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The Philippine Journal of Science is a journal on basic sciences published quarterly by the Science and Technology Information Institute - Department of Science and Technology (STII - DOST) with editorial office in Bicutan, Taguig, Metro Manila.

OUR COVER. The cover, taken from the second manuscript in this issue, is a sanger sequencing gel obtained from the first round of purification of mutants. It is the complementary strand (containing anti-codons) which is being read here. Only C, G and T lanes were run. The first three lanes confirm the mutation of Lys 41 to Ala. The anti-codon for Lys-41 (wild-type CTT) has been changed to CGC for Ala. The last three lanes are the C, G and T lanes for the Thr 45 (GGT) to Asp (GTC) mutation.

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THE LEAF VOLATILE OIL OF *NOTHOPANAX FRUTICOSUM* (L.) MIQ.

LUZ OLIVEROS-BELARDO[3], ROGER M. SMITH[4]
LINDA COLLINS[5] and ROBERT MINARD[5]

ABSTRACT

Hydro-steam distillation of the fresh leaves of Nothopanax fruticosum (L.) Miq. gave a 0.32% yield of volatile oil. GC-MS analysis of the oil showed the presence of α-bergamotene, an oxygenated sesquiterpene $C_{15}H_{16}O_2$, 1-ethenyl-1-methyl-2-(1-methyl-ethenyl)-4-(1-methylethylidene)-cyclohexane, α-elemene, β-bourbonene, β-cubebene, β-bisabolene, α-farnesene, -elemene, β-elemene, α-cadinene, α-elemene and α-copaene.

INTRODUCTION

Nothopanax fruticosum (L.) Miq. and several related species are collectively called *papua* in the Tagalog dialect. Of the *papuas*, the most widely used for decorative purpose are *Nothopanax fruticosum* (L.) Miq. and *Nothopanax ornata* (Bailey) which are quickly distinguishable from the color of their leaves-the former has purely dark green leaves; hence is called green *papua*, and the latter, with its yellow leaves, is known as the golden *papua*.

Nothopanax fruticosum (L.) Miq. is the subject of this research paper. The plant is described (Quisumbing, 1987) as an "erect shrub growing from 1 to 2.5 meters in height. The leaves are decompound, 3-pinnate, and up to 30 centimeters long. The pinnate are 6 to 10, the upper ones being shorter. The leaflets and ultimate segments are very diverse in form, mostly lanceolate, 5 to 10 centimeters long; the terminal segments are usually larger than the others and more often lobed, pointed at the tip, and sharply and irregularly toothed. The flowers are numerous, umbellately arranged, short stalked, borne on terminal inflorescences in the upper axiles of the leaves, and up to 15 centimeters long. The fruit is very broadly ovoid, compressed and about 4 centimenters long."

The plant is used as vulnerary, astringent, febrifuge, sudorific, inhalant, and as relief for neuralgic and rheumatic pains (Pancho, J., 1989).

In the Philippines, it is used as an ornamental plant grown as hedges. The leaves were extensively used as background support in wreaths and sprays until fern fonds became more available for the purpose. When the leaves are thickly inter-twined around a long abaca rope and flowers are stuck thereto, there is built a long floral chain. During certain ceremonial events the chain is held by a line of boys and girls who walk solemnly to the tune of some appropriate music to symbolize unity and cooperation in reaching a common worthy goal.

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The interest in the study of the leaf oil of *Nothopanax fruticosum* (L.) Miq. was aroused by an observation that the leaves repelled cockroaches. The abandoned leaves were left intact with no signs of having been bitten or chewed. This situation created some speculation that the leaves might have emitted some aroma that kept the cockroaches away. Because plant aromas reside in essential (volatile) oils it was decided to subject the leaves of *N. fruticosum* to hyro-steam distillation in order to find out if they contain volatile oil. Sure enough a volatile oil was obtained - a new essential oil from Philippine plant. The oil was found to have pesticidal action on some house insect pests like flies, ants, mosquitoes, dog fleas, head lice, and cockroaches. (Oliveros-Belardo and Z. Galpa-Sadiwa, 1991).

The object of this study was to identify components of the volatile oil of the leaves of *Nothopanax fruticosum* (L.) Miq., particularly sesquiterpenes for which the oil gave a remarkably positive test.

MATERIALS AND METHODS

Collection and identification of the leaves

The leaves of *Nothopanax fruticosum* (L.) Miq. that were used in this investigation were gathered in some districts of Metro Manila and in the province of Cavite, Philippines. They were authenticated by Professor Juan V. Pancho, formerly of the Systematic Botany Laboratory of the University of the Philippines at Los Baños, Laguna, and by Dr. Wilfredo Vendevil, Chief Botanist of the Philippine National Museum, Metro Manila.

Obtention of the oil from the leaves

The fresh leaves were weighed and loaded in a laboratory hydro-steam distillation set-up that was provided with a Clevenger receiver. Distillation was carried out until no more oil distilled over. The oil was separated from the aqueous distillate, then dried with anhydrous sodium sulfate, transferred into nitrogen-filled amber colored bottles and stored under refrigeration until used for analysis.

Some properties of the oil

Samples of the oil were observed for properties like color, odor, refractive index, presence of sulfur, nitrogen and sesquiterpenes.

Preliminary gas liquid chromatography (GLC) of the oil

In order to have a rough profile of the oil in terms of the number of major components, a preliminary GLC of the oil was performed. Three tenths microliter of the oil were dissolved in hexane and isothermally run through Varian Aerograph Model 600-d gas chromatograph equipped with a flame ionization detector under the following operating conditions: a 5' x 1/8" stainless steel column packed with 10% Carbowax 20M on 60/80 Chrom-W; nitrogen (carrier gas), 25 mL/min at 14 psi; air, 300 mL/min at 2.5 psi; and hydrogen, 25 mL/min at 2.5 psi. Column temperature was kept at 190°C. Injection temperature was 205°C. The recorder was a Varian Aerograph Model 30 with a chart speed of 120 inches per hour.

Gas liquid chromatography coupled with mass spectroscopy (GC-MS)

The GC-MS analysis of the oil was carried out on a Hewlett Packard 5890 gas chromatograph that was linked to a V6 Trio-2 mass spectrometer with voltage 70 eV ionizing voltage. The sample of the oil was run on Hewlett Packard Ultra column, 5% methylphenyl silicone 12m x 0.2mm i.d. Detector temperature, about 250°C; ignition port, 180°C, split ratio, 20:1; carrier gas, helium at about 2 mL/min; temperature programming, 50°C for one minute increasing to 8°C per minute up to 300°C.

The GC-MS was duplicated in another laboratory for the purpose of comparing results. The gas chromatograph used was a Carlo Erba Model 5163 GC. it was coupled with a mass spectrometer which was a Kratos MS-25 magnetic sector instrument. The ionization was electron impact with an electron voltage of 70eV. The column was a J & W Scientific DB5 Phase (5% Phenyl/95% Methyl) 60m x 0.254mm I.D. - 0.25 um film. Four microliters of the sample were diluted in 2.5 mL of methylene chloride. 0.2 microliter of this was injected into a split injector (260°C) with a split ratio of 30:1 Helium was the carrier gas with the flow rate through the capillary column of 1 to 2 cc/ min. The oven temperature was ramped from 30°C-270°C at 5°C per minute and held at 270°C until all compounds eluted from the column.

Identification of the spectra

Because authentic samples of substances that could have been used for direct comparison of mass spectrograms were not available, spectra herein obtained were identified by comparison or "matching" with those spectra that are recorded in literature.

RESULTS

Yield and properties of the oil

Fresh leaves of *Nothopanax fruticosum* (L.) Miq., on hydro-steam distillation, gave a yield of 0.32% of volatile oil. The oil was light yellow in color with a grassy note odor. Its refractive index was 1.5001 at 25°C. It gave negative tests for the presence of nitrogen and sulfur, but an impressively high positive test for sesquiterpenes.

