Alpha-Ionone has also been separated from dihydro-alpha-ionone by hydrolyzing the Girard-P hydrazone of the later at pH 5. Girard-T was used on a micro-scale to concentrate and separate carbonyls in the orange essential oil before analysis by gas chromatography. The ketones were conveniently regenerated by treating the Girard solution with formaldehyde. Sterically hindered ketones such as benzophenone and camphor do not react with the Girard reagents, and appear in the 'non-ketonic' fraction in a separation.

Aldehydes react very readily also with the Girard reagents, in some cases without addition of acetic acid. Girard and Sandulesco (1936) reported that Girard hydrazones of aldehydes were difficult to hydrolyse but this is not substantiated by later work which indicated that the hydrolysis occurred at room temperature. Some aldehydes polymerised under the acid conditions of hydrolysis but can be regenerated safely with formaldehyde. The low molecular weight aldehydes are not easy to extract from aqueous solution but can be recovered by precipitation as their 2-4-dinitrophenyl-hydrazones. With the

advances of gas chromatography, Girard reagents have found less use in the separation of carbonyl components from essential oils for analytical purposes; however, they are widely employed in synthetic organic chemistry

3.0 THE TARGET GENUS *THUJA*

3.1 Distribution and growth area

The common cedar, *Thuja*, is a member of the gymnosperm and belongs to the group of softwoods used extensively in construction. It is an evergreen which can be either a tree or a shrub with pyramidal crowns, conical in outline. The tree has flat, fan-shaped, 2 ranked sprays of foliage with horizontal and ascending branches. The bark is thin and fissured on older trees. The outer bark is scaling in patches of irregular shape, while the inner bark is fibrous. The genus *Thuja* resembles most closely to the genus *Chamaecyparis*, (cypress) though it differs principally in the shape of the cones and the larger leaves. The genus *Thuja* embraces six species and is confined to China, Japan, Formosa and North America. Two species are indigenous to North America. The western species, *Thuja plicata*, Donn ex D. Don is a valuable timber tree of the Pacific Slope and Northern Rockies. The eastern species, *Thuja occidentalis* L., subject of this dissertation, ranges from the maritime provinces and northern parts of the United States including central Minnesota, Wisconsin, Vermont, New Hampshire, Maine and southern New York and westward to Lake Winnipeg and northward to the southern end of James Bay (Johnston 1970). The other name of *Thuja* is arbovitae, "tree of life" which dates from

the 16th century when French explorer Cartier learned from the Indians how to use the tree's foliage to treat scurvy and save his men from dying.

Thuja occidentalis L is a medium-size tree commonly 12 - 15 meter (40-60 ft) tall and 30-60 cm (12-24 in) in diameter at breast height (d.b.h.). In Michigan a record tree measures 34 meter (113 ft) in height and 175 cm (69 in) in d.b.h.(Curtis 1946). *Thuja* species grow in relatively humid climate with annual precipitation range from 710-1170 mm (28 - 46 in), and extreme precipitation range of 510-1400 mm (20-55 inches) in the southern Appalachians. One-third to one-half of the precipitation occurs during the warm season. Snowfall ranges from about 100-380cm (40-50 in) annually. Temperatures are often cool during *Thuja*'s moderately short growing season. The northern limit of the *Thuja* range extends to the forest-tundra transition (sub-arctic zone) in Canada. There, the average January temperature ranges from -12 to -4 ^{0}C (10 -24 ^{0}F) and July temperature ranges from 16-22 ^{0}C (60-72 ^{0}F).

Thuja occidentalis L. grows on a wide variety of organic and mineral soils. It does not develop well on extremely wet or extremely dry sites. Where *Thuja* grows, the site is often associated with streams or other drainage-ways or calcareous mineral soils. *Thuja* leaves last three to four years on the tree, therefore shedding of brown leaves is a continuous process throughout the life of the tree. *Thuja* foliage turns yellow or brown from unfavourable winter weather, de-icing salts and drought (Foster et. al. 1978). At times the damage is sufficiently severe, such as during very dry winter, to cause death to the tree (Ramsey 1936). The tree is relatively free from serious insect injury (Curtis

1946, Rose and Lindquist 1980). Damage is principally from carpenter ants and leaf-miners. The tree provides valuable shelter and browse for wildlife habitat, particularly for deeryard during severe winter (Verme and Johnson 1986). *Thuja* is also widely planted as ornamental and hedges in the United States (Hepting1971) and in Europe since the 16th century.

The wood is rot-and termite-resistant and used principally for products in contact with water and soil (Johnston1970). It is also used for making Kraft pulp and particleboard. A closer look is focussed on the leaves and branchlets because these tissues are used in this research.

3.2 The leaves and branchlets

The leaf has a flattened scale-like shape and grows at the apical meristem zones. The phyllotaxis of the scales are opposite to each other at one node and shift ninety degrees at subsequent node around the orthostichy of the stem. The longitudinally flattened shape of the stem causes one pair of the leaves to fold at the axis to form the bend, and the next pair on the node to lay flat adaxially and abaxially. The adaxial side of the leaves is shiny while the abaxial side is dull. The leaves grow out of nodes so closely spaced that it is difficult to see clearly the internode. At a node where a leaf is formed, a confluent of an extra pair of scales are formed in parallel abaxially and adaxially so that the apical meristem of the branch can continue to develop while at the same time the leaf is formed. A leaf primodia is predetermined internally by periclinal division of a mother cell in the peripheral zone of an apical meristem (Guttenberg, 1961).

The branchlet has the same arrangement as the leaf though with a clearer boundary of the nodes because of internodal elongation The branchlets are displaced close to the leaf base or even onto the leaf itself by subsequent growth adjustments. Auxiliary buds that form branchlets are commonly initiated later than the leaves subtending them. Transversely, the branchlet is oval in shape and not as flattened as the leaves. A protrusion near the tip of the leaf and branchlet exhibit yellowish green reflection which may be a resin duct. When pierced, there seems to be an enhanced emission of a fragrance characteristic of 'cedar leaf oil'. The easy release of aroma is a clear indication of the presence of significant essential oil.

Anatomically, the leaf contains three types of tissues:

1. Epidermis,
2. Mesophyll and
3. Vascular tissues.

The epidermis layer is the thick wall of the cells covered with cuticles and stomata on all sides, especially on the abaxial side of the leaf. The mesophyll layer is parenchymatous in nature; its walls have characteristic ridge-like invaginations and contain chloroplasts and resin ducts.

The vascular system has one or two vascular bundles close to each other in the centre of the leaf. The vascular bundle is surrounded by transfusion tissue consisting of tracheids and living parenchyma cells. Spongy parenchyma are polyhedral shaped and connected in a row. Pallisade are stake-like parenchyma found directly below and right angle to the epidermis and water is transported towards the epidermis more through them than through the spongy parenchyma. Pallisade parenchyma are one of the last tissues to stop growing and dividing and they often continue to function as a meristem for some time even after the cells of the spongy parenchyma and epidermis have ceased to divide. Parenchyma cells play a part in wound recovery, regeneration and short distance transfer of solutes. They resume meristematic activity when the environment is artificially changed. Some of the parenchyma cells are packed together with no intercellular spaces, while others have intercellular spaces. Intercellular space is formed when the middle lamella between two newly formed primary walls of new cells comes in contact with the primary wall of another cell and not with the middle lamella between it and the neighbouring cells. A small space develops where the new lamella comes in contact with the mother cell wall. That space disintegrates and forms the intercellular space. It enlarges if similar space is formed in the neighbouring cells. The intercellular space is

lined with the substance of the middle lamella. This development is called schizongenous, and resin ducts are formed schizongenously. The parenchyma cells contain tannins, resins and starch which vary according to season (Fahn 1990). The tracheids in the leaf are from regions further away from the bundle and possess lignified walls and bordered pits. Some tracheids from the vicinity of the vascular bundle are unusually long with one end tapered and the other end forked. The tracheids close to the bundles are long while those further away are more parenchyma-like in shape and have relatively thin, slightly lignified walls and bordered pits.

In median longitudinal cross sectional cut, the leaf shows a thick epidermis layer and messophyll area with chlorophyll parenchyma cells which appears to intersperse with whitish albuminious cells. The epidermis layer of the leaf consists of a very thin two layer cutin. The outer layer called the cuticle proper which can be peeled off, and the layer below it the cuticular layer which consists of cutin and wall materials. The cuticle proper is formed by cuticularization, a secretion of cutin or its precursors to the surface cell wall. The cuticular layer is formed by cutinization, a deposition of cutin between the cellulose microfibrils of the outermost wall layers, where pectin and hemicellulose may be present. Deposition of wax is in a continuous layer on the surface of the cuticle to give "bloom" to the leaves and to reduce wettability of the surface. The epi-cuticular wax reduces damage to photo-synthesis and heat load by reflection of light (McClendon, 1984). A direct solvent extraction will result in these waxes being present in the essential oil and causing more contamination. Then again a mixture of wax and essential oil might in itself be of value.

In the transverse cross sectional cut, the leaf reveals a single resin duct close to the abaxial side of the leaf. Stomata are in abundance at the abaxial side of the leaf. Stomata are interruptions on the surface of the epidermis layer and they look suspended from the subsidiary cells arching over them. The guard cells of the stomata are either in elliptical or fully opened position. Guard cells accumulate potassium from adjacent cells and control the size of the stomatal apertures. The stomatal aperture leads to a substomatal space or chamber which is continuous with the intercellular spaces in the mesophyll. The walls of the guard cell, like the epidermis, is cutinized in the outer layer and extends from the stomatal aperture to the substomatal chamber where it joins the inner layer of cutin (Fahn 1990).

Cross sectional cut of the branchlet shows a thick layer of epidermis and a distinct layer of endodermis. In between these two layers the mesophyll area is filled with numerous small intercellular spaces. Resin ducts can be seen all around the orthostichy line. Collenchyma, prism like or long fibres with tapered ends, and parenchyma, narrow elongated polyhendrals connected to each other and containing chloroplast and tannin are situated below the epidermis (Esau 1953).

Tannins are valued products in their own right but only if available in commercially interesting amounts. Tannins are polymeric flavones and are essentially non-volatile with steam..

In the collection of the cedar foliage for this thesis, no distinction is made between leaves and branchlets. The isolated essential oil is derived from a mixture of both.

3.3 The essential oil of *Thuja*

Cedar leaf oil has been in public use before 1900. In United States of America alone, the yearly volume of cedar leaf oil reported is approximately 10,000 lb. Cedar leaf oil has been granted GRAS (Generally Recognised As Safe) status by FEMA (Flavour Extract Manufacturers Association) in 1965 and approved by the FDA (Food and Drug Administration) for food use (21 CFR 121.1163). In 1970 the Council of Europe has included cedar leaf oil (*Thuja occidentalis L*) in the list of flavouring substances temporarily admitted for use, possibly with a limitation on the active principle in final product. The Food Chemical Codex (1972) has a monograph on cedar leaf oil. In biological data tests, cedar leaf oil did not show any irritation after a 48-hour closed-patch test on human subjects (Kligman 1966) nor any sensitization in the test that was carried out on 25 volunteers. The oil, at a concentration of 4% in petrolatum did not exhibit phototoxic effect either (Urbach and Forbes 1973). Physical properties of *Thuja* are described in the Journal of Essential Oil Association # 86. Perfume companies depend heavily on the major constituent, the *alpha* thujone (Gildemeister and Hoffman 1956; Guenther1952) which is found in the leaves and branchlets or branch ends of *Thuja occidentalis L*. Currently percentage concentration of cedar oil in perfumery final products ranges from a low of 0.001 % to a high of 0.4%.

CONCENTRATION (%) IN FINAL PRODUCTS

	Soap	Detergent	Creams, lotions	Perfume
Usual	0.01	0.001	0.005	0.2
Maximum	0.1	0.01	0.03	0.4

Essential oil of the 'leaf' of *Thuja occidentalis* L is distilled from the leaves and branchlets and used in medicines and perfumes. The odour and taste of the leaves strongly resemble camphor. The leaf oil is relatively well-known and may regularly be found as an essential oil of commerce. It is the most important conifer leaf oil of North America (Simon and Beliveau, 1987). The volatile oil of cedar leaves, (*Thuja occidentalis L*) was chosen as a test mixture because this oil is readily available commercially, its chemical composition is well known and it is available locally for preparation of small quantities of the fresh oil. Also, its composition is sufficiently complex to tax the technique. The major constituents of the cedar leaf oil is *d-alpha* thujone and *l*-fenchone. Also isolated are *d-alpha* pinene and *l*-borneol (free and as the acetate) (Wallach 1893)

Essential oil of the 'wood' of *Thuja occidentalis* L can be obtained by steam distilling from the sawdust which is a by-product from the sawing of cedar wood and has a strong characteristic odour. Wood oil is used for its miticide properties in storage of furs in Canada. Knowledge of wood oil is limited (von Rudlof, 1964).

3.4 Fractionation methods

Essential oils derived from *Thuja occidentalis L* have not been reported to be tested with the Girard reagent. Other 'chemical tools' to separate essential oils on the basis of functional group content have been devised. A need to segregate a neutral fraction into ketonic and non-ketonic cuts of the pharmacologically active constituent of the ayurvedic crude drug (folk medicine) resulted in another reagent being developed. The motivation was that with Girard P reagent, the acid-labile diterpenoids in the non-ketonic portion, underwent extensive dehydration / isomerization, even under the rather mild conditions involving use of an acid ion-exchange resin as the catalyst for the condensing (grabbing) of the carbonyl by the Girard reagent. Dr Sukh Dev (Sukh Dev et. al. 1980) in India created a reagent used under neutral or slightly basic conditions and supported on a solid matrix so that the non-ketonic and non-aldehydic material would stay in the solution phase when the condensation is carried out in a non-polar solvent such as hexane /toluene in which the reagent is invariably insoluble. The carbonyl components would be bound with the reagent on the insoluble solid silica. This permits separation of the carbonyl compounds from other neutral components in an expeditious manner, even on a large scale by simple filtration. The reaction was carried out by heating the material with the reagent in an appropriate non-polar solvent such as hexane. In the Sukh Dev experiments the reagent gave gratifying results. This methodology appear attractive to us to use in our effort to develop new technology applicable to *Thuja* oil.

It is of interest to know whether aldehydes could be separated and concentrated. The classical chemical reagent , aqueous bisulfite solution has generally been employed to separate the aldehyde compounds from a mixture. Aqueous sodium bisulfite is far from an ideal reagent. Incomplete extraction, troublesome formation of precipitates, difficulty in liberating the aldehyde from the resulting sodium bisulfite adduct or extracted solution are frequent problems. I was attracted to the possibility that a new and extremely cheap and easy to use reagent developed by Okamoto and Ohta (1979) might give surprising results when applied to separating aldehyde from a complex essential oil mixture. As part of this thesis I have applied this Okamoto reagent to the cedar oil mixture even though only very low level of aldehyde has been reported in *Thuja*.

