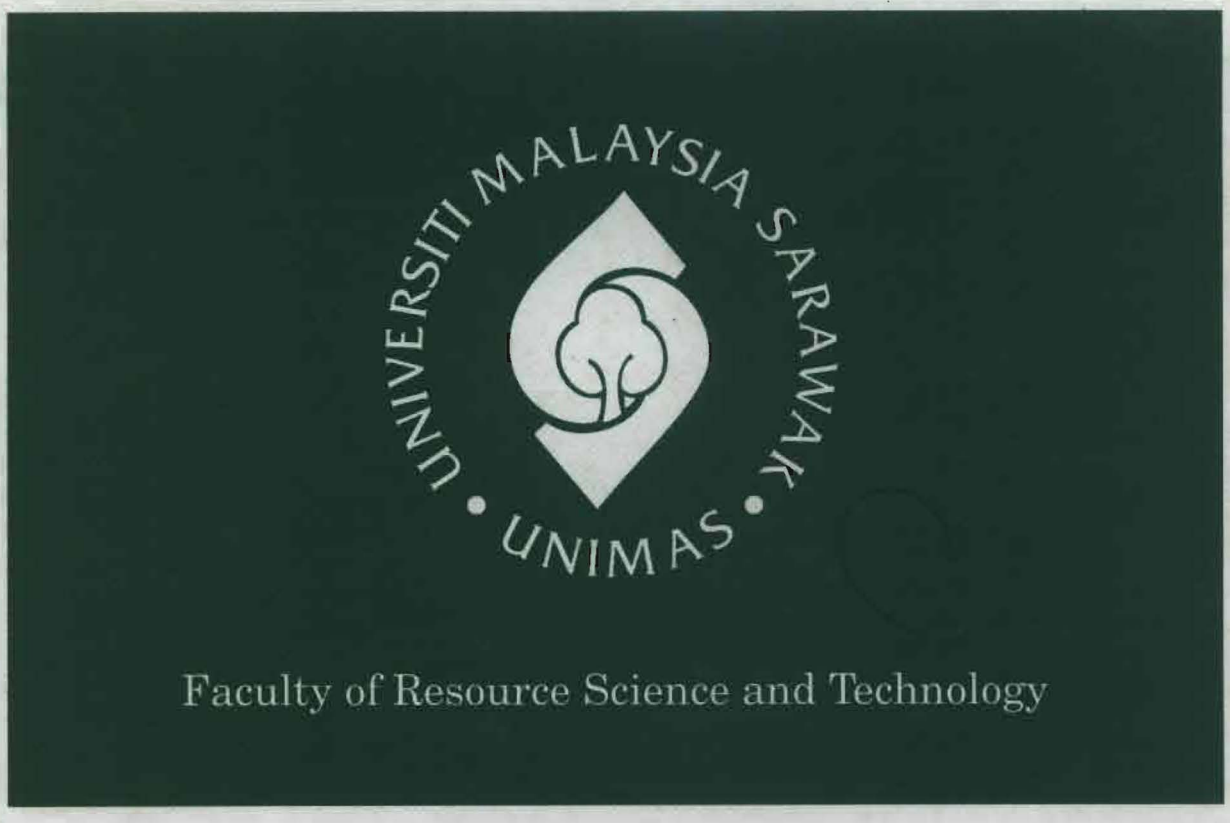

ISOLATION AND CHARACTERIZATION OF GUM FROM *AGATHIS BORNEENSIS*

Aini Faizi Jaslan

Bachelor of Science with Honours
(Resource Chemistry)
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This project is submitted in partial fulfillment of
the requirements for the degree of Bachelor of Science with Honours
(Resource Chemistry Program)

Faculty of Resource Science and Technology
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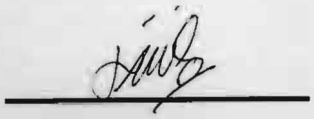
DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.



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DEDICATED
TO
'Mak' and 'Abah'
WITH LOVE

ACKNOWLEDGEMENTS

'Bismillahirrahmanirrahim...'