Preliminary gas liquid chromatography (GLC) of the oil

Isothermal GLC of the oil gave a chromatogram that reveals essentially 15 peaks as shown in Fig. 1.

GC-MS analysis of the oil

The gas chromatogram of the oil (programmed temperature) shows 40 peaks of which 20 are numbered and the rest unnumbered lesser peaks. Figure 2 shows the peaks in the gas chromatogram. From the numerous mass spectra that were obtained, 13 components were identified by means of matching with spectra of known substances. They were predominantly sesquiterpenes. In the order of their elution, they were: peak 2, α-elemene, 0.7%; peak 4, β-bourbonene, 5%; peak 5, β-elemene, 1.5%; peak 7, α-elemene 5.2%; peak 11, β-cubebene, 5%; peak 12, α-bergamotene, 41%; peak 13,

α-farnesene, 2.3%; peak 15, ∝-cadinene, 1.1%; peak 16, β-bisabolene, 1.4%; peak 17, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclopropane, 6.9%; peak 23, ∝-elemene, 2.1%; and peak 28, $C_{15}H_{16}O_2$, 7.8%. Alpha copaene was identified among the spectra. Two sesquiterpenes, namely, β-farnesene and α-humulene were tentatively identified. The mass spectra of the identified sesquiterpenes are shown in Figures 3 to 13. Table 1 lists the components in the descending order of their percentage in the oil.

DISCUSSION

There are several ways of separating and identifying the components of mixtures like essential oils. Fractional distillation and chromatographic methods (GLC, HPLC, thin layer, column and paper chromatography) are well known and are widely used. The use of gas chromatography has been expanded by coupling it with mass spectroscopy in what is called GC-MS analysis. It has become a method of choice of those who are engaged in the identification of volatiles in natural products.

Briefly, mass spectroscopy involves exposing a compound to electron bombardment by which the sample is fragmented into ions that are indicated by peaks in the mass spectrum. The mass spectrum shows the mass of the molecule and the masses of pieces from it. From the mass ions and the corresponding relative abundance, one gets an idea of the fragments into which the unknown or sample was broken. These fragments when fitted together, as in a jigsaw puzzle, help in reconstructing the chemical structure of the unknown.

Two ways of facilitating the identification of a mass spectrum are usually resorted to. The quicker way is by comparing the spectrum with spectra that are recorded in literature. If a matching spectrum is found, the identification is practically finished. The other way is by doing a parallel MS run with an authentic sample. If the spectrum of the unknown and of the authentic sample agree, the identification may be considered confirmed.

GC-MS enables the analyst to have a "quick look" on the chemical nature of an unknown compound. And, since it requires only a small amount of sample to produce an array of different spectra, it is like shooting several birds with one stone. However, the method is not without limitations. The "quick look" becomes a "hard look" when one is dealing with new or hitherto unknown compounds of which essential oils are rich reservoirs. Also, there are instances when the mass spectra produced are unusual, making their identification quite difficult.

Unusual spectra are brought about by any of the following: (1) faults in the fractionation of a mixture; (2) irregularities that are caused by the inherent nature of some compounds such as those emanating from isomerism; (3) unresolved samples from incomplete GLC resolution; (4) lack of effective separate spectra; (5) spectral overlap due to closely positioned related compounds; (6) certain compounds follow a common fragmentation pathways, so they give similar spectra. A case in point is delta-cadinene and gamma-cadinene both of which give closely similar spectra; (7) presence of a mixture of reaction products that are formed from peculiar organic chemical reaction mechanisms that take place during electron bomardment. Examples are

formation of a terpene peak from dehydration of a monoterpene alcohol; demethylation and/or loss of water from a terpene alcohol containing branched methyl groups; cleavage of the acetyl group and rearrangement of a single hydrogen atom in an ester; etc. Many types of organic reaction mechanism come into play making it occassionally difficult to fathom and correlate the message that each peak in the mass spectrum carries.

Libraries with mass spectral data base and other mass spectral reference literature will be of tremendous help in identification work, but access to this kind of library facility is not always possible specially when its location is in a foreign land. Likewise, reference standard substances for comparison work enhance a great deal the progress of identification, but those materials are not always available.

Some of the aforementioned possible setbacks might have been the reasons why in the present research work there remained several unidentified spectra.

The present study of *N. fruticosum* revealed that the major components of the oil are sesquiterpenes which, fortunately, are known compounds for which matching their spectra in available literature accomplished their identification. The minor peaks which were not identified are worth looking into. It becomes apparent, though, that in view of the very small percentage of the minor peaks and on account of their being closely located, GC-MS analysis needs to be supplemented with other methods of fractionation. For this purpose, the method of preparative gas chromatrography is highly recommendable. The method can be so controlled and manipulated as to move the peaks farther apart, ensuring the obtention of eluates that will satisfy purity requirements for subsequent testings. At the same time, repeated GLC runs will provide sufficient amounts of fractions that will be needed for identification by means of time-honored methods of elementary analysis, UV, IR, NMR, X-ray diffration, preparation of derivatives, degradation, synthesis, etc., all of which will help establish the absolute configuration of the isolates, particularly of new substances of which essential oils are known to contain.

As mentioned earlier, the oil was found to have pesticidal property. It would be interesting to find out which component or group(s) of components in synergetic association would be fatal to house insect pests. Finally, toxicity tests on the oil will help assess either the safety and benefits or the risks and hazards of the leaf oil of *Nothopanax fruticosum* (L.) Miq. if ever it is used as a spray insecticide in the homes.

SUMMARY

Fresh leaves of *Nothopanax fruticosum* (L.) Miq., upon hydro-steam distillation, gave a yield of 0.32% volatile oil.

The oil was light yellow in color with a grassy note scent. Its refractive index was 1.5001 at 25°C. Tests were negative for the presence of sulfur and nitrogen, but highly positive for the presence of sesquiterpenes.

Preliminary gas liquid chromatography (isothermal) showed 15 peaks. GC-MS analysis showed about 40 peaks. There were essentially 21 significant peaks from which

eleven sesquiterpenes were identified by matching their respective spectrum with spectra that are recorded in available literature. An oxygenated sesquiterpene derivative was detected, possibly a sesquiterpene alcohol or a sesquiterpene lactone.

The separation and identification of the minor peaks, specially the very small ones, may be facilitated by the use of preparative gas chromatography.

On account of the search for botanical pesticides that would be more desirable than synthetics, it would be worth extending the study of the oil of *Nothopanax fruticosum* (L.) Miq. to toxicity tests in order to determine whether or not the oil would be acceptable as a legitimate spray pesticide for house insect pests.

ACKNOWLEDGEMENTS

We extend thanks to: The Department of Chemistry of the Loughborough University of Technology, Loughborough, Leicestershire, England, for the use of its library and laboratory facilities; to Dr. A. Payne of Kodak Co., Leichestershire, England, for his help in spectral determinations; to The Department of Chemistry of Pennsylvania State University, State College, PA., USA, for the use of its library facilities and its Mass Spectrometer Facility; the National Academy of Science and Technology, Philippines, for a research fellowship granted to the senior author; to Mrs. Zenaida Galpa-Sadiwa for her assistance in many ways; to Miss Alma O. Belardo for her help in the typing work; and to Mrs. Amelia Belardo-Cox for her assistance in the preparation of the table and figures for this publication.

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Table 1. Main constituents found in the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. in the descending order of their percentage in the oil.