The Okamoto/Ohta reaction is as follows:

$$R\text{-}CHO + H_2N\text{-}(CH_2)n\text{ - }COONa \text{ ---- } R\text{-}CH = N\text{-}(CH_2)n\text{ -}COONa \text{ -----} R\text{-}CHO$$

Based on what is reported in the literature, the manner in which the reagent achieved its selectivity for aldehydes only and not other carbonyl is not adequately understood. However, the discoverers' results were extremely encouraging. Using several amino acids where n=1, 2, 3, 5 and 10, the amount of aldehyde extracted tended to increase with the value of n but of great practical importance the readily available and inexpensive reagent 6-amino-*n*-caproic acid (n=5) work well. This reagent, sodium 6-amino-*n*-caproate, which is made simply by mixing together equal molar amounts of the amino acid and sodium hydroxide, is stable at room temperature. None of the extraction experiments using this reagent yield a precipitate and the extraction percentages were consistently higher than those done by the saturated sodium bisulfite method. Unlike

sodium bisulfite which can extract some sterically unhindered ketones, the Okamoto reagent reacted only with aldehyde when used according to the described protocol.

3.5 Objectives

My objective and orientation were different from the prior researchers in this area. For the most part these people were providing descriptive data about the essential oil, the analysis of that oil and the variability of that oil versus some parameter. My central objective asks the question what new ways can be found to manipulate the essential oil to get out from it components with modified properties which made these fractions more suitable for a wide range of application.

One objective is to lay the ground work for uses of the essential oil from the northern white cedar (*Thuja occidentalis L*) in new value-added products either in the perfumery, pharmaceutical, or aroma-therapy industries. The main emphasis is to preserve and prolong essential oil from going rancid. The more stable the essential oil becomes, the greater the chance for the product to stay or increase in market share, because it can be used in more varied products.

A problem with oil is rancidity. Oils undergo oxidation which cause off taste and off aromas. It is reasonable to associate this oxidation with the conversion of aldehydes in the oil to acids by the action of air. One objective of this study was to determine whether

the Okamoto treatment could remove aldehydes, even just traces of aldehydes, from cedar oil and improve the oil property.

Oil from cedar is valued for its ketone content. A second objective was to determine whether the application of the Sukh Dev reagent could produce a new oil from the carbonyl fraction with ketone content much higher than the original, which would be superior in old uses and/or applicable to some new uses.

A third objective was to investigate the properties and yield of the fraction of oil containing just the non-carbonyl components of *Thuja*. To examine these issues we had to collect cedar and make essential oil as a starting material. The collection of cedar allow us to answer or to solidify and confirm answers to the questions: Is there a seasonal variation in the quantity of oil from a given population of trees? Is there a variation in the water content, in collections done at different times of the year? If a piece of research is carefully thought out, its execution may provide useful understanding on a range of questions.

The experiment was designed with the knowledge that prior research have been done on inter-tree variation of essential oil composition of *Thuja* (Kamdem 1993), comprehensive analysis of cedar leaf oil (Kamdem 1993, Shaw1953), and methods of distillations to isolate the oils (Simon 1987). My objectives and orientation were different from all these authors. Currently percentage concentration of cedar oil in perfumery final products ranges from a low of 0.001 % to a high of 0.4%.

In this research, the focus was on ways to separate carbonyl and aldehyde compounds from the whole essential oil in simple and easy methods so that *Thuja occidentalis L* oil can be more marketable and attractive for use in perfumery and pharmaceutical preparations.

4.0 EXPERIMENTAL MATERIALS AND METHODS

4.1 Sample location and collection

A grove of about twenty five *Thuja Occidentalis L* trees was chosen for the source of material. The grove is located in the town of Portland in eastern Ontario. The location was selected so as to be accessible both in summer and in winter. Permission to prune was obtained from the owner of the land. The location is about 2 km from a bus parcel depot so freshly collected sample could be immediately shipped to Toronto. Collection was done around the beginning of each month for a year, starting in October 1997 and ending in September 1998. One extra collection was made in February 1999 because the previous year eastern Ontario experienced a severe ice storm and the distillation result was inexplicably low. Leaves and branchlets were clipped randomly from all the trees in the clump so that variability between trees was overcome. Care was also taken to make sure that clipping was done at random heights from one foot above the ground to as high as the arms can reach. In this way the sample encompassed all possible age groups of the leaves and branchlets. Once the samples arrived in Toronto, the cedar leaf and branchlets

were further cut into manageable sizes to be steam distilled. The final essential oils collected were stored refrigerated except when sampled for analysis or chemical separation. A sample obtained in February 1998, soon after the ice storm in eastern Ontario produced significantly less oil than any other sample. Because of this anomaly or outlier, a February 1999 collection was done and the yield was in line with the overall annual yield data and this datum is substituted in the table. A causal relationship between the low oil yield and the ice storm has not been established. The assertion is merely anecdotal. It would be interesting to spray cedar with water under freezing conditions and try to confirm the observation. Of course a correlation with some parameters which increased yields would be more interesting still.

The monthly collection of *Thuja* foliage (leaves and branchlets) can only be distilled three to four days later because of shipping time. The collection was packaged in plastic to avoid drying and the material was kept at room temperature all the time. From the literature it is known that there is little effect of temperature on oil stability.

4.2 Steam distillation and azeotropic determination of water

Steam distillation was done every month. Between 800 and 1000 grams of cedar leaves/branchlets were treated to yield roughly 5 gram of oil. The leave and branchlets were stored in a closed plastic bag between the time of collection and the time of distillation. A five litre three-necked round bottomed flask equipped with a pressure release valve, a 500 ml pressure-equalised dropping funnel for adding water, and boiling chips was partially filled with water and heated with an electric heating mantle. The

steam generated in this vessel was led through a bent glass tube out of the boiler flask into a 12 litre four-necked flask mounted in an appropriately sized heating jacket. The large centre neck was used to load the cut cedar pieces and then was capped with a glass stopper. One neck contained the glass steam inlet pipe passing through a rubber stopper which fit snugly into the glass joint. A third neck contained a thermometer adapter and a thermometer to monitor the temperature inside the flask. The fourth neck led through a goose-neck joint into a spiral water-cooled condenser. The downward end of this condenser was connected by another glass adapter to a 1 litre graduated Erlenmeyer flask where the water and oil were collected. The cedar was placed in the 12 litre flask with a small amount of water. The heat was turned on the 12 litre heating mantle enough to keep water at 100 C but not sufficient to char the cedar leaves. The cooling water was turned on so it passed through and cooled the condenser. The water was set boiling vigorously in the 5 litre flask which acted as a steam generator. Water was added to the generator from the dropping funnel when needed to replenish the water which was turned into steam. The vigorously produced steam from the boiler passed rapidly into the glass tube and through it into the bottom of the 12 litre flask where it heated the cedar parts to 100^{0} C. The steam forced the oil out of the leaves and branchlets and swept it up through the goose-neck connection into the spiral condenser where it was cooled by the flowing cold water. The steam and oil condensed and the steam pressure are forced them downwards and out of the condenser into the collection flask. When a predetermined volume of distillate has been collected the apparatus was cooled and the distillation ceased.

To determine the percentage of water in a sample of cedar leaves and branchlets, the following azeotrope experiment based on TAPPI standard T208 om-84 was used. An azeotropic as opposed to simple oven drying method was used to determine the water content because in standard oven drying the volatile oil may escape and be counted as the percentage of water loss.

A two-litre three-necked round bottomed flask was mounted securely in an oil bath and a magnetic stirring bar was placed at the bottom of the glass container 100 grams of accurately weighed cedar leaves and branchlets were placed into the flask through the large centre neck and this neck closed with a glass stopper. One side neck was also stopped and into the other side neck was placed a Dean-Stark trap with a bottom stop-cock for removing a higher density liquid from a lower density one. The Dean-Stark trap was connected to a reflux condenser which in turn vents through a bubbler to prevent moisture from the outside to enter the condenser. About 1000 ml of toluene was placed in the 2 litre flask and the toluene refluxed by the application of heat from the bath. When the toluene boiled the vapours extracted the water from the cedar and transported this water vapour up into the condenser where it liquefied and fell back into the Dean-Stark trap (separator). Both toluene and water vapours passed up into the condenser and both condensed and fell back. Toluene and water as liquids were immiscible and the two liquid phases separated in the apparatus. The water, being heavier than toluene, settled to the bottom and was drawn off through the stop-cock every half hour into a collection flask, while the toluene was allowed to flow back to the flask and re-boil. The collecting flask was capped tightly to prevent moisture loss. When no more water was observed to

separate, the heating was stopped. The whole experiment took 7 hours to complete. The volume of water separated was carefully measured. The % water in the cedar could then be calculated by measuring the total volume of the aqueous phase separated, multiplying by 1.0 (the density of water) to give the grams of water separated. Since the starting sample of cedar leaves and branchlets weighed 100 grams the percentage water is the the weight of water recovered divided by the sample weight times 100.

4.3 Fractionation

4.3.1 Girard reagent experiment

The volume of Girard reagent used in this experiment was significantly more than the mixture tested to ensure thorough and complete bonding of the carbonyls. The formula weight of Girard reagent $H_2NNHCOCH_2N(CH_3)_3$ Cl is 167.64 and a multiplier of two was used. One gram of cedar oil with an atomic weight of 152 (calculated as thujone) known to contain approximately less than 70% ketone compounds or 0.0046 mole or 4.6 mmole. (0.70/152=0.0046 mole). Therefore for the Girard reagent used to mix with the one gram of cedar oil and to be in 100% excess, was

2 x 0.0046 x 167.64 = 1.542 gram.

The essential oil was mixed with

10% Girard reagent (1.542 gram),

10% acetic acid(CH_3C - OH), density of 1.049, and

80% ethanol with a density of 0.794,

the resulting mixture became

1.542 gram Girard reagent, (10%)

1.542 gram acetic acid (10%) or 1.47 ml (1.542/1.049)

12.33 gram ethanol (80%) or 15.54 ml (12.33/0.794)

The experiment was done by setting an oil bath on a heating element with stirring capability. Each component (ethanol, acetic acid, essential thuja oil and Girard reagent) was weighed separately and put into a 50 ml round flask containing a magnetic stirrer and 20 ml cyclohexane. The Girard T reagent was the last one weighed because it is hygroscopic. The round bottomed flask was fitted with a condenser and the condenser in

turn was fitted to a nitrogen bubbler which provided an inert oxygen free atmosphere during heating. The flask was immersed into a pre-warmed oil bath, thermostatted at a temperature of 100^0C as a precaution even though cyclohexane boils at 87^0C. The stirrer was then switched on and the mixture was left to stir and reflux for one hour for the reaction to work. The carbonyls (aldehydes and ketones) reacted on heating to form hydrazones. After an hour, the round bottomed flask was cooled in an ice and water bath. When the contents cooled, it was poured into a beaker and about 30 ml of water was added (water used to be twice the amount of ethanol, 2 x 15.54)

Next the pH of the mixture was adjusted to be around pH 7. Adjustment was first made with saturated sodium carbonate to prevent the pH from over shooting above 10 (sodium carbonate can only go as high as pH10). When the increase was not fast enough, a 10% concentration of sodium hydroxide was added to make the pH adjustment faster.
Once the desired pH 7 was obtained, the mixture was ready to be separated.

The mixture, which was about 70ml, was put into a separatory funnel and 20 ml cyclohexane was used to rinse the beaker. The separatory funnel was capped and shaken vigorously a couple of times., Every few shakes, the funnel was tipped upside down so that the valve could be opened to release the built up pressure. After the shakes, the funnel was left to settle for a couple minutes for the layers to separate. The lower layer was poured out into a flask. The upper clear layer, which contained cyclohexane was poured into a second flask. The lower layer was then poured back into the funnel and a second 20 ml cyclohexane was added. The separatory funnel was shaken again for a

couple of times, making sure the valve was released to reduce pressure, and then it was left for the layers to separate and collected again. A third time addition of 20 ml cyclohexane was added.

At the end of the third cut, the lower layer portion containing the grabbed carbonyl in water and ethanol was adjusted to a pH below 4. The adjustment was done using hydrochloric acid. The mixture was then left to sit for one hour, giving time for the carbonyl to be released into the cyclohexane. Then, the mixture containing the ungrabbed carbonyls was separated again, using cyclohexane. The carbonyl migrated into the cyclohexane and the Girard reagent stayed in the other aqueous phase. The wash, using separatory funnel, cyclohexane, shaking, and settling was done three times, each time using 20 ml cyclohexane for rinsing the beaker as well as addition to the mixture to make sure as much carbonyls as possible migrated to the cyclohexane. The collected cyclohexane solution containing the carbonyls was poured into a round flask with its weight recorded first. The round R.B. flask was put on to a rotary evaporator. After each 10 minutes on the rotary evaporator, the flask was removed and weighed and recorded. It was kept being put back unto the evaporator for another 5 minutes until the noted weight did not differ from the previous reading by no more than 0.05 grams.

The aqueous solution containing the non-carbonyls was also rotary evaporated and the weight noted.

4.3.2. Sukh Dev reagent experiment

Once it was established that the collections in all months were essentially the same and at the same time Sukh Dev experiment takes a substantial amount of time to do, it was decided that only one experiment was performed on a single typical month's cedar oil collection.

All equipment used in this experiment was washed with methanol and blown dry with air.

To determine what the amount of Sukh Dev Reagent - Semicarbazide on Silica-gel to use, an assumption was made that one gram cedar oil contains less than 70% ketone and the known ketone is thujone with molecular ion of 152; therefore one gram of cedar oil contains 4.6 millimoles of ketones (0.70 / 152 = 4.6 mmoles).

Sukh Dev recommends that at least 2.5 times the substrate moles be used. Sukh Dev reagent contains 0.723 mmoles of semicarbazide per gram of solid. If we use 2.7 times the substrate, we will use 17.14 g of the Sukh Dev reagent (4.6 / 0.723 x 2.7 = 17.14g). A small flask was tared and 1 gram of cedar oil weighed into it using a disposable glass pipette to transfer the oil. 17.14g of the reagent, semicarbazide on silica-gel, was weighed in a tared plastic container. The 17.14g of solid semicarbazide on silica-gel reagent was poured into a round 250 ml flask with the aid of a wide mouth funnel, and the flask was placed on a ring to keep it steady. With a pipette, the 1 gram essential oil was siphoned from the small flask and dripped into the flask, taking care that the pipette do not touch the semicarbazide. Then 35 ml cyclohexane were used to wash the small flask which

contained the cedar oil as well as the funnel that was used to pour the reagent into the round 250 ml flask. The cyclohexane liquid was poured into the round flask and set with a stirrer in an oil bath. The oil bath was put on a heater equipped with stirrer. The top of the flask was capped with a water condenser and the water tap was opened to keep the water flowing for cooling. The condenser was topped with a nitrogen bubbler to keep an inert atmosphere inside the flask. The batch was left stirring overnight with the temperature set to 80^0C. The next day, the round flask was removed from the bath and cooled down with a bucket of ice and water. A Buchner filter and filter flask was set up for suction filtration through paper. The slurry was poured from the reaction flask into the filter and the round flask was washed with 35 ml of cyclohexane. A second 35 ml of cyclohexane was used for another wash to make sure the reagent was thoroughly washed. The solid was kept as this contained the carbonyl containing compounds. The filtrate was put into a clean round flask and evaporated under vacuum on a rotary evaporator until the cyclohexane solvent was removed and the non-carbonyl oil recovered. This oil amounted to 0.154 gram or 15.4 % of the original 1 gram. It was still distinguishable as having a cedar-like smell. [The material on the filter is the reagent chemically binding carbonyl. This was to be treated with oxalic acid to enable the semicarbazide on silica gel to release the carbonyl]. This filtered solid was put into a clean 250 ml round flask with 6% (4.46 gram) oxalic acid and 80 ml distilled water and 50 ml cyclohexane. The round flask was set up into an oil bath with condenser and nitrogen on the top and heater/stirrer on the bottom . It was heated and left to stir for 6 hours. After 6 hours the round flask was cooled with an ice pack. Here it was noted that the flask contained 3 phases, the cyclohexane layer on the top, the water layer in the middle and the semicarbazide on

silica gel with the oxalic salt at the bottom. The carbonyl was in the first layer with the cyclohexane. To get the carbonyl, the first layer was siphoned out even with a bit of the second water layer taken. The siphoned liquid was put into a separatory funnel. This round flask was rinsed with 25 ml cyclohexane to make sure all the carbonyls were out of the reagent and into the cyclohexane layer. Then the cyclohexane layer was siphoned out again and put into the same separatory funnel. The water layer was separated and removed. The cyclohexane solution was put into an Erlenmeyer flask and 3-5% by volume anhydrous sodium sulphate was added to absorb any left-over water. The solution was left for 10 minutes. Then this solution was filtered by gravity with a fluted filter paper into a round 250 ml r.b. flask. The flask was put onto a rotary evaporator for 10 minutes to reduce the cyclohexane. Then with a disposable pipette it was transfer to a smaller but tared r.b. flask. With a small amount of cyclohexane (1-2 ml) each time the large R.B. flask was cleaned, making sure all around the walls of the large flask was rinsed. This was done 2 to 3 times, using altogether around 6 ml of cyclohexane. This small R.B flask was put on to the rotary evaporator for 10 minutes. Then the flask was weighed, and put back on again to the rotary evaporator for another 10 minutes. This was done for a couple of times, each time the weight of the flask was read until two consecutive weights did not differ by more than 0.02 gram. The final weight recorded was the carbonyl extracted from the original 1 gram cedar oil.