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ABSTRACT

Studies were conducted to determine the physico-chemical properties and organic compound of gum from *Agathis borneensis*. The proximate compositions studied include percentage of moisture, ash, nitrogen and protein. The results showed moisture and ash content of 1.76% and 2.2%, respectively. Nitrogen content is lower compared to standard specification of nitrogen from Joint Expert Committee and Food Additives, JECFA/FAC, which is only about 0.23%. Percentage of protein is about 1.31%. Isolation and identification of organic compound was carried out by extraction, thin layer chromatography fractionation and subsequently analyzed on Gas Chromatography/Mass Spectrometry. The organic component identified are arabinose, rhamnose, glucuronic acid, 2,6-dimethyltridecanenitrile, N-isopropyl-3-phenylpropanamide and 6-azaspiro-2,5-octa-4,7-diens-6-carboxylic acid. A consideration amount of arabinose provides an early indication to similarity to Arabic gum.

Key words: *Agathis borneesis*, proximate composition, organic component.

ABSTRAK

Kajian telah dilakukan bagi menentukan sifat fizikokimia serta sebatian organik gam dari Agathis borneensis. Diantara kandungan proksimat yang dikaji adalah peratus kelembapan, abu, nitrogen dan protin. Keputusan menunjukkan peratus kelembapan dan abu adalah sebanyak 1.76% dan 2.2% masing-masingnya. Kandungan nitrogen adalah rendah jika dibandingkan dengan spesifikasi piawai bagi kandungan nitrogen yang dikeluarkan oleh Joint Expert Committee and Food Additives, JECFA/FAC, dimana hanya sekitar 0.23%. Peratusan protin pula adalah sekitar 1.31%. Pemencilan dan pengenalpastian sebatian organik telah dilakukan dengan kaedah pegekstrakan, pemfraksian pada kromatografi lapisan nipis dan seterusnya dianalisis menggunakan Kromatografi Gas/Spektrometri Jisim. Komponen organik yang dikenalpasti adalah seperti arabinosa, rhamnosa, asid glukuronik, 2,6-dimetiltridekanenitril, N-isopropil-3-fenilpropanamida dan 6-azaspiro-2,5-octa-4,7-dien-6-asid karboksilik. Kehadiran arabinosa yang agak banyak memberi petunjuk awal terhadap persamaan dengan gam Arabik.

Kata kunci: Agathis borneensis, kandungan proksimat, komponen organik.

1. INTRODUCTION

The Araucariacea tree (*Agathis borneensis*) is native to Malaysian 'kerangas' forest, growing at altitudes greater than 30 m. Its distribution is wide, varying in Peninsular Malaysia and throughout Borneo to the state of Sabah and Sarawak. This tree is also found in Brunei and Kalimantan (Whitmore, 1972 and Yii, 1995). In Malaysia, *Agathis borneensis* is known by variety of vernacular names such as 'Tumu' in Kelabit Highlands and 'Bindang' in Sarawak (Yii, 1995). It is generally called 'Damar minyak' in Peninsular Malaysia (Whitmore, 1972) and 'Tulong' in Brunei (Wong, 1997).

Agathis timber is well known and is classified in botany and in trade as softwood, a term used to refer to wood where a type of water-conducting tissue called 'vessels' is absent. Whitmore (1972) stated that 'Damar minyak' is the only commercial Malaysian softwood. It is particularly useful to produce beautiful softwood, which fetch a very good price. Its pleasing yellow timber has a natural sheen that is very suitable for furniture and cabinet making, wall and ceiling paneling. *Agathis borneensis* occur in small quantities and thus the export of this species is controlled. The gum exudates from this tree are harvested for domestic use throughout Sarawak (Ismail *et al.*, 2003).

Resinous material exuded by woody plants as a metabolic product but useful for protective purpose often congeals relatively rapidly to form a solid (Lambert *et al.*, 1999). Even though, the surface stickiness can be lost, yielding a translucent and glassy

substance. It is dissolves in some organic solvents but partially dissolve in water. As the material ages, it becomes harder, less soluble and less combustible.

Resins are composed of the molecular class of organic compounds known as terpenes. This class is built up from the five-carbon compound isoprene (CH_2=CH-C(CH_3)=CH_2). Molecules made of two units contain 10 carbon atoms and are called monoterpenes, with a variety of molecular skeletons. Monoterpenes give rise to the fluidity of the initially formed resin and are lost over time through volatilization. According to Lambert (1999), distillation of natural pine resin produces a liquid mixture of monoterpenes known as oil of turpentine. The solid residue is composed of larger molecular weight constituents and is called rosin or colophony. Liquid monoterpenes are lost during the process of solidification. Due to this, a lot of study is interest for the resin with higher molecular weight. Molecules based on six isoprene units and 30 carbon atoms are called triterpenes are high in molecular weight and usually are solid. This occured in triterpenoids resins such as 'damar'.