Components	Percent
1. α -Bergamotene	41.00
2. $C_{15}H_{16}O_2$	7.8
3. 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	6.9
4. α -Elemene	5.2
5. β -Bourbonene	5.0
6. β -Cubebene	5.0
7. α -Farnesene	2.3
8. ∝ -Elemene	2.1
9. β -Elemene	1.5
10. β -Bisabolene	1.4
11. ∝ -Cadinene	1.1
12. ∝ -Elemene	0.7
13. α -Copaene	–

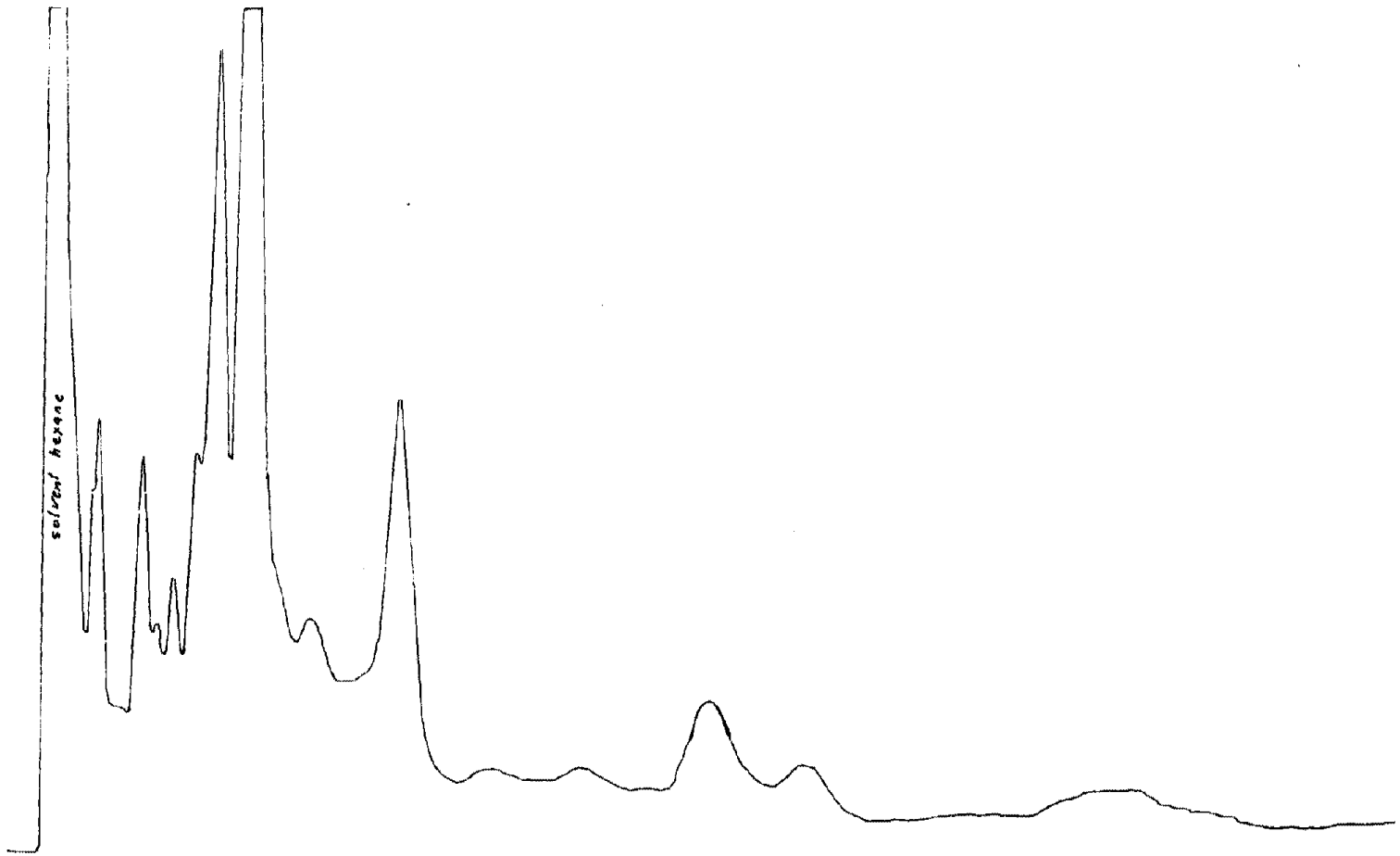

Figure 1. Gas chromatogram (isothermal) of the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. Conditions: Column, carbowax; FID detector; nitrogen (carrier gas), 25 mL/min at 14 psi; hydrogen, 25 mL/min at 2.5 psi; air, 300 mL/min at 2.5 psi; injection T, 205°C.

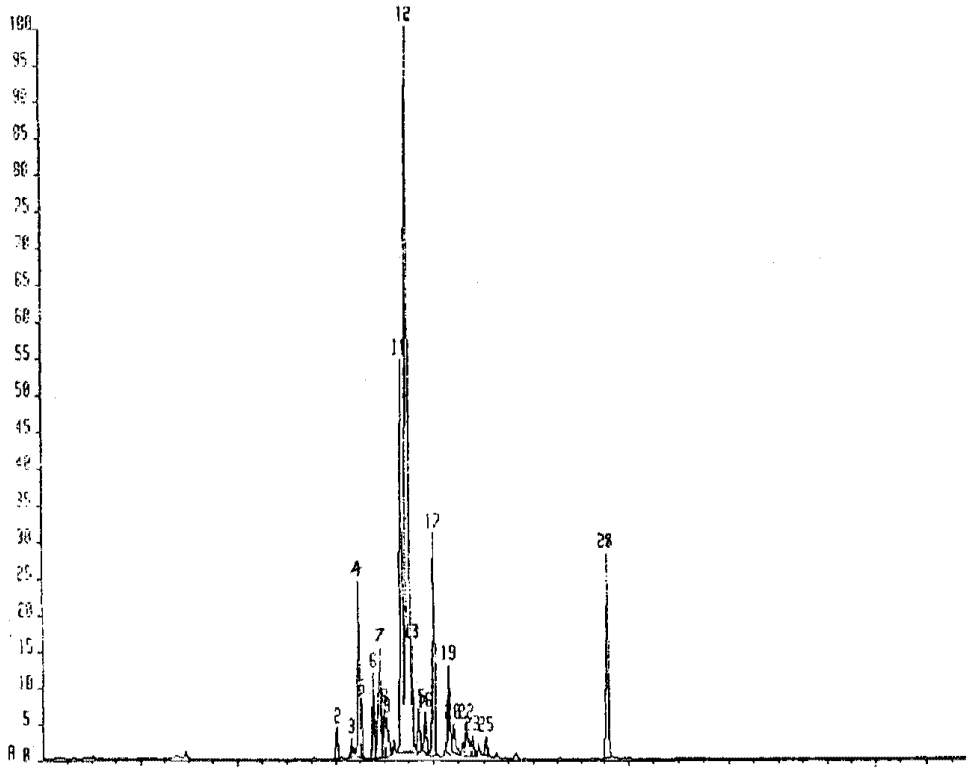

Figure 2. Gas chromatogram of the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq.
Conditions: Column, 5% methyl phenyl silicone 12M x 0.2 mm i.d.; carrier gas, helium 2 mL/min; detector T, about 250°C; ignition port, 180°C; split ratio, 20:1; Temperature programming; 50°C for one min increasing to 8° per min up to 300°C. Peak 2, α-Elemene; Peak 4, β-Bourbonene; Peak 5, β-Elemene; Peak 7, β-Elemene; Peak 11, β-Cubebene; Peak 12, α-Bergamotene; Peak 13, α-Farnesene; Peak 15; α-Cadinene; Peak 16, α-Bisabolene; Peak 17, 1-ethenyl-1-methyl-2-(1-methylethenyl)1-4-(methylethylidene)-cyclohexane; Peak 23, α-Elemene; Peak 28, $C_{15}H_{16}O_2$.