4.3.3. Okamoto reagent experiment

Okamoto reagent contains 6-amino caproic acid and sodium hydroxide.

Each mole of 6-amino caproic acid (98%) has a formula weight of 131.18g/litre.

Each mole of Sodium hydroxide has a formula weight of 40.0g/litre.

Therefore 1.2 mole of reagent contained

157.42 g/l of amino caproic acid (1.2 x 131.18)

48.00 g/l of sodium hydroxide (1.2 x 40.00)

In this essential oil experiment, where only 3-5 gram of cedar oil was used, the amount of reagent needed was only one quarter (1/4) of what the paper suggested, which was 39.36 g 6-amino caproic acid and 12.00 g sodium hydroxide. These two compounds were put into a long neck flask, topped with distilled water to make 250 ml.

3 gram of cedar oil was weighed and put into a small tared 25 ml flask. Using 50 ml of diethyl ether, the content of the flask was transfered into a 125 ml separatory funnel. 20 ml of the reagent was measured and poured into the separatory funnel and capped. Then the capped funnel was shaken for two and one half minutes, and the valve was released a few times for any built up pressure. Then the content of the funnel was left to settle. The darker liquid which was the water and grabbed aldehydes separated at the bottom. This liquid was collected into a 125 ml flask. Another 20 ml of the reagent was measured and poured into the funnel. It was shaken for another 2½ minutes and left to settle. The bottom liquid was separated into the same flask that collected the first shaking. It was decide to err on the excess because otherwise the Okamoto solution might still be in the upper part. The left over which contain all the mixtures except for aldehydes was kept

apart. The separatory funnel was cleaned with distilled water, then with acetone to dissolve any organic matter and finally with methanol to rid any left-over acetone. It was then blown dry with air. The water solution with the aldehydes grabbed by the reagent, was then poured back into the separatory funnel. It was washed with 10 ml diethyl ether, which was measured out by graduated cylinder. The separatory funnel was well shaken. The bottom layer, which was the aldehyde solution, was collected in a 50 ml flask and the ether solution (top layer) poured out into the flask for non-aldehyde. Then the pH of the solution containing the aldehyde was adjusted to between pH 4 and 6 using 12% HCl to release the aldehyde. The separatory funnel was cleaned with distilled water, acetone and methanol and blow dry. The pH 4 aqueous solution was poured back into the funnel and extracted with 20ml diethyl ether to separate the aldehyde. Standard shaking was done with pressure released and then the layer was let to settle and separate. The bottom water layer was collected in a beaker and the top layer containing the ether and aldehyde was saved in an Erlemmeyer flask. A second extraction with another 20 ml diethyl ether wash was done to make sure all aldehydes were absorbed into the ether. The top ether layer was again collected and poured into the same Erlenmeyer flask.

To remove water from the ether solution a 4.0 gram of sodium sulphate (Na_2SO_4) was put into the flask and let stand for 15 minutes. To recover the aldehyde, a r.b. flask was tared, and the contents of the Erlenmeyer flask was poured into a suction filter connected to the tared r.b. flask. The Erlenmeyer flask was washed with a bit of diethyl ether. The r.b. flask was put onto a rotary evaporator and as the amount reduced, it was put into a

smaller tared flask for further evaporation. The amount of aldehyde in essential oil is known to be very minute and an accurate record of the weight has to be done.

4.3.4 Gas Chromatography

To examine the oil qualitatively, each month's essential oil obtained was fed into a Hewlett Packard 5890 Gas Chromatography (GC) machine as a dilute solution in cyclohexane. The use of cyclohexane as diluent is important. Hexanes or another low boiling petroleum fraction will not work because these solvents are actually mixtures of many compounds which confuse the gas chromatographic trace. The twelve month cedar oils were analysed qualitatively (peak shape and position) and quantitatively (relative peak area)

An arbitrary month of November 1997 oil was used for the fractionation. First, gas chromatography / mass spectroscopy (GC/MS) was done on the untreated oil to provide some characterisation of the major components in the oil. Chemical literature data were used to compare the identities of these components. From Kamdem (1993) the major ketone components are thujone, fenchone and a much smaller quantity of camphor. The aldehyde component is abietal and in Simard (1988) it is e-2-hexenal.

Samples to go in for Gas Chromatography (GC) were readied by using one (1) ml cyclohexane and two drops of the sample. The GC is Hewlett Packard HP 5890 GC. Run was done on column A, which is a 15 meter long column SPB-1 by Sepelco. The parameter was set with an initial temperature of 60^0 Celsius for a 3 minutes duration.

Then the temperature was increased at the rate of five degree Celsius per minutes (5^0C/min) until it reached two hundred and fifty degree Celsius (250^0C). When the machine was ready for the run, a "Hamilton" 10 µl needle was used. The needle was cleaned at least 4 times with cyclohexane to rid the column in the needle of any foreign mixture. Then a 4 µl of the sample was drawn. Once an amount of 4 µl was obtained, the needle was further drawn higher until a bubble was seen at the beginning of the reading. This was to ensure that all the 4 µl liquid was in the needle and not dripping anywhere else. The needle was then inserted upright into the injection column A and a swift injection was done to allow the liquid to flow in fast and around the same time the 'start' button was pressed to initiate the run.

5.0 RESULTS AND DISCUSSION

5.1 YIELD OF CEDAR OILS

The seasonal variation in the yield of cedar oils of leaves and branchlets is shown in Table 1.

Table 1

Sample dates	Fresh weight of leaves & branchlets (g)	YIELD Total oil (g)	YIELD Oil/ fresh wt. (%)	YIELD Oil/ dry wt. (%)
October 1997	550	4.04	0.735	n/a
November 1997	865	7.41	0.857	n/a
December 1997	827	8.81	1.060	n/a
January 1998	865	8.54	0.987	n/a
February 1999*	1007	8.45	0.839	1.824
March 1998	1170	10.43	0.892	n/a
April 1998	1180	7.95	0.674	1.643
May 1998	1019	6.97	0.684	1.927
June 1998	1025	6.49	0.633	1.667
July 1998	989	5.81	0.588	1.506
August 1998	1001	6.52	0.651	1.589
September 1998	1007	8.62	0.856	1.991
Average		**7.50**	0.788	
Standard deviation		1.674	0.150	

*replace 1998 data (Ice storm in Eastern Ontario)

(1998 February	768	3.33	0.434)

From the distillation data collected for a year, there is distinct difference in yield when a comparison is made between the cooler months of November to March and the warmer months of April to August. The difference in yield is as much as 0.282 g or 30% less yield in the warmer months.

Table 2

Comparison of yield (%)

(Oil/fresh material)

	Cooler months Nov. - March	Warmer months April - August
	0.857	0.674
	1.065	0.684
	0.987	0.633
	0.839	0.588
	0.892	0.651
	---	---
Number (n)	5 (n1)	5 (n2)
Average	0.928 (T1)	0.646 (T2)
Std. Dev.(s)	0.0955 (s1)	0.0380 (s2)

Applying T-test to the difference, it yield 6.135 which is highly significant at more than 99.5%.

T-test = $(T_1-T_2)/[\text{square root of } (s_1^2/n_1 + s_2^2/n_2)]$
Degrees of freedom(df) = $(n_1 + n_2 -2) = 5 + 5 - 2 = 8$
from table of T Distribution, df of 8 at $t_{.995}$ is 3.355

This indicates that harvesting for essential oil in the winter months will be more productive and it confirms established practice and present knowledge. A possibility of variation in oil yield may relate to the variation of water content of the biomass. Study of growing sites for *Thuja* show one-third to one-half of precipitation occurs during the warm season (Johnston 1970).

Azeotropic drying was done on the last 6 months' samples to obtain data on moisture content. Unfortunately the azeotrope data do not cover the whole year because the experimentation was well underway when this point was kindly drawn to my attention.

Table 3

Results of Azeotropic Drying

	Moisture (%)
April	59.0
May	64.5
June	62.0
July	61.0
August	59.0
Sept	57.0
February'99	54.0
Average	59.4

The warmer (April to August) months' moisture content was 61%. Compared to the February 1999 datum of 54% it was wetter by 11.5%. This shows that harvesting in winter months is more productive because bulk volume to distil is less and oil yield is higher.

5.2 Carbonyls by the 'Sukh Dev' and 'Girard' methods

In the carbonyl fractionation, 'Sukh Dev' carbonyl rich oil fractions were obtained in two separate experiments, each time using the same 'Sukh Dev' method. The first experiment to separate the carbonyls was obtained by using one (1) gram of the November 1997 untreated essential oil. The second experiment also used a "November 1997" oil, but the Sukh Dev procedure was applied to the residual oil after an aldehydic extraction using "Okamoto" method had already done. This second residual oil still contained carbonyls though presumably only the ketonic type. The carbonyls recovered in these experiments were 67.4% and 69.0% respectively which is consistent with the expectation of the previous literature that the cedar leaf essential oil contains about 70% carbonyls. The 'non-carbonyls' fractions obtained from the two experiments were 15.4 and 14.8% respectively. There is about a 17% mass balance discrepancy for each experiment. This deviation can be assumed because both the non-carbonyl and carbonyl fractions were volatile and the compounds can to a certain extent codistil under the vacuum which was required to remove the cyclohexane solvent used in the separation. Fortunately it would be expected that the loss would come mainly from the more volatile non-carbonyl fraction as demonstrated by the fact that these compounds have the shorter GC retention times. Qualitatively the 'non-carbonyl' fraction has a much more prominent proportions of short retaining peaks than the carbonyl fraction and the non-carbonyls contain the long retaining materials which elute around 30 minutes. Eventhough the fractionation looked successful, it cannot be denied that the 'non-carbonyl' fraction still contains prominent and significant peaks in the areas where thujone, isothujone, fenchone elute. Further work would have to be done to establish whether these peaks are residual ketone arising

because the amount of the Sukh Dev reagent was less than optimal or whether these peaks arise from non-carbonyls which co-elute with the carbonyls.

The control fractionation done with the traditional Girard T reagent showed the same behaviour: a dramatic reduction in the intensity of the early eluting peaks and the late eluting doublet. In similar fashion the 'non-carbonyl' fraction from Girard T still contained strong signals where the typical carbonyls come. In the results from the Girard T fractionation, there was 48% carbonyl, 34% non-carbonyls and 18% mass loss. The very high 34% residual 'non-carbonyl' fraction from Girard T contrasts strongly with the Sukh Dev fractions of 15.4% and 14.8%. Perhaps some sterically hindered carbonyls were reluctant to react with the Girard T reagent.

Neither the treatment with Sukh Dev or Girard T reagent have been optimised with respect to formation or decomposition; so not much should be made of the comparative yields and purities other than to say that the Sukh Dev method is competitive as a starting point for a process.

Extracted Sukh Dev carbonyl fractions from untreated and residual oils and the Girard T carbonyl fractions were analysed by GC.