2. LITERATURE REVIEW

2.1 Introduction

Agathis borneensis is emergent tree to 55 m tall and 100 cm diameter (Yii, 1995). Moreover, this tree is *monoecious* or rarely shrubs. Sabah and Sarawak represents a single genus of *Agathis* with 5 species. Bark of *A. borneensis* is warty lenticellate, papery

scaly or flaky with maurish gray in color. The inner bark is granular with pale brown color. In addition, *Agathis* tree produce seeds that are not in any kind of specialized fruit structure, but which instead are attached to woody scales arranged into cones. *Agathis borneensis*, like other conifers, are wind-pollinated and its seed is wind-dispersed. Wong (1997) stated that an individual tree bears both male cones (as small as a little finger) and female cones (as large as a fist). Even though, these developed at different times, facilitating cross pollination between trees.

2.2 *Agathis* species in Sarawak

A genus of thirteen species, *Agathis* is represents in Sarawak by five species, *A. borneensis, A. endertii, A. kinabaluensis, A. lenticula* and *A. orbicula.* Exudate resinous of Agathis borneensis is colourless or slightly whitish (Yii, 1995). This resin is deposited into a wound and a dark coloured wood due to accumulation of induced secondary metabolites in the form of gum (Azizol, 1992). Ismail *et al.* (2003) reported that the gum exudates from this tree are harvested for domestic use throughout Sarawak.

Agathis borneensis, known in Sarawak as 'bindang' by Malay. The native Iban called it 'bulu' but generally it is also known as 'kayu jadi' by Malay and Iban (Yii, 1995). In the term of ecology, *A. borneensis* is infrequent in the lowlands, although found in sandy margins of some peat swamps and 'kerangas' and lower montane forest to 1200 m. This tree prefers more acidic soils and often forming dense. In addition, *Agathis borneensis* is typically found nearly pure stands on certain areas of low-lying wet 'kerangas' forest and dry 'kerangas' forest on sandstone formations at higher altitude.

2.3 Role of gum in plants

Gummosis is a common wound response that results in the exudation of a plastic gum sealant at the site of cracks in bark. The exudate is a composite of polysaccharides and glycoproteins structurally related to cell wall components such as galactans and hydroxyproline-rich glycoproteins (Goodrum *et al.*, 2000). Plants hydrocolloids can be defined as a long chain and high molecular weight polymers that dissolve or disperse in water to give a thickening, stabilizing or gelling effect. It is generally polyuronides composed of more than one type of monosaccharides unit (Sharma and Zapsalls, 2003). Hydrocolloids share similar characteristics that are responsible for their ability to form viscous solutions in water and dispersions that possess suspending and stabilizing properties. The polymers are used to improve or manipulate the texture of food products because of their ability to retard flow, modify gelling characteristics and preserve emulsions and suspensions (Balmaceda *et al.*, 2003).

The effectiveness of hydrocolloids in modifying the rheological properties of solutions depends on the distribution of molecular weight in the given polymer, the degree of hydration of the molecules and the extend of interaction with different molecules or molecules of the same type. Moreover, the effectiveness is also affected by concentration of the polymer and environmental conditions, such as temperature and the presence of other types of molecules (Krumel and Sarkar, 2003).

An understanding of the structure and functional properties of the gum would be necessary in order to most effectively exploit the gum. An understanding of the properties

of the monosaccharides of *Agathis borneensis* should contribute towards understanding of the origin of adhesiveness in monosaccharides.

2.4 Gum analysis

Study of enantiomeric composition of monoterpenes of conifer resin has been reported by Wong *et al.* (1997) and showed that α-pipene was the dominant monoterpenes for all species of *Agathis* except *Agathis moorei*. Classification of resin from *Agathis* by nuclear magnetic resonance spectroscopy verified that the representatives *Agathis* spectrum with all carbon exhibits resonance in three general areas. The alkenes region (unsaturated carbon) is characterized of most modern or recent resins to have strong exomethylene resonances. The second general region is resonance of alkanes (saturated carbon) which is not take part of multiply bonded functionalities. The third region is the carbonyl (C=O) that is not strongly populated in the *Agathis* spectrum (Lambert *et al.*, 1999).

In the recent years, *Agathis* species have been received considerable attention because of their reputation throughout the world. However, there is very limited report on analysis of the resin from *Agathis borneensis*.

3. OBJECTIVE

The main objective of this project is to identify the major compounds of the resin from *A. borneensis* that may have potential in commercialization. In addition, this studies emphasized on physicochemical properties and characterization of*Agathis* exudates gum.