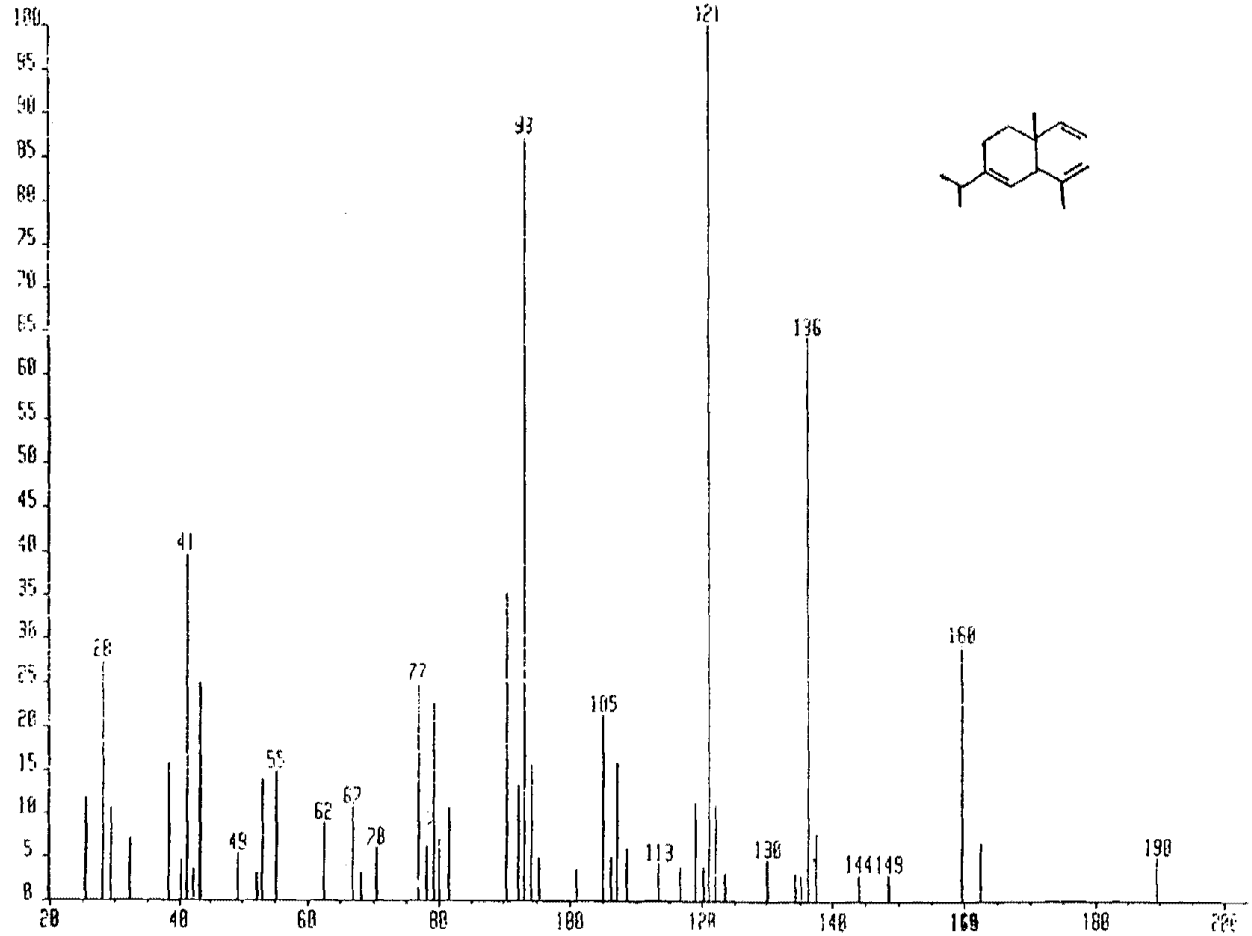

Figure 3. Mass spectrum of delta-Elemene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

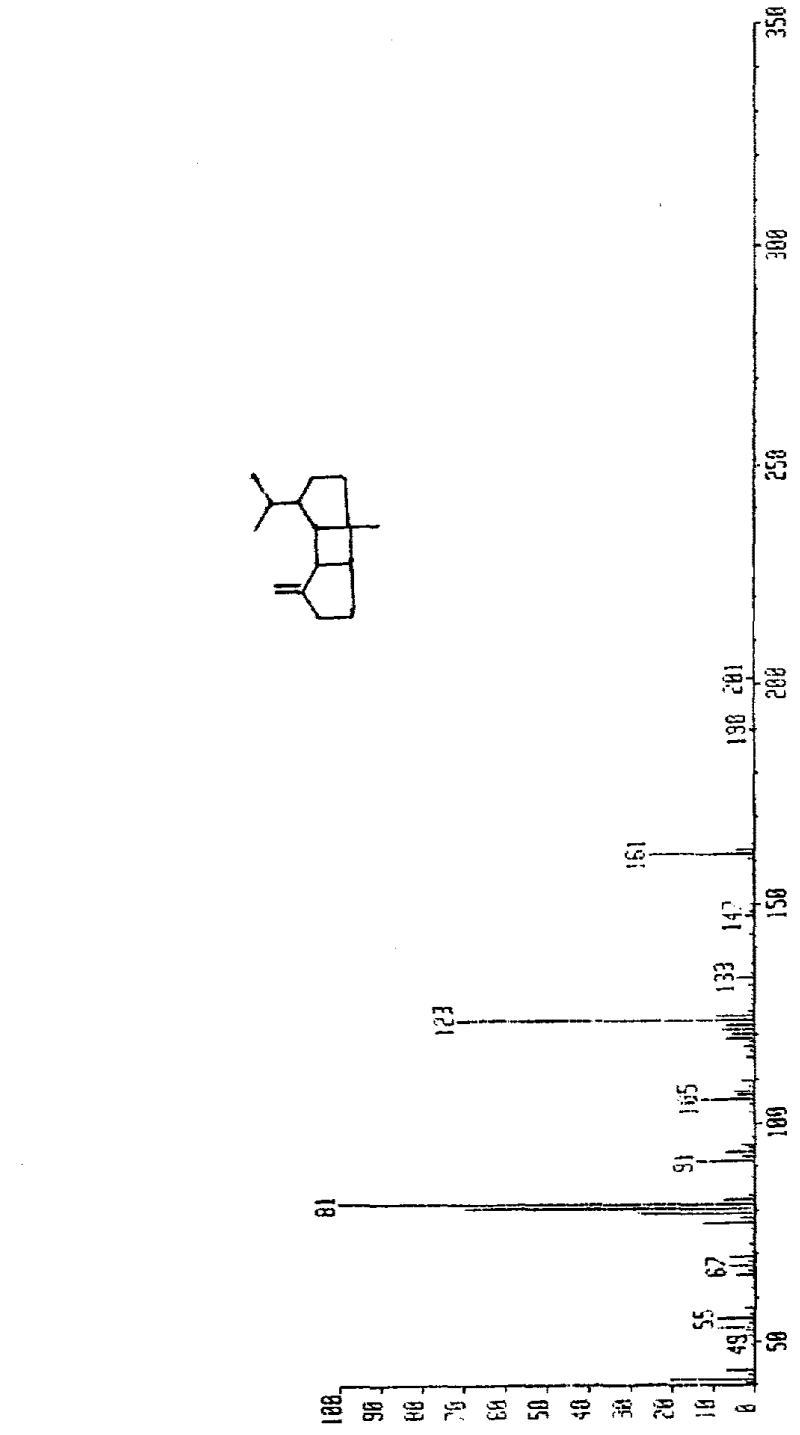

Figure 4. Mass spectrum of beta-Bourbonene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