Figure 4 G.C. of Carbonyls extracted by Sukh Dev method from November 1997 oil

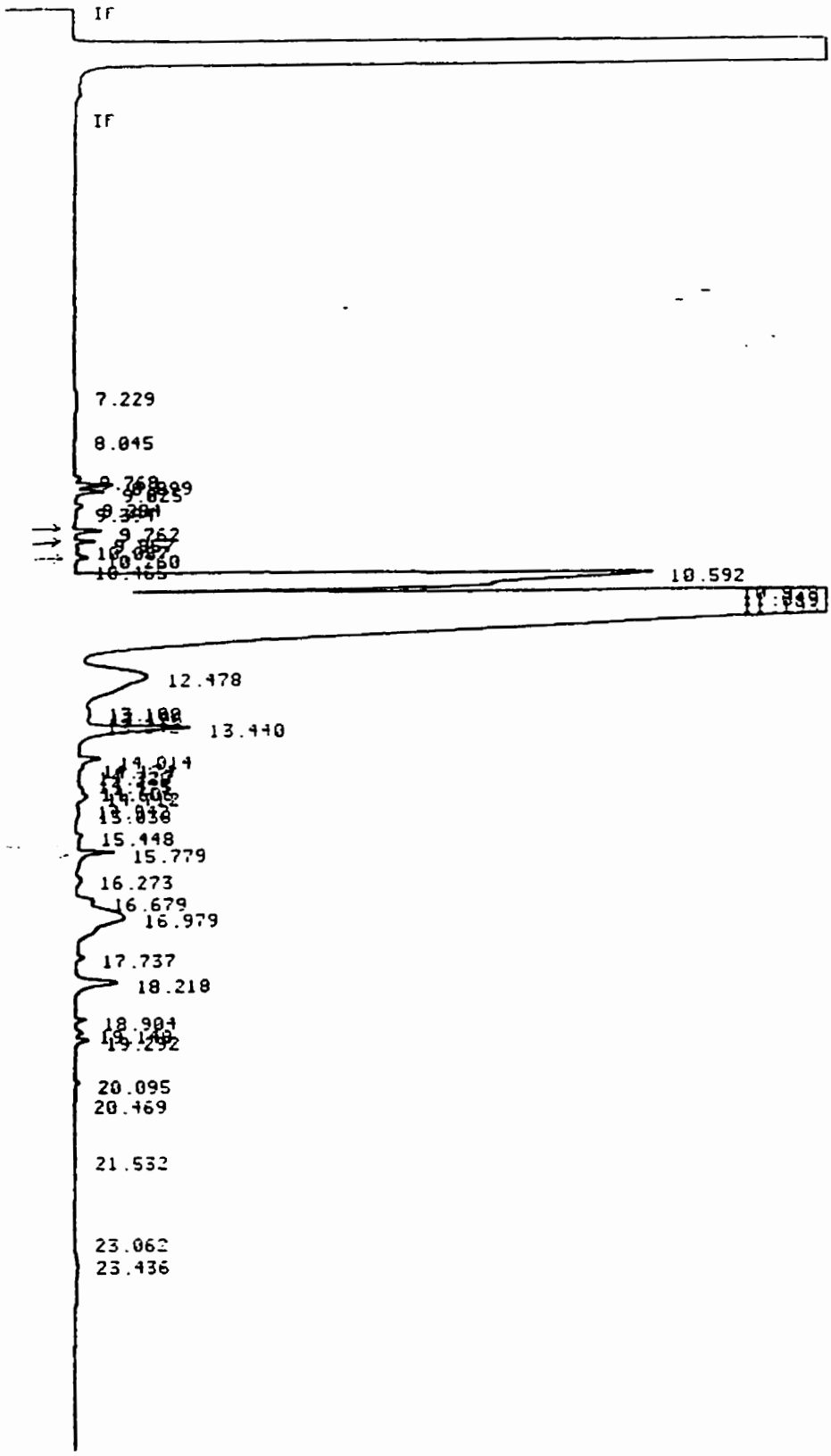


Figure 5 G.C. of carbonyls extracted by Sukh Dev method from residual of Okamoto

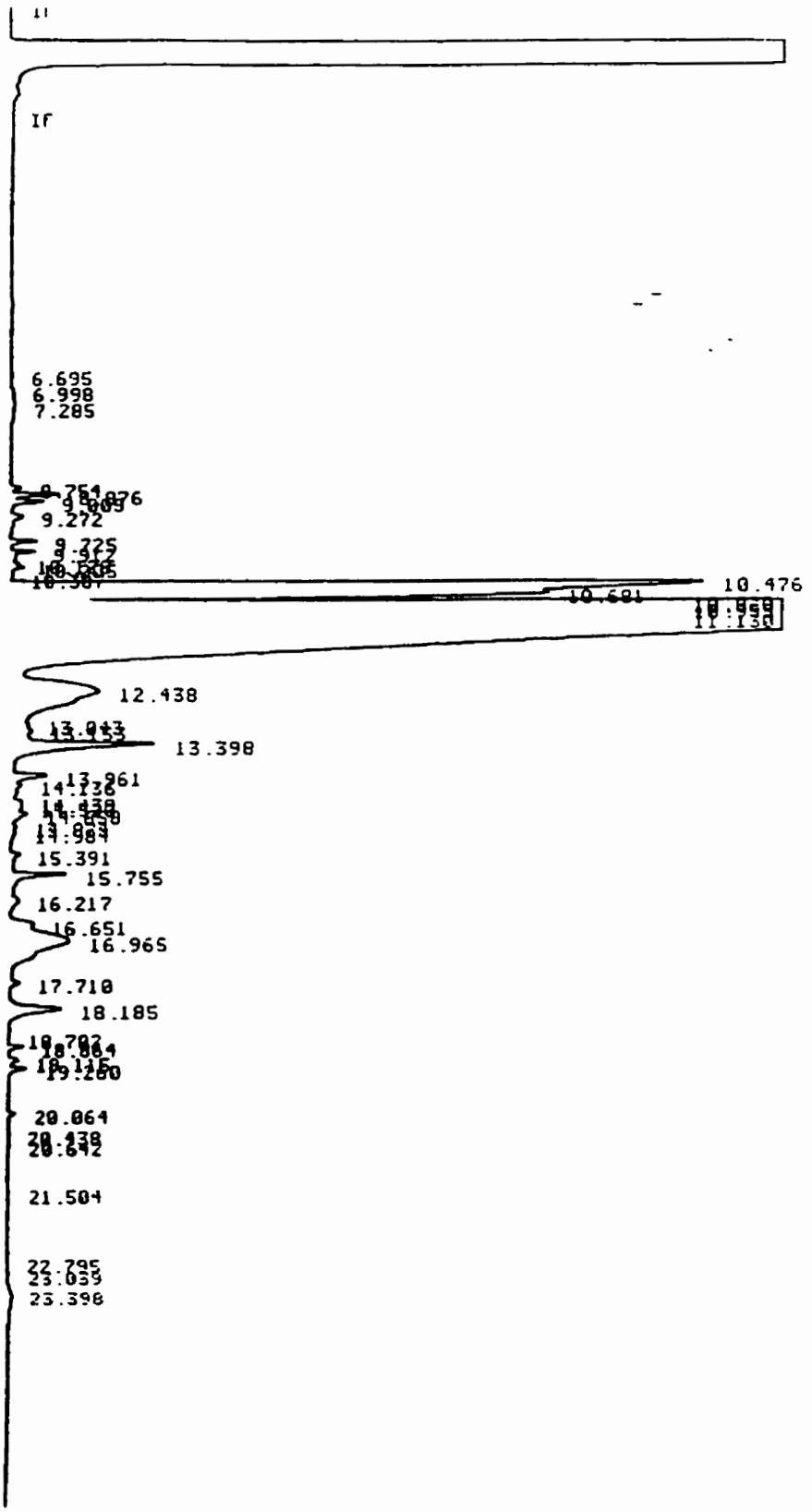
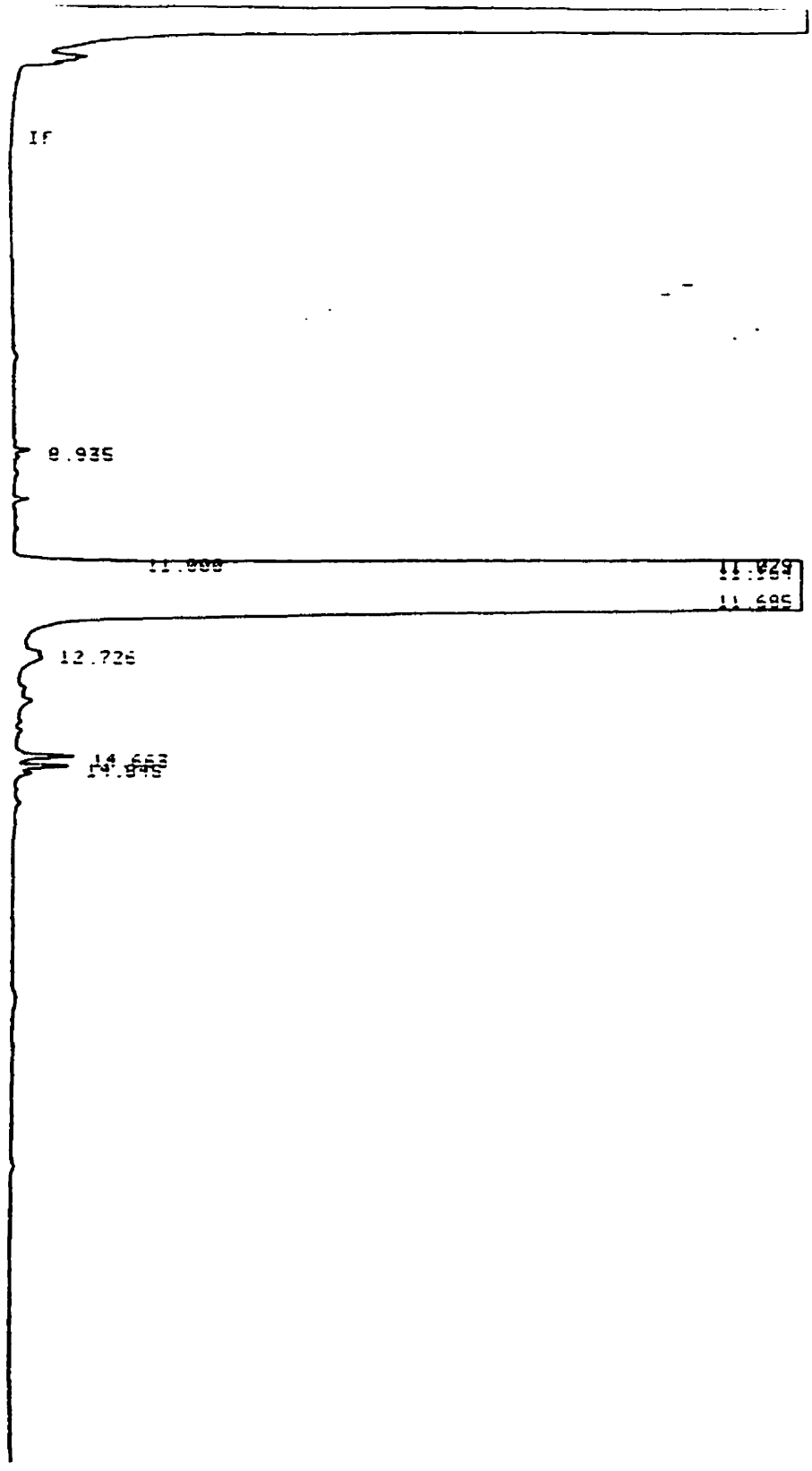


Figure 6 G.C. of carbonyls from Girard T method from November 1997 oil



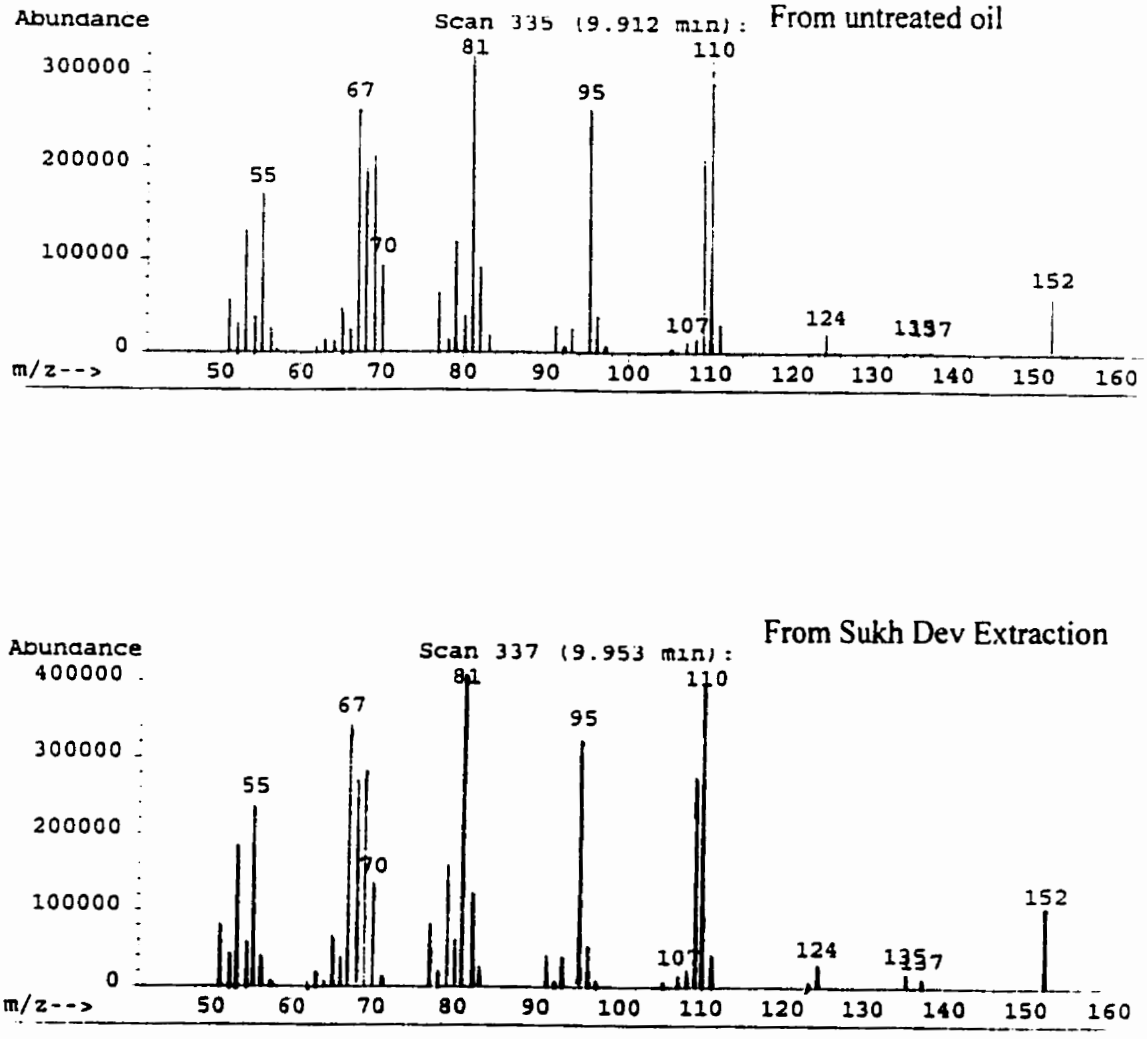
On analysis with GC/MS, it was possible to identify Thujone, Fenchone and Camphor in the original untreated oil as well as in the Sukh Dev carbonyl extraction.

There is no distinction between thujone and iso-thujone because they are inter convertible under acidic or basic conditions with the intermediate enol. From the chemical data base thujone mass specs shows fragmentation pattern with initial molecular ions at 152 and peaks at 124, 110, 95, 82, 81, 79, 69, 68, 67, 55, 41, 20.

The fragment peak at 124 can be ascribed to a stable molecule in thujone that is easily knocked off. That molecule is carbon monoxide (CO) with a mass of 28 (C=12, O=16). Therefore the initial molecular ion of 152 minus 28 is 124. The fragment peak at 110 can also be another stable molecule, ketene (C_2H_2O with mass of 42).

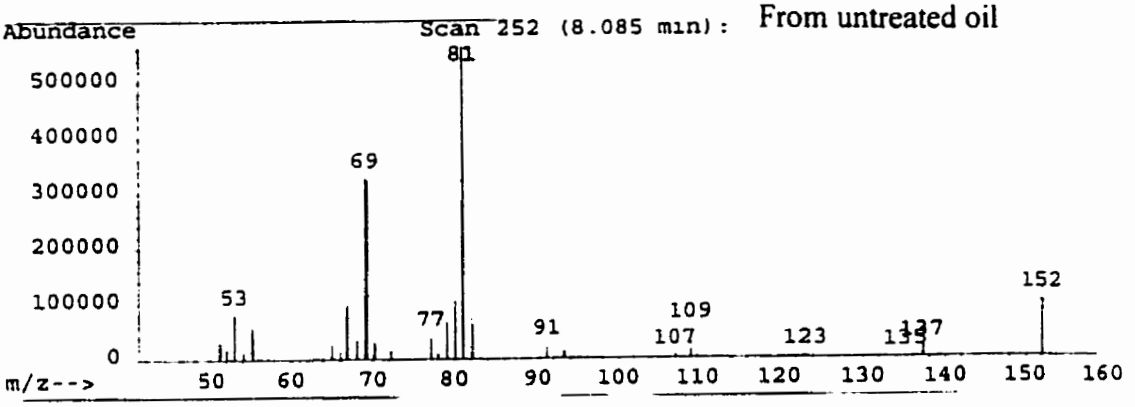
In the untreated oil, thujone was found between retention time of 8.9 - 9.9 minutes. The extracted thujone with the Sukh Dev method appeared between 9.0- 9.96 retention time.