4. MATERIALS AND METHODS

4.1 Origin and extraction of gum sample

A. borneensis gum was collected from Bario of Kelabit Highlands, Sarawak in June 2003. Approximately, 20 g gum was extracted in 60 ml of 95% ethanol for two days where two layers were formed. The ethanol layer was pipetted out before adding new batch of ethanol and extracted again for another two days. This process was repeated 3 times. Subsequently, the supernatant solution was concentrated using rotor vapor prior to characterization testing.

4.2 Thin Layer Chromatography (TLC)

Thin layer chromatography, TLC was performed on a prepared 20 x 20 cm silica gel F_{254} 0.25 mm plate Merck. The gum spotted 1 cm from the bottom of the plate by using capillary tube. The plate was then allowed to dry. The solvent system used in TLC were:

(a) 3:18:1:4 (v/v) AcOH-EtOAc-HCO_2H-H_2O

(b) 1:5:3:3 (v/v) 1-butanol-EtOH- H_2O

(c) 10:5:1 (v/v) EtOH-0.1M HCl-1-butanol

(d) 4:1:5 (v/v) 1-butanol- EtOH- H_2O

as used by Martinez (1996). The plate was removed from the tank and was allowed to dry again. Subsequently, the plate was examined under UV light at 254 nm (UVP model CC-10) and the positions of spots were marked. The R_f (retardation factor) value was then determined by using the following equation:

$$R_f = \frac{\text{Length of mobile component, cm}}{\text{Length of mobile solution, cm}}$$

4.3 Proximate analysis

4.3.1 Moisture content

The moisture content was determined by using gravimetric method (Smith, 1967). Briefly, 5 g of samples were weighed in the crucible plate and stored in oven for 4 hours at 105°C. The sample was cooled in decicator for 30 minutes and weighed. The percentage of moisture content was calculated based on equation below:

$$\% \text{ Moisture content} = \frac{\text{Dry sample weight, g}}{\text{Sample weight, g}} \times 100\%$$

4.3.2 Nitrogen content

The nitrogen content of gum was analyzed using the Kjeldahl method as described by Martinez (1996). Briefly, 1.0 g of sample was digested using 12 ml H_2SO_4 for 1 hour at 420°C. Sample was then distilled for 4 minutes after the addition with 75 ml of distilled

water, 30 ml 4% solution of boric acid and 50 ml of 40% sodium hydroxide. The sample was then titrated with 0.1 N hydrochloride acid. The nitrogen percentage was calculated using the following equation:

$$\% \text{ Nitrogen} = \frac{(B - A) \text{ x normality HCl x } 14.007}{\text{Sample weight}} \text{ x } 100\%$$

where,

A = titration volume 0.1 N HCl for reference

B = titration volume 0.1 N HCl for sample

The protein content was calculated using a conversion factor of 6.25.

4.3.3 Ash content

Ash content was determined using the method described by Smith (1967). Briefly, 5 g of gum was weighed in a preweighed crucible plate. Sample was then heated until the entire sample turn black. It was then heated in furnace at 550°C until it produced white or gray ash. The sample was then allowed to cool in decicator and weighed. The percentage of ash content was calculated using the equation below:

$$\% \text{ Ash} = \frac{\text{Ash weight, g}}{\text{Sample weight, g}} \text{ x } 100\%$$

4.4 Structure identification

4.4.1 Chemical compositions

The gas chromatography chromatography-mass (GC-MS) is the most powerful instrument for analyzing mixture of organic compounds (Popl *et al.*, 1990). The extract from gum was analyzed on Shimadzu QP-5000 GC-MS fitted with DB-5 capillary column (internal diameter of 30 mm x 0.25 mm) with the film thickness of 0.25 μm. It was used to capture the presence of an organic compound in the extract gum with helium as the carrier gas. 1.0 μm of the sample was injected using an indivisible mode. The temperature for the injector and detector was set at 280°C and 230°C, respectively. The mass spectrum was then interpreted and the interpretation was then being compared to store in the Wiley Incorporated Spectral database (WIS). The mass spectrum for the sample that was gives similarity more than 85% compared to mass spectrum in the library was accepted and authenticated as the individual component.

5. RESULTS AND DISCUSSION

5.1 Proximate analysis

Proximate content including moisture, ash, nitrogen and protein are assumes as non-carbohydrates substances. The determination of proximate content is important because it has an effect on physicochemical properties of the polymer. Table 1 shows the percentage of proximate content of gum from *A. borneensis*.

Table 1: Percentage of proximate