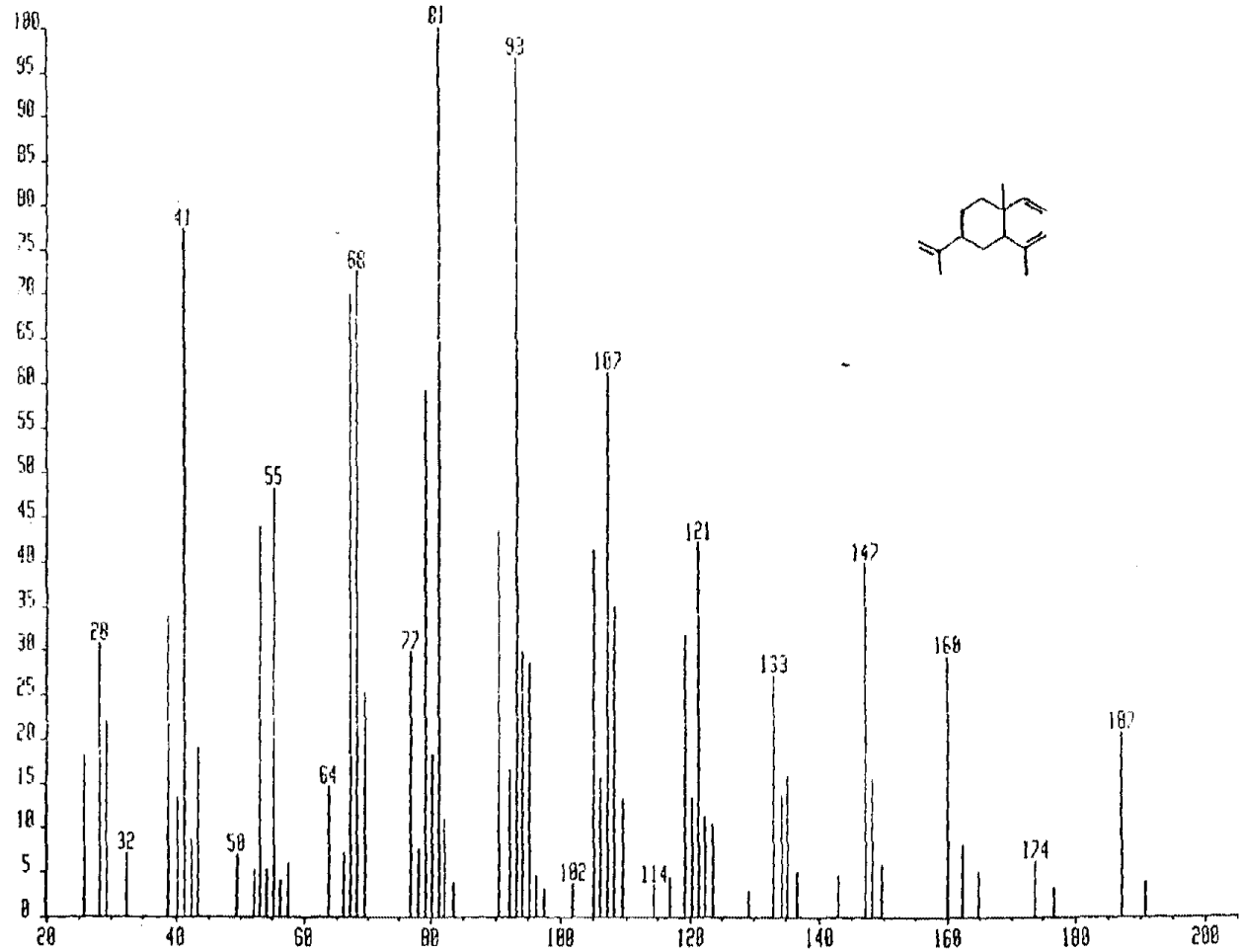

Figure 5. Mass spectrum of beta-Elemene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

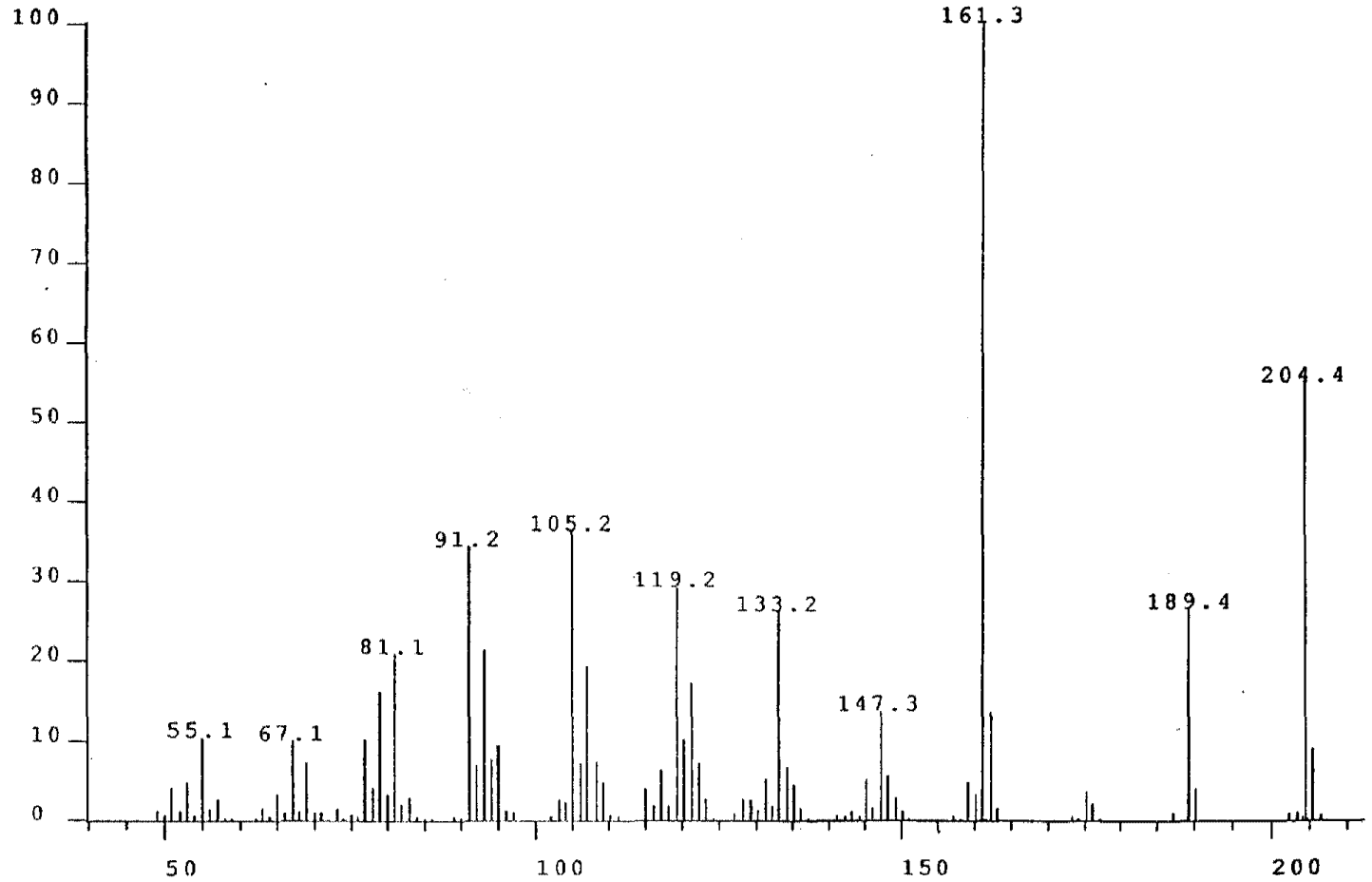

Figure 6. Mass spectrum of alpha-Elemene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

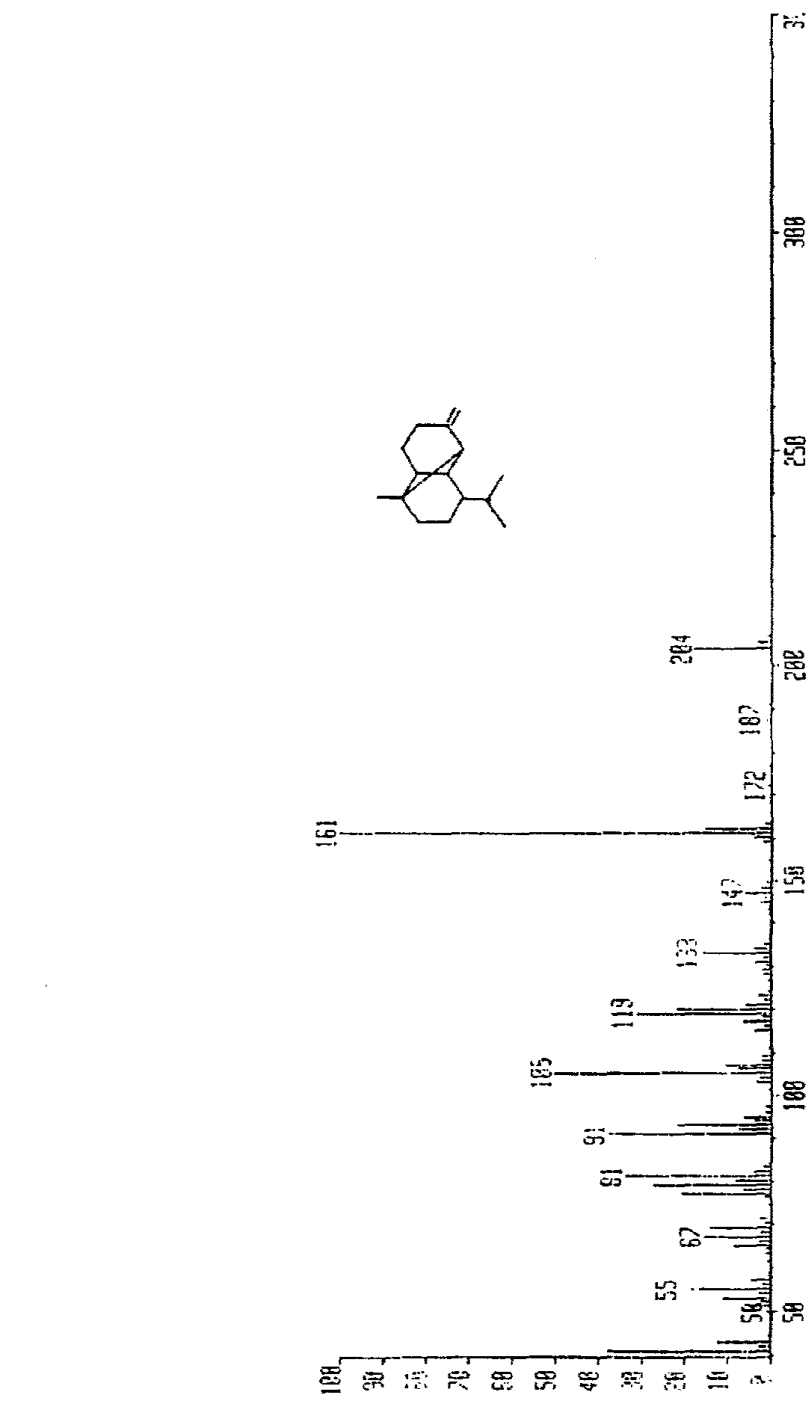

Figure 7. Mass spectrum of beta-Cubebene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

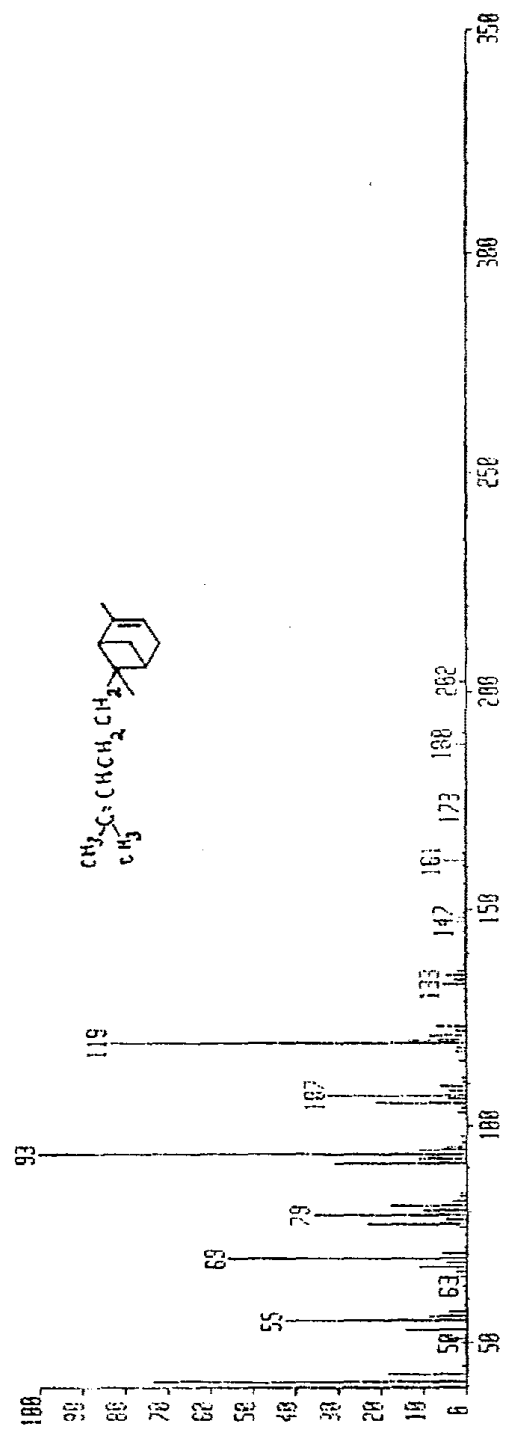

Figure 8. Mass spectrum of alpha-Bergamotene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

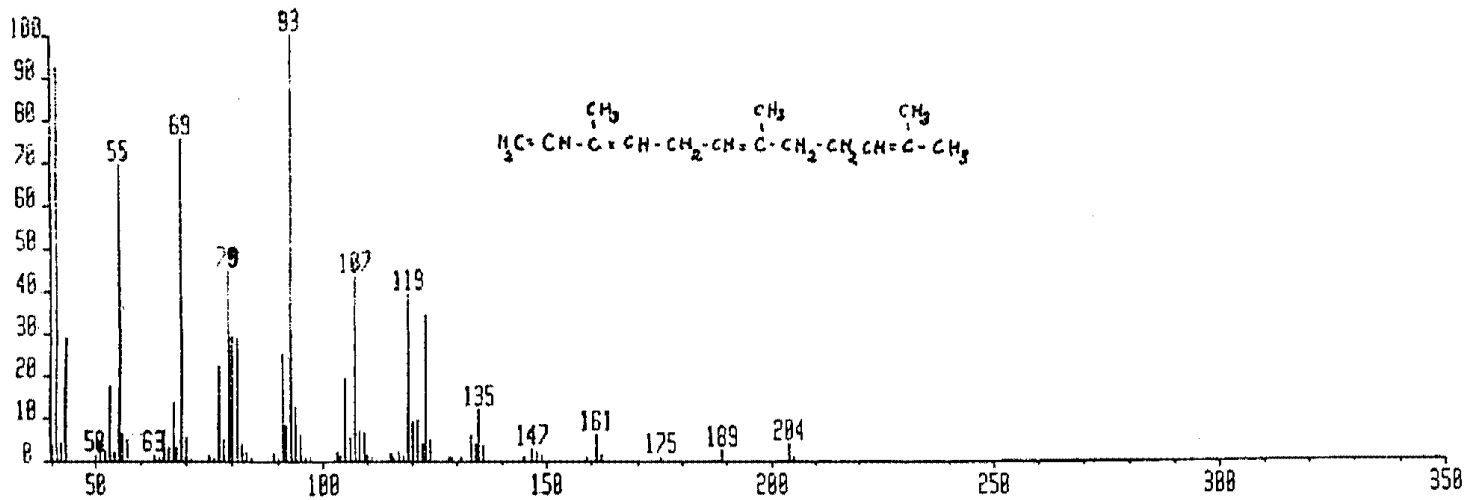

Figure 9. Mass spectrum of alpha-Farnesene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