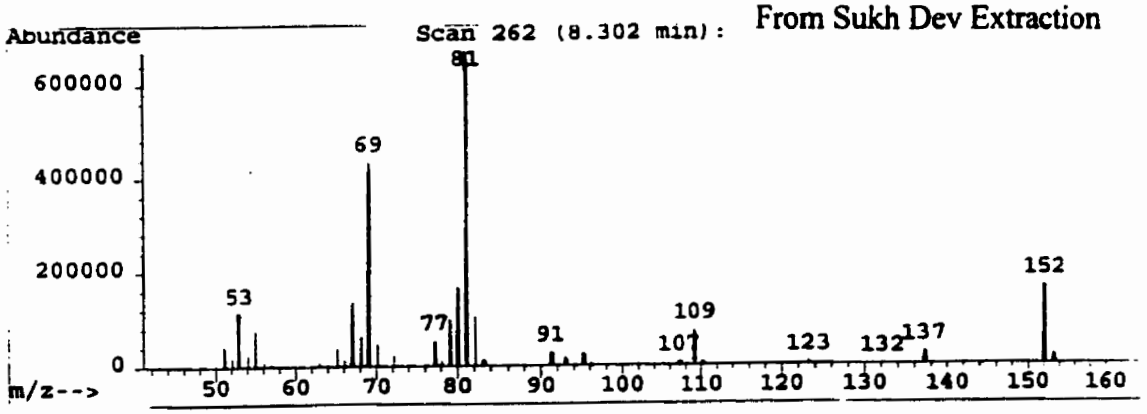
Figure 7 GC/MS of Thujone - November 1997 oil



Fenchone has methyls at all its α positions and is a rearranged terpene that do not follow the head to tail isoprene rule discussed in the introduction. In Buchbauer (1981), the fenchone mass spectrum has fragmentation ions at 152, 123, 108, 95, 81, 80, 79, 69, 67. The commonest or most prominent (highest) peak of fragmentation is at 81. In the original untreated oil, fenchone was observed between 8.085 and 8.415 minutes retention time. In the carbonyl extraction by Sukh Dev, fenchone was found between 8.0 to 8.7 minutes retention time.

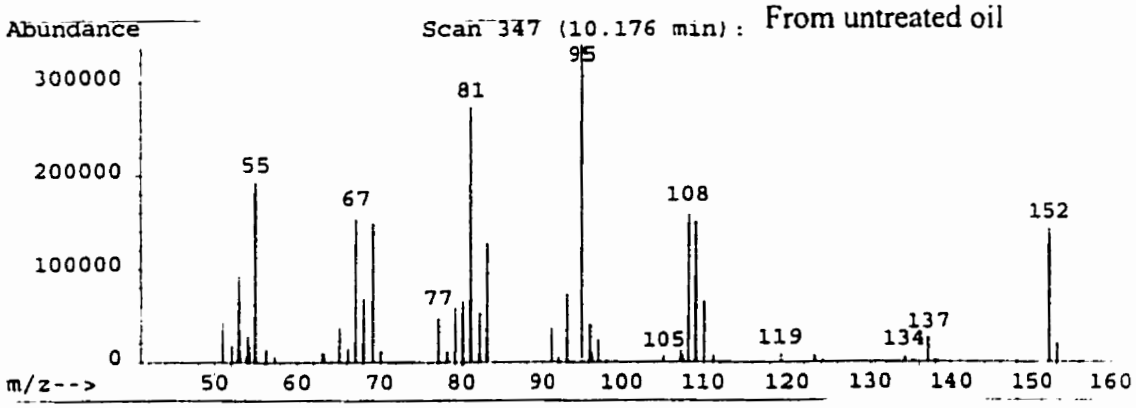
Figure 8 GC/MS of Fenchone - November 1997 oil

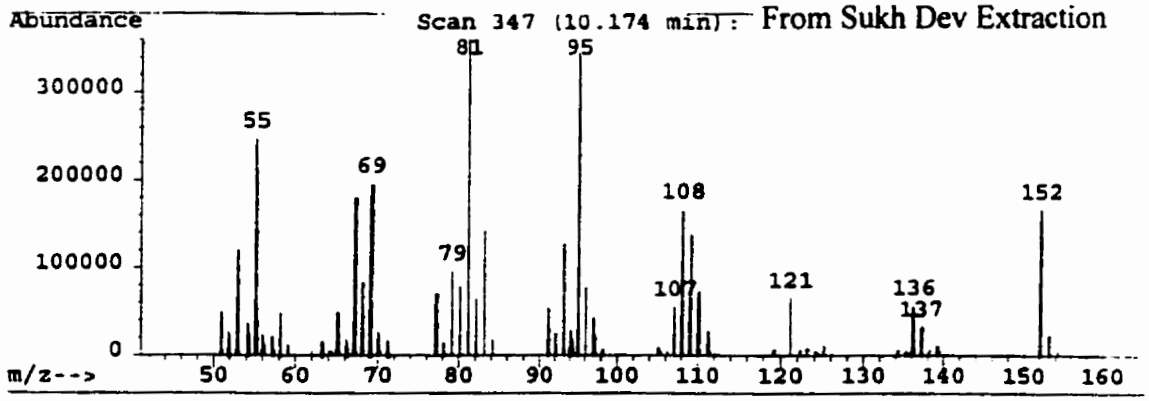




Mass specs data from Weinberg (1966) indicates fragmentation pattern for camphor at 152, 137, 110, 109, 108, 95, 83, 81. In the untreated oil camphor was located between 10.117 and 10.176 retention time. In the extracted carbonyl by Sukh Dev, camphor was found at 10.174 minute.

Figure 9 GC/MS of Camphor - November 1997 oil



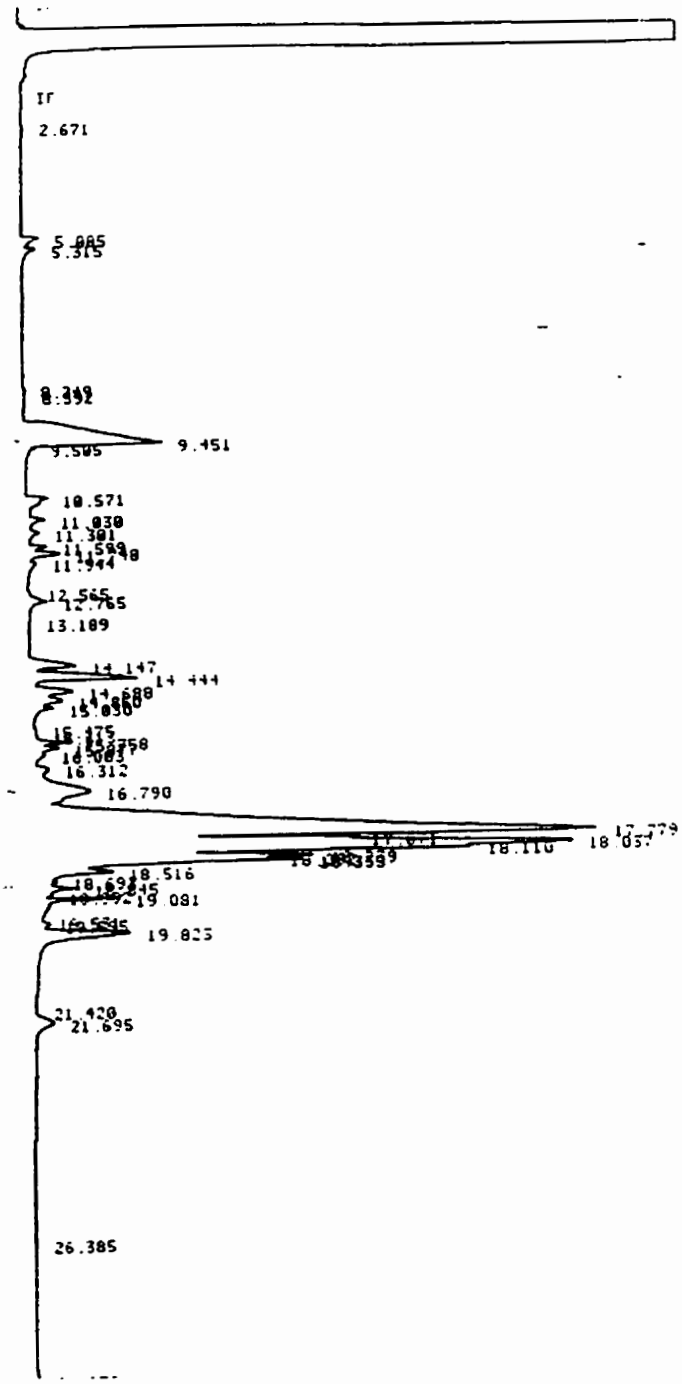


5.3 Aldehydes by the 'Okamoto' method

The aldehyde extraction using the 'Okamoto' method was also made using the November 1997 cedar oil. Out of three gram of cedar oil used, the extracted aldehyde fraction amounted to only 0.0018g. and had an 'off' unpleasant odour. The residual 2.6368g 'non-aldehyde' oil differed in no apparent way from the original when the simple GC trace was examined. This is not surprising considering the very low level of aldehydic material extracted. At this level, there was a serious risk that all that the extract might have contained was artifact. What I mean by artifact here was possibly a small quantity of substances which may be the reagent, or the compound derived from the reagent, or the compound derived from the reagent reacting with the sample, or the impurities in the solvent, etc. To test the possibility of an introduction of artifact, a "blank": that is, using the procedure in Okamoto without adding cedar oil was performed. The blank yield 0.0001g but no odour was detected. This proved that Okamoto method did extract something with an "off" odour from the cedar oil.

The GC graph on this small residual sample shows a series of peaks, the larger observed peaks comes between seventeen and twenty (17-20) minutes.

Figure 10 G.C. of Aldehyde from Okamoto extraction from November 1997 oil



A GC/MS was run and sample of mass specs were done around the peaks, but none showed any semblance to aldehyde. A scan to find abietal on the untreated oil yielded a hit around 35.6 -35.76 minutes, but looking at the GC trace, I cannot see any real peak at this retention time

Aldehyde is a compound where the next fragmentation peak should be a loss of 29 mass (CHO). In the GC/MS of the untreated and Okamoto oils, all the scans taken showed highest peaks of molecular ions with even number M/e peaks at relatively high mass, such as M/e 168, M/e 170. In order to further confirm that we were not seeing artifacts, we looked in the original November 97 untreated cedar oil in the GC/MS for all the peaks that contain M/e 168 or M/e 170. The software associated with the GC/MS instrumentation allowed the search through the total ion current for all the peaks looking for particular M/e peaks. We found eight (8) retention times, one at the start of the run, one at more than 28 minutes and the rest between sixteen and eighteen minutes. The span of this latter group of retention times was the same as the major substances found in the Okamoto fractions (Fig. 20). Although there were marked similarities between the mass spectra of some of these components of the untreated oil and the trace Okamoto extract, in no case were identical spectra identified. One can only say that Okamoto did extract some compounds from the cedar oil during the experiment, but some transformation must have occurred in the process. Acid components would be expected to pass through the separation and contaminate the aldehyde fraction (Okamoto and Ohta 1979). This contamination would not be expected to have any substantive effect if the aldehydic fraction was substantial but since the aldehyde fraction is so minute, a small amount of

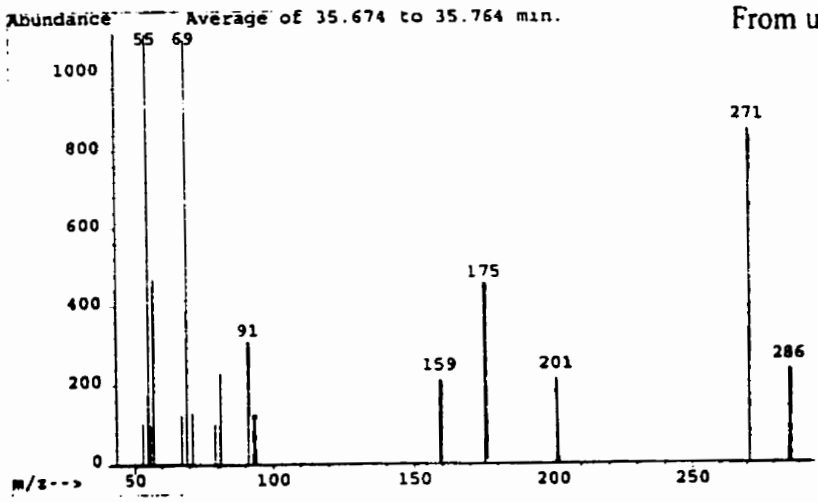
contamination would have a serious affect. But again, aldehyde is very volatile and can easily form into other compounds. The six-amino caproic acid used in this experiment is very polar, and breaks readily into ions and ions do not go into gas, so it is hard to see it in the GC. Most of the terpene components are C_{10}, a molecular weight of 120. If the dehydrating product of 6-aminocaproate is caprolactam $C_6H_{11}N0$ (molecular weight of 113), any combination with terpene component will result in molecular ions of more than 300. Large aliphatic aldehyde may also not show molecular ion on the CG/MS; however, the highest mass ion in the mass spectra might then be expected to show loss of ethylene (McLafferty).

Something should be said about aldehydes which have previously been reported *in Thuja occidentalis L.* The distillation did not produce E-2_hexenal, a very volatile compound which decrease steadily with prolonged storage, especially at room temperature (Simard 1988). Perhaps the prolonged period between cutting and distillation compromised this substance.

Since the aldehyde abietal had been reported (Kamdem and Hanover 1993) a search was made in the untreated oil to locate a mass of 271 which Tabacik (1971) reported as a typical principal fragment of abietal. A match was found at around 35.6 minute retention time. In this untreated oil to the naked eye there was no material eluting at this retention time so if abietal is present it is very much a trace component. The mass spectrum was taken covering the time between 35.67 to 35.76 minutes which exhibited a pattern characteristic of abietal, containing a base of 286 and a substantial ion at 271. No such

match was found in the Okamoto treated oil showing that abietal if present had not been extracted and definitely had not been concentrated.

Figure 11 GC/MS of Abietal - November 1997 oil



5.4 GAS CHROMATOGRAPHY

The twelve month cedar oils were analysed qualitatively (peak shape and position) and quantitatively (relative peak area). All of them show similar pattern with first distinct high peak around seven (7) minute and a group of higher peaks from nine to twelve (9-12) minutes and the last high peak at around 29 minutes.

Chemical literature data were used to compare the identities of these components. From Kamdem (1993) the major ketone components are thujone, fenchone and a much smaller quantity of camphor.

The aldehyde component is abietal and in Simard (1988) it is E-2-hexenal.

Figure 12 G.C. of October 1997 *Thuja* oil

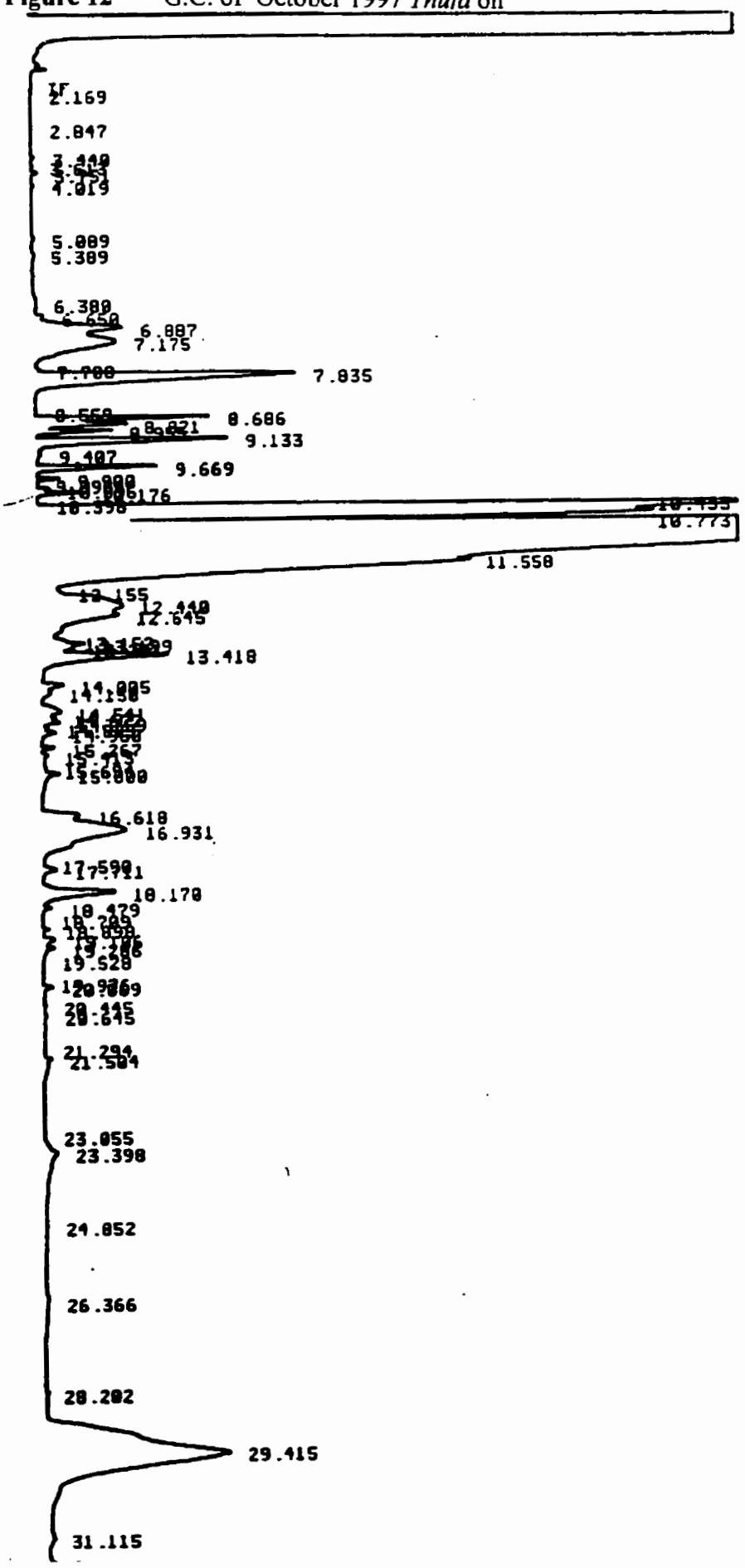


Figure 13 G.C. of November 1997 *Thuja* oil

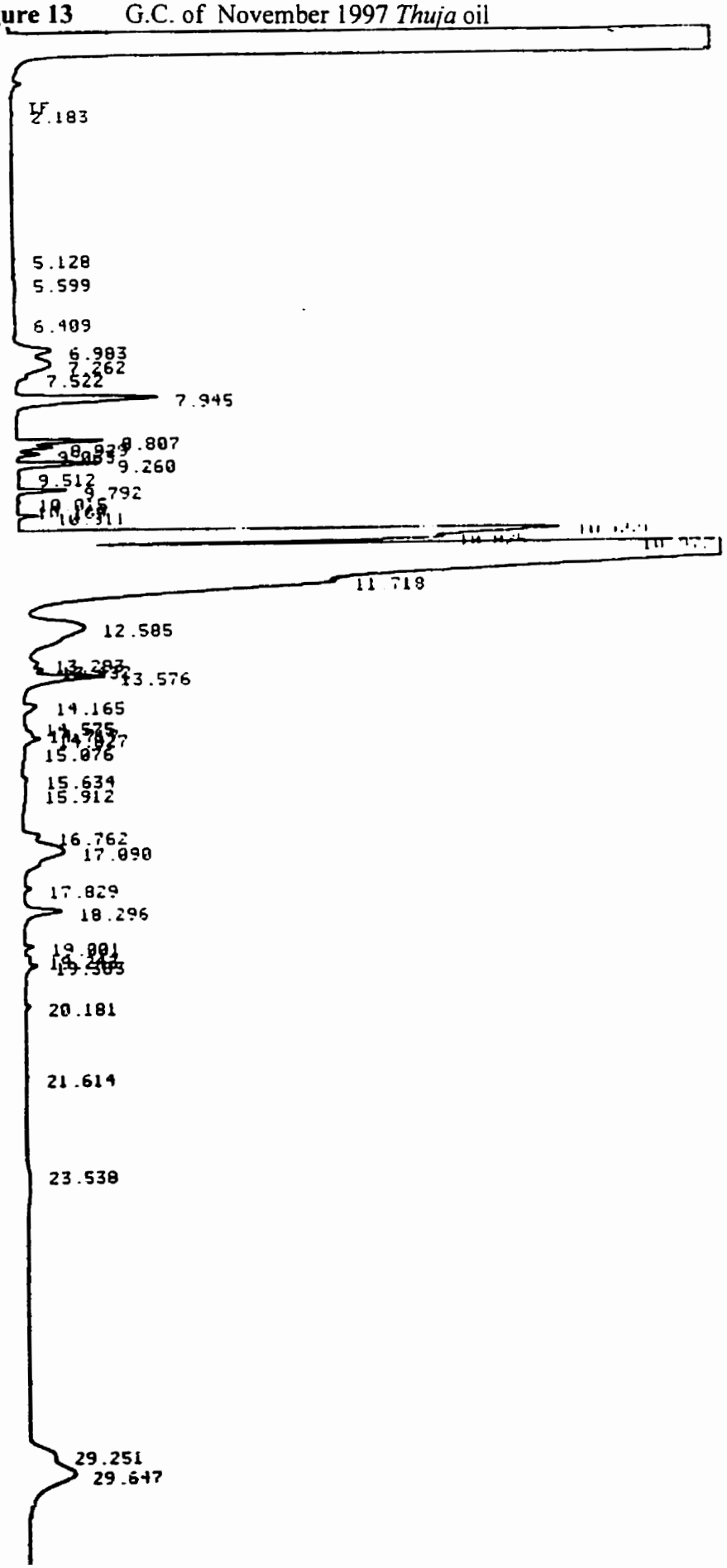


Figure 14 G.C. of December 1997 *Thuja* oil

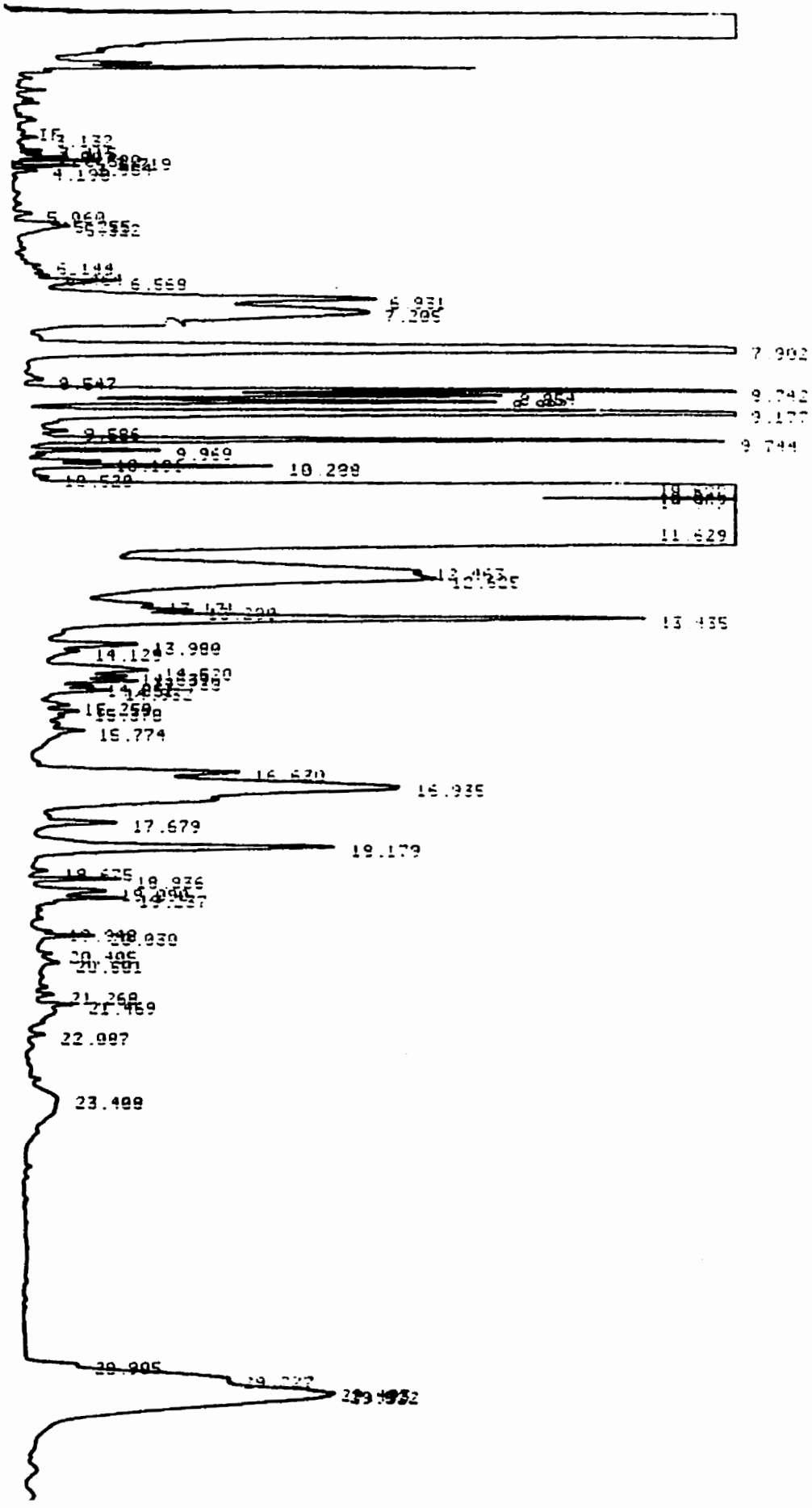


Figure 15 G C. of January 1998 *Thuja* oil

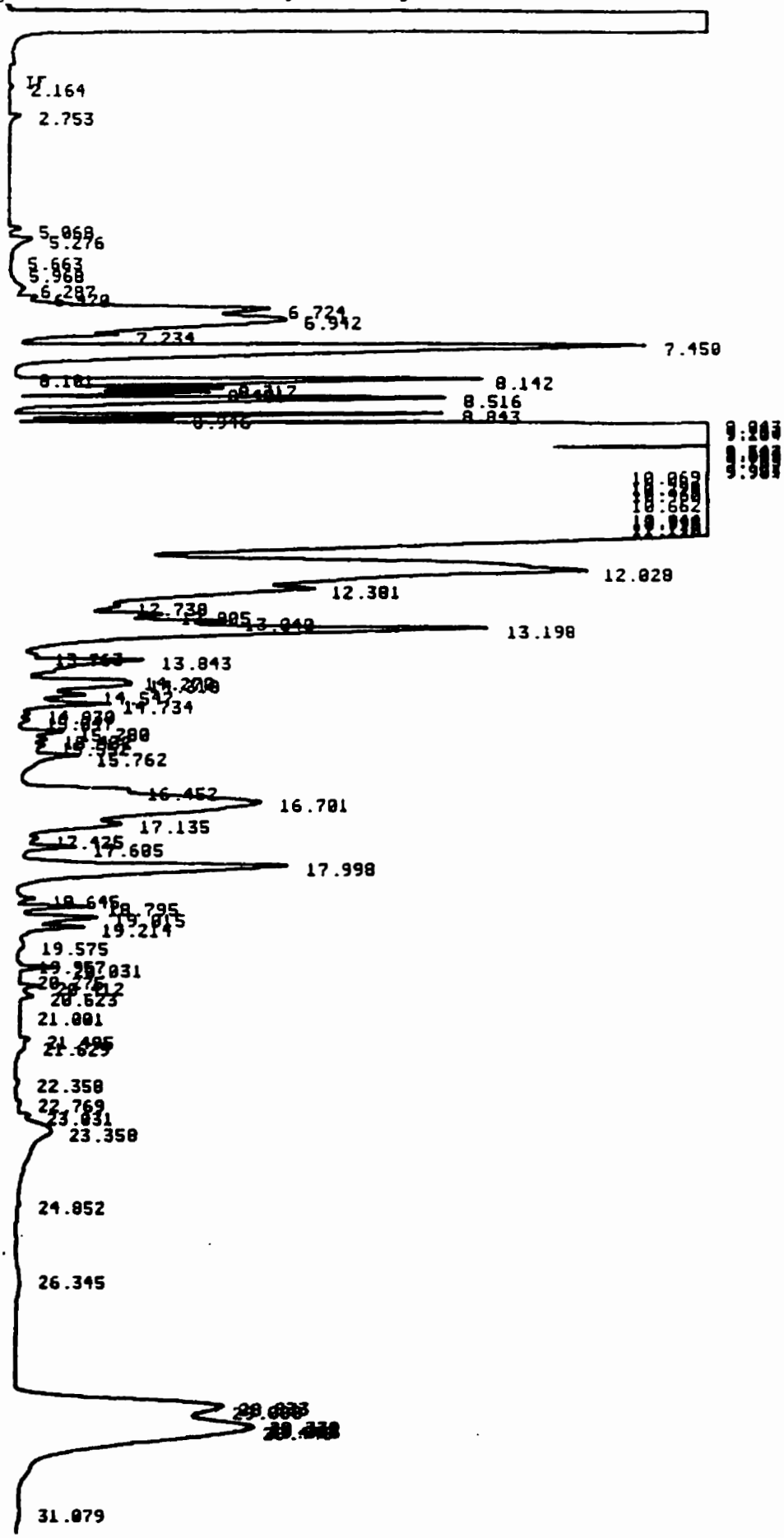


Figure 16 G.C. of February 1999 *Thuja* oil

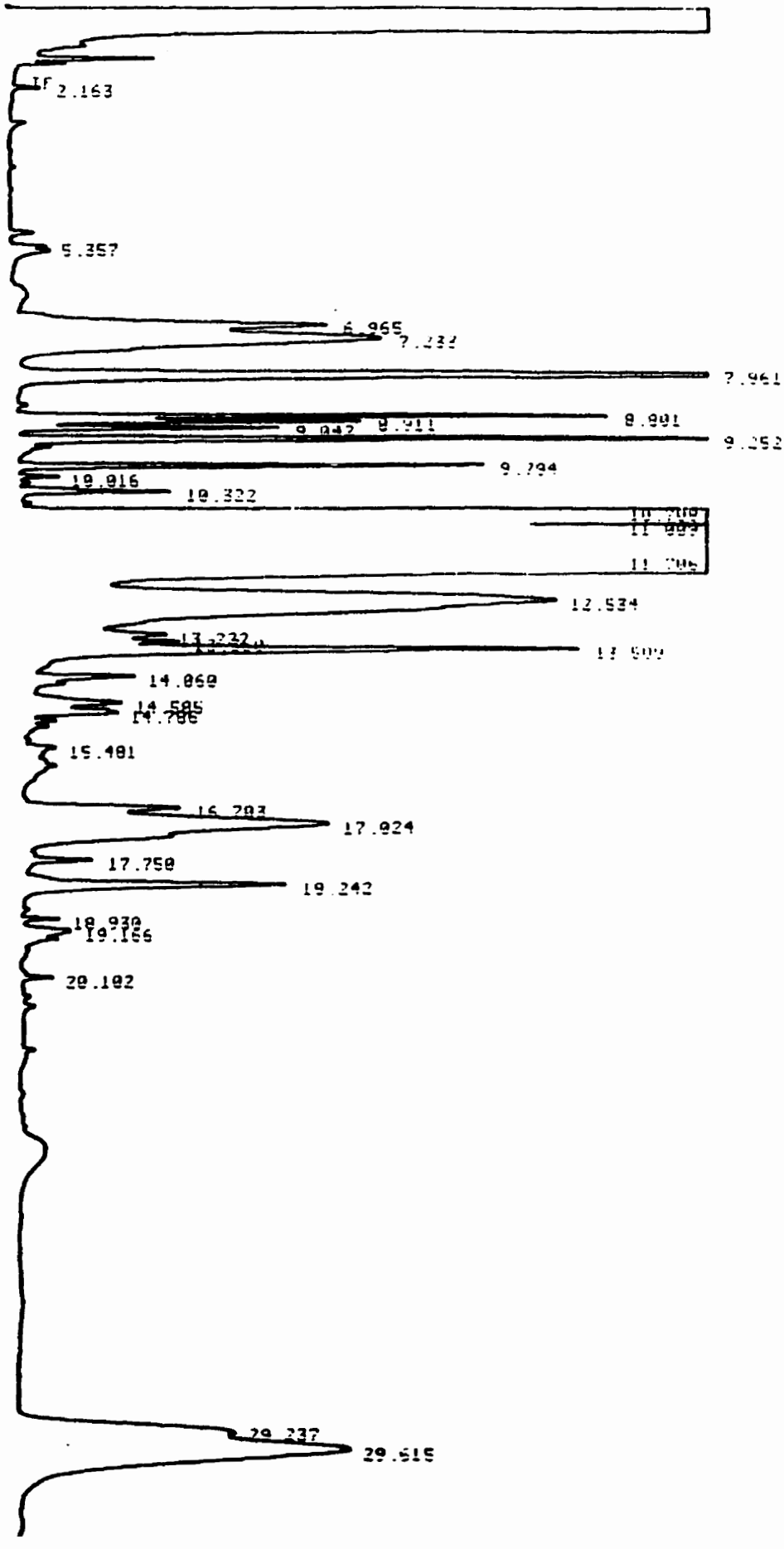


Figure 17 G.C. of March 1998 *Thuja* oil

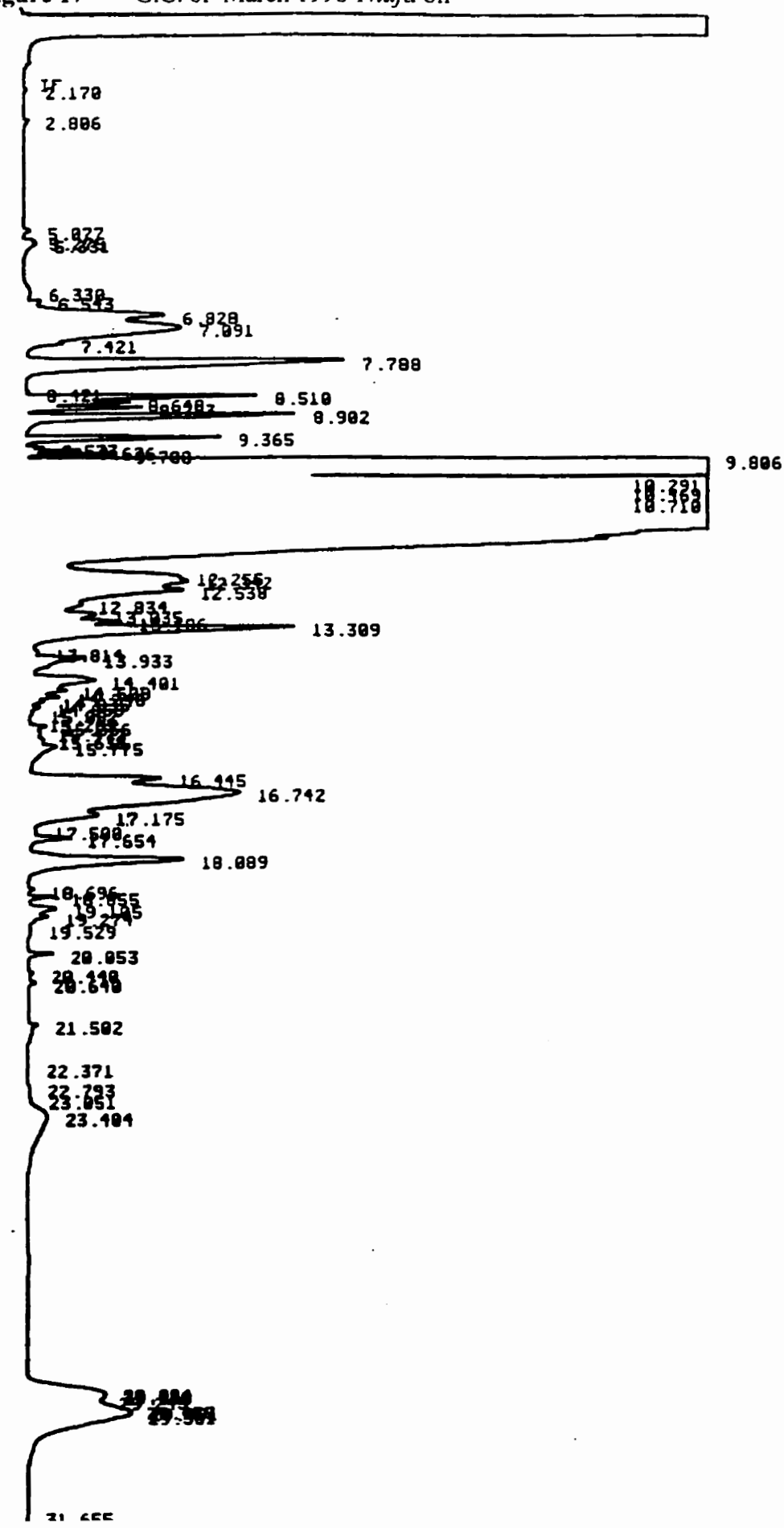


Figure 18 G.C. of April 1998 *Thuja* oil

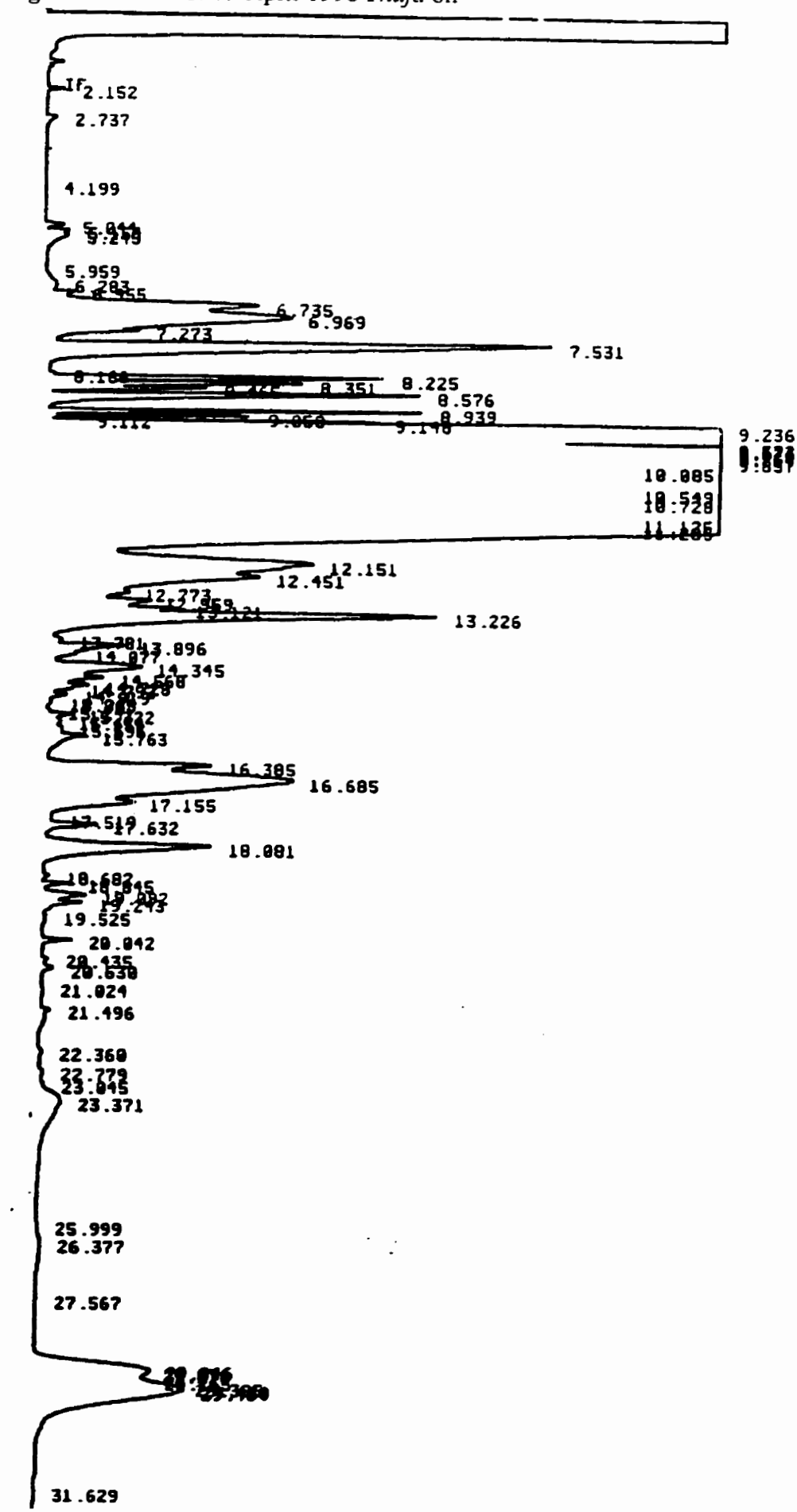


Figure 19 G.C. of May 1998 *Thuja* oil

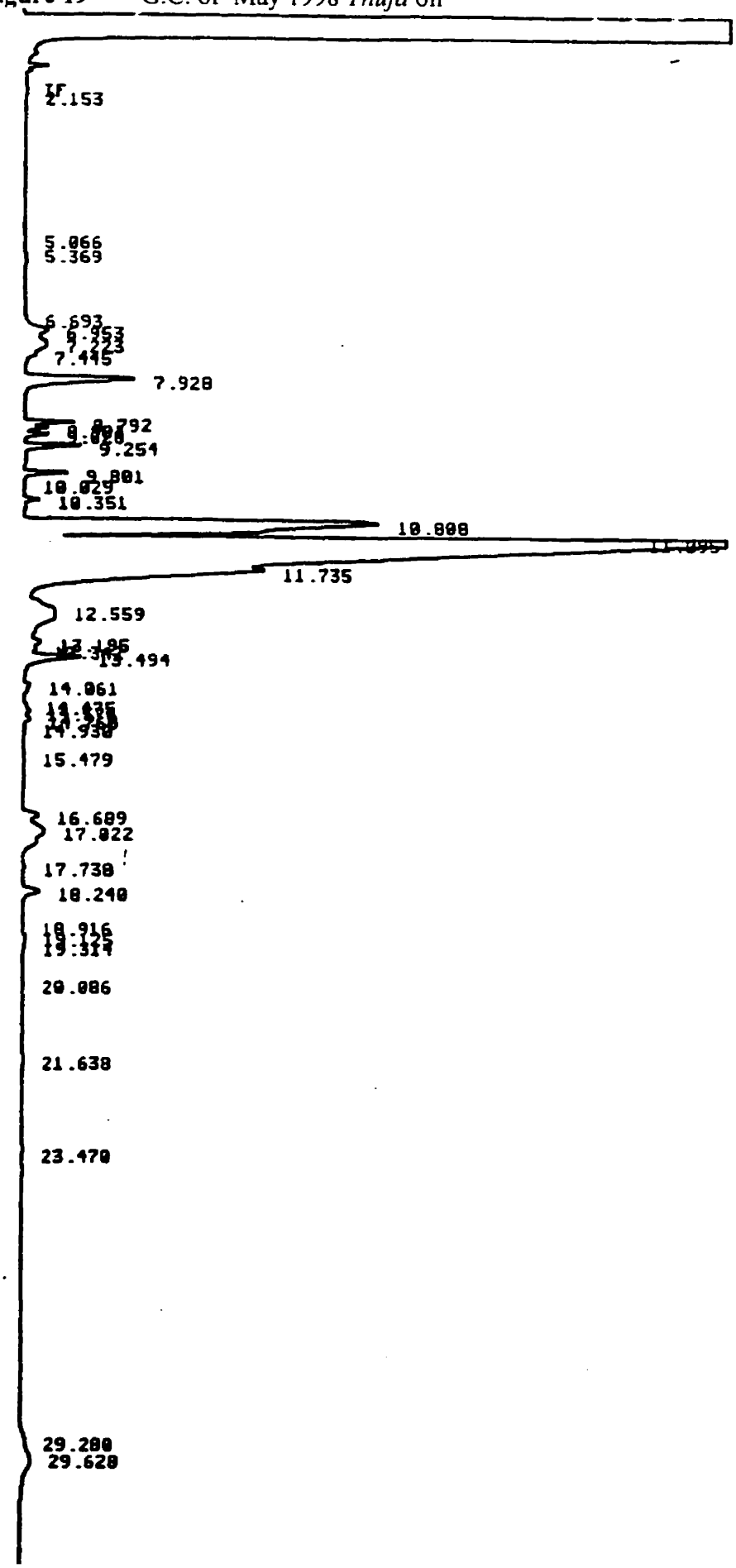


Figure 20 G.C. of June 1998 *Thuja* oil

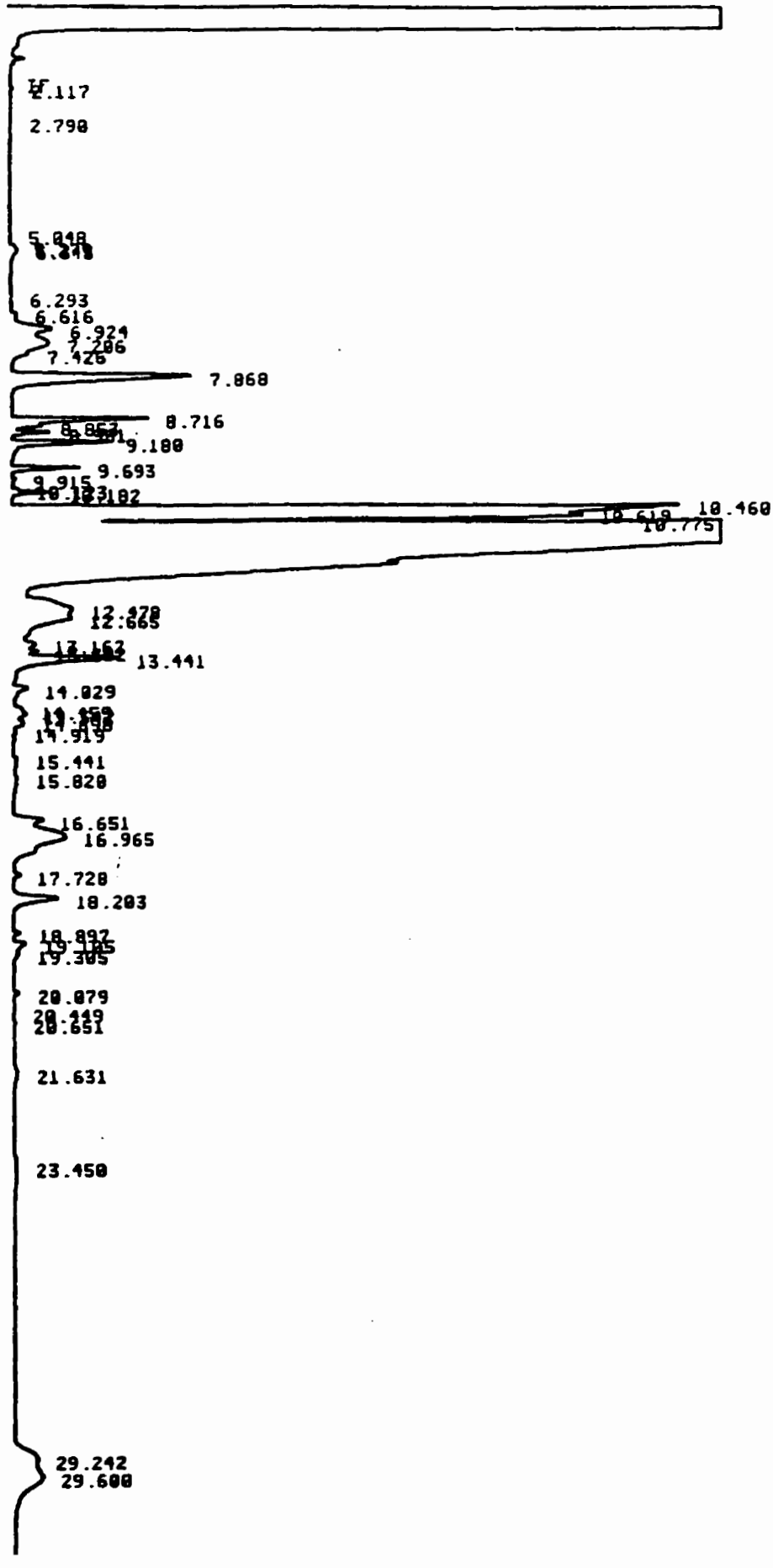


Figure 21 G.C. of July 1998 *Thuja* oil

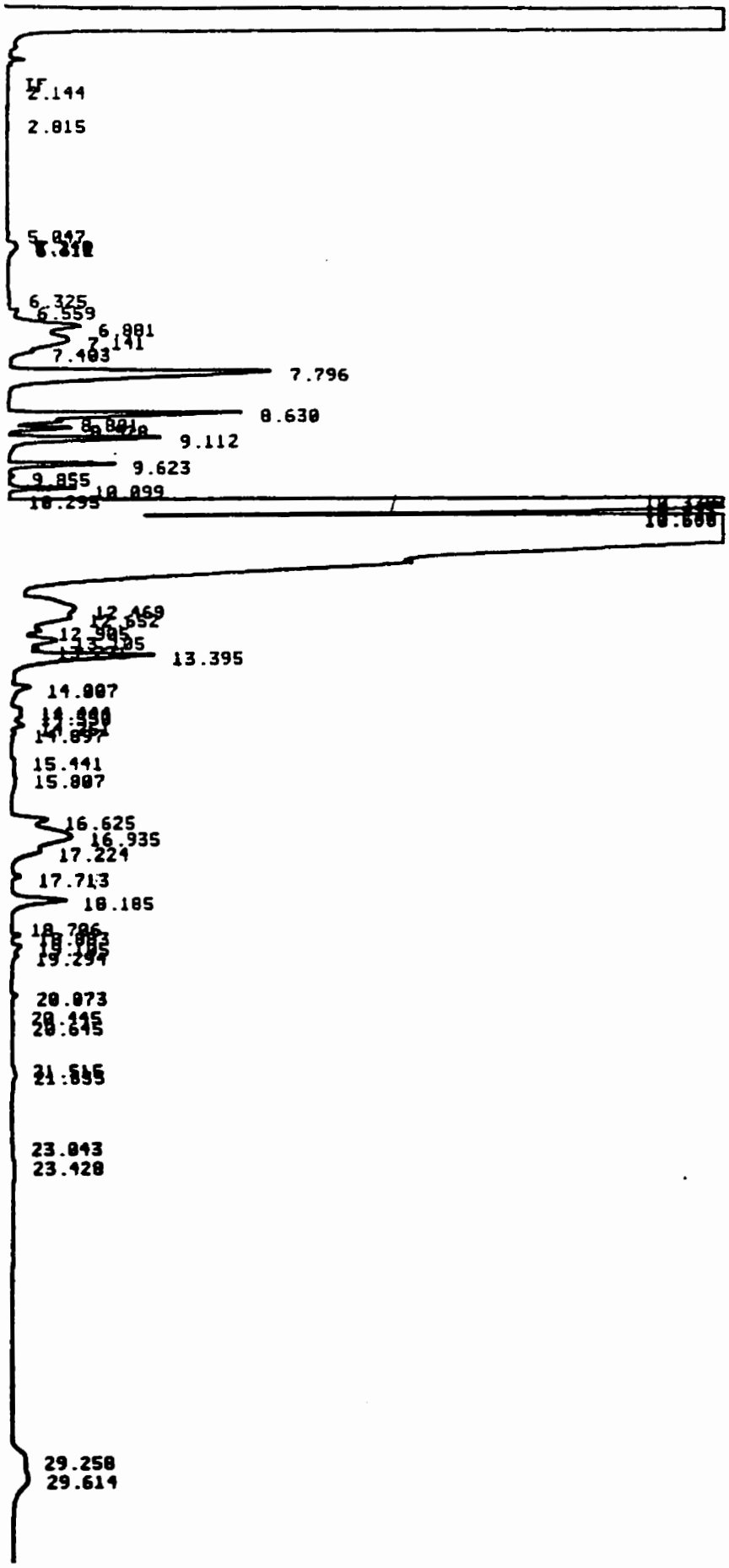


Figure 22 G.C. of August 1998 *Thuja* oil

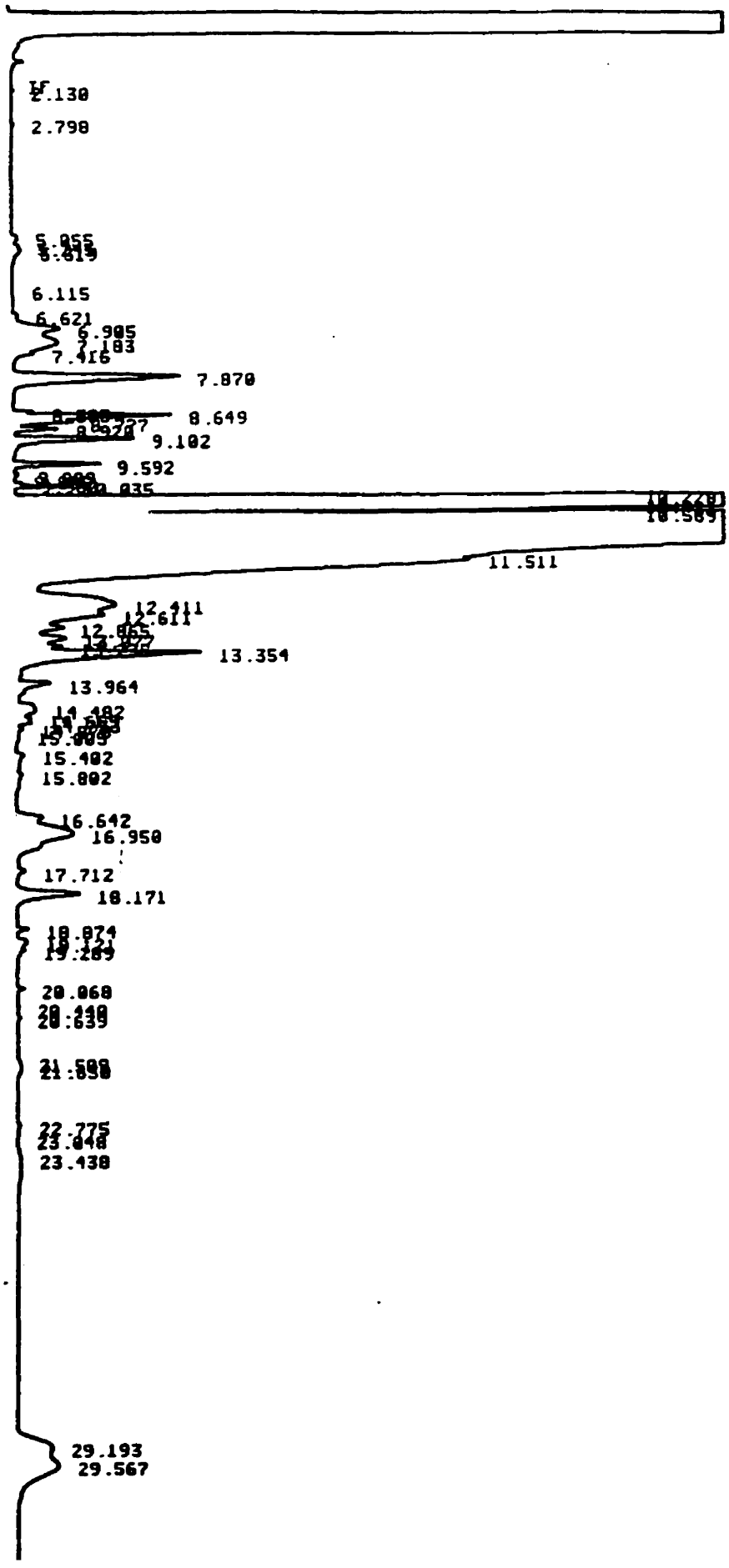