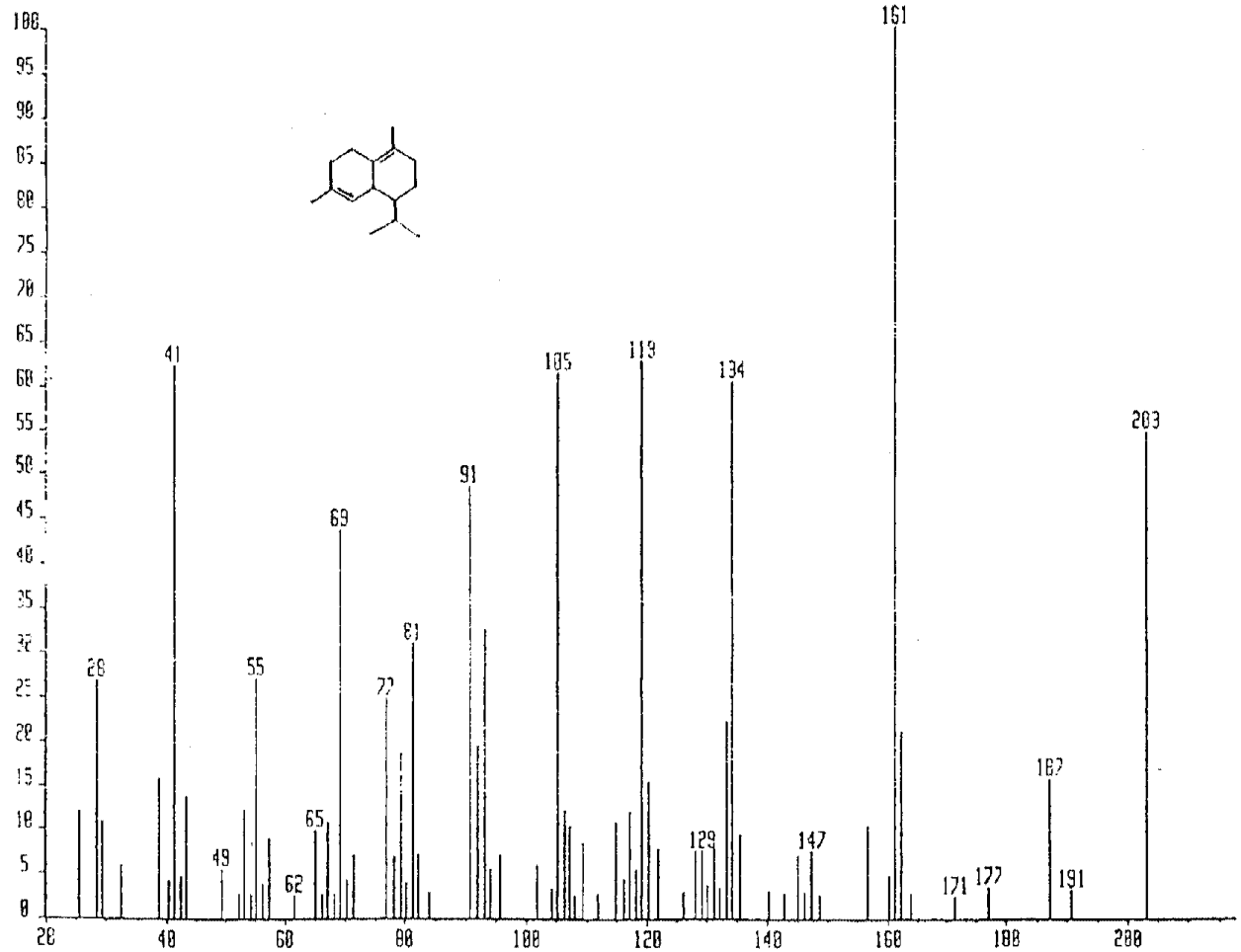

Figure 10. Mass spectrum of delta-Cadinene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

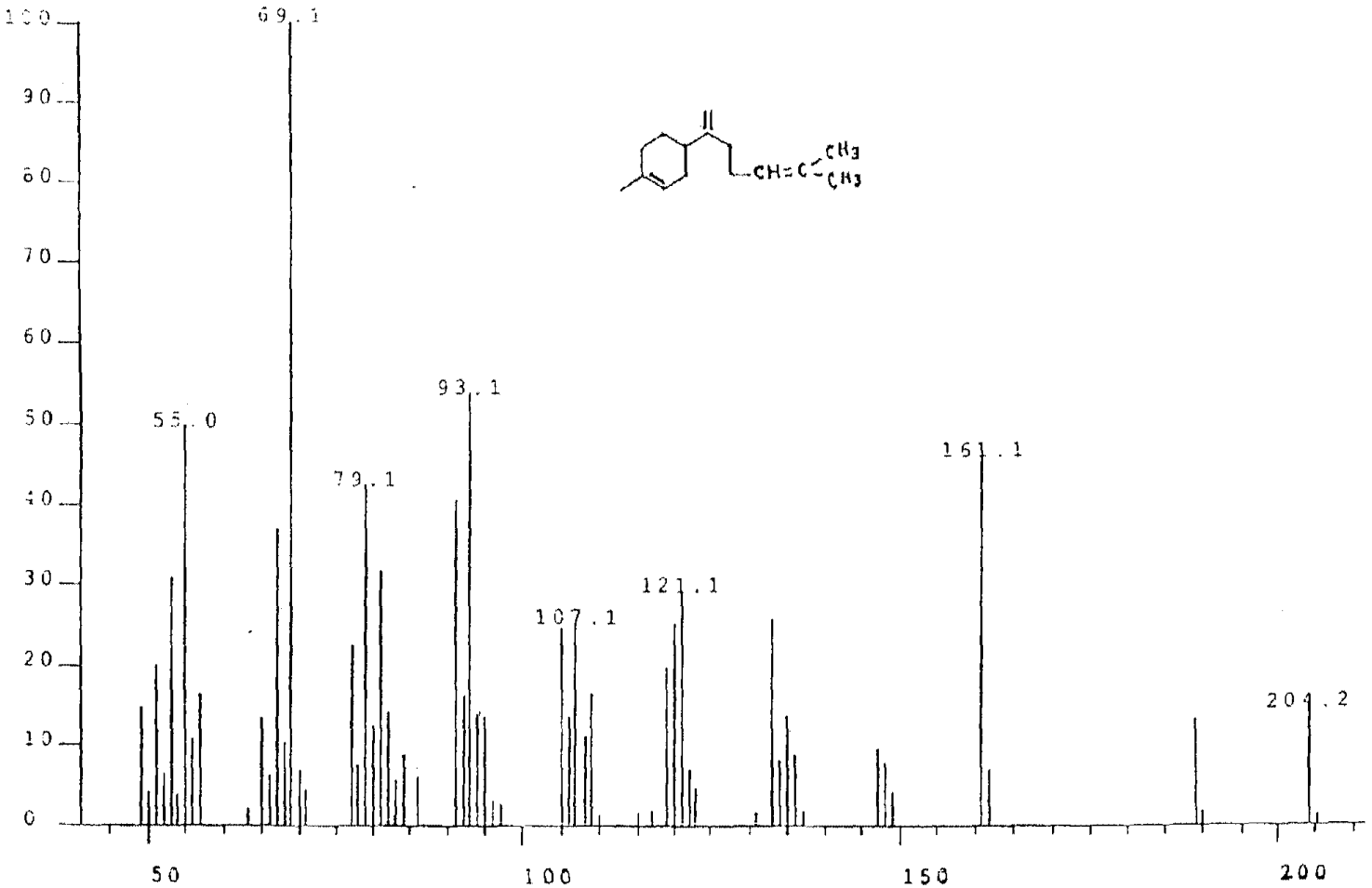

Figure 11. Mass spectrum of beta-Bisabolene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

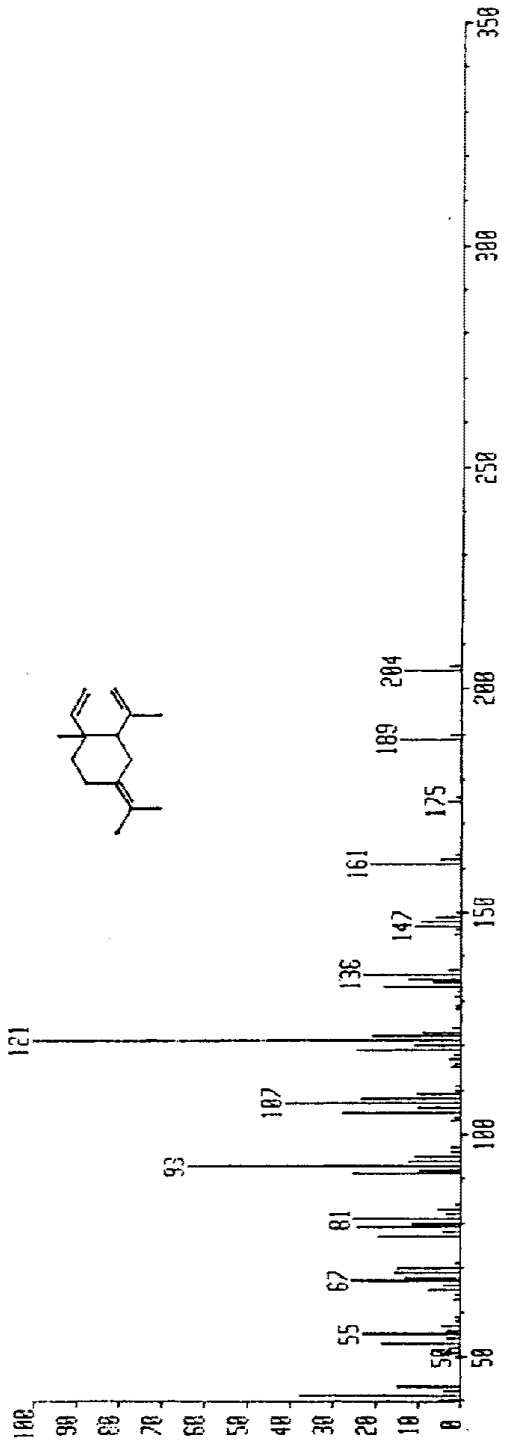

Figure 12. Mass spectrum of gamma-Elemene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.

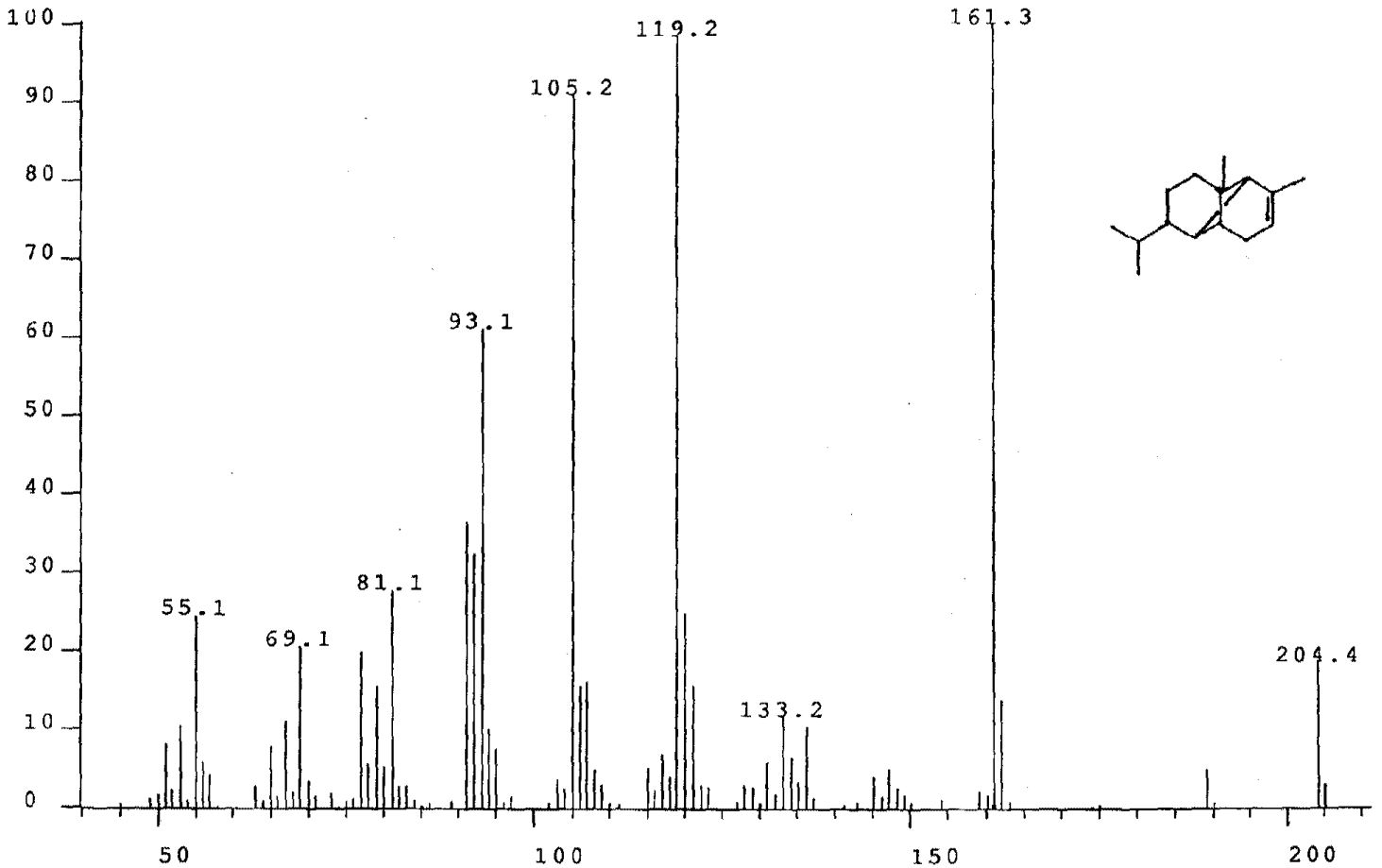

Figure 13. Mass spectrum of alpha-Copaene from the leaf volatile oil of *Nothopanax fruticosum* (L.) Miq. from the Philippines.