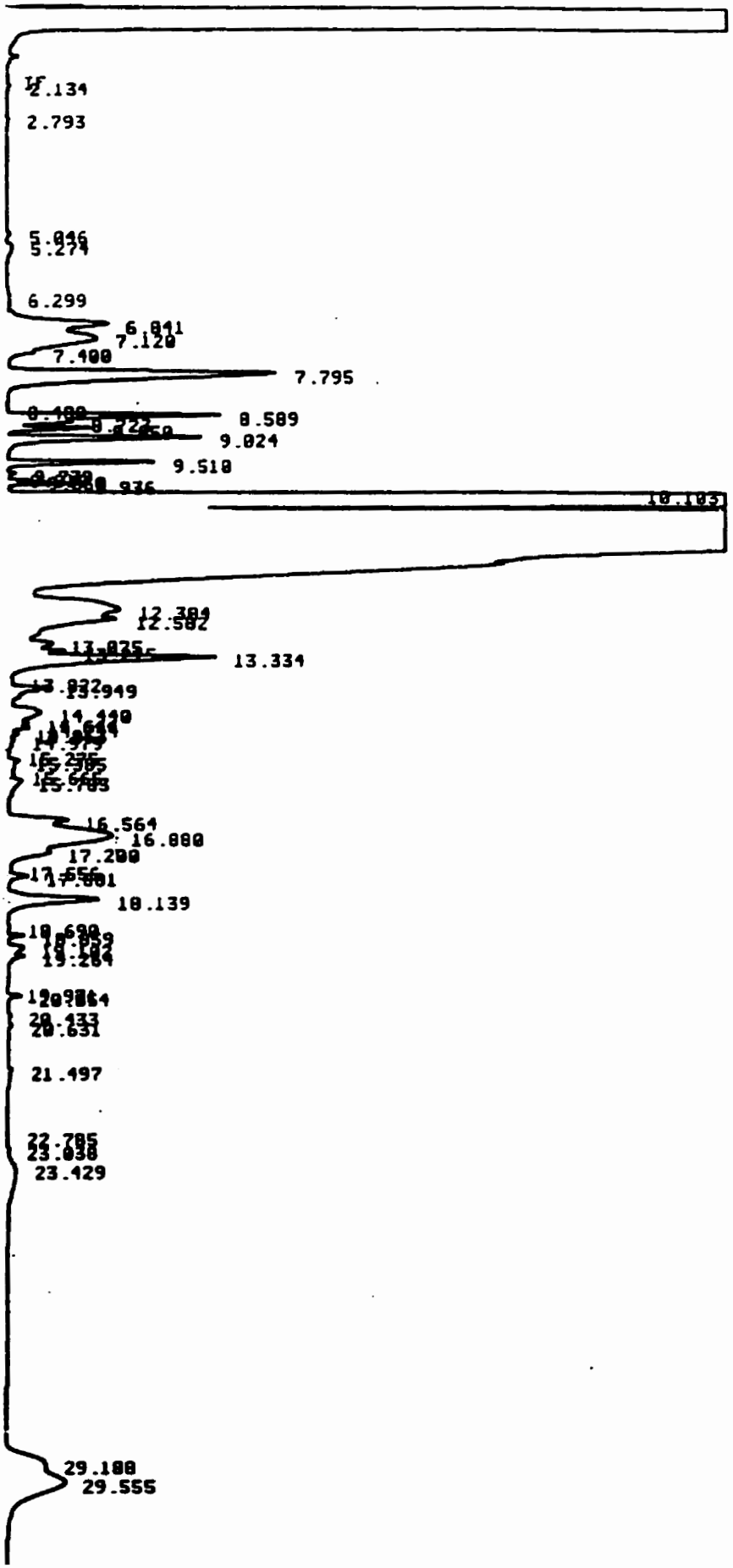


Figure 23 G.C. of September 1998 *Thuja* oil



6.0 CONCLUSIONS AND RECOMMENDATIONS

The seasonal yield of cedar leaf oil ranged from a low of 0.59% for July to a high of 1.06% in December (fresh weight basis). In the summer months the yield was approximately 30% lower than in the winter months. results which recommend in favour of winter harvest and extraction.

Analyses for isolating carbonyls using the Sukh Dev technique yielded promising results. Comparison in carbonyl extraction between the Sukh Dev and the known Girard-T reagent showed that both techniques can extract carbonyls in similar quantities. However. the Sukh Dev approach may be preferable for industrial application because of its simplicity.

The non-carbonyl fraction of the "Sukh Dev" extraction was also analysed by GC/MS. Thujone. fenchone. and camphor were still present though in much reduced volume. This indicates that the Sukh Dev reagent did not capture all the available carbonyl containing molecules.

After fractionation the oil was also analysed by gas chromatography and mass spectroscopy to determine its main chemical components. The main chemical components of the oil were thujone and fenchone.

The 'aldehydic' extraction of the oil using the Okamoto technique, showed some very minute yield with a distinct off smell. It is difficult to state what type of aldehydic molecule was extracted since there was no matching to the known abietal aldehyde or to the mass spectrum from any particular retention time of the untreated oil..

One possibility for future experiments is to first treat the oil with a mild base such as aqueous bicarbonate before subjecting the oil to the Okamoto type analysis, in case there are acidic components. The biosynthetic pathway for oil synthesis would also be of considerable scientific interest.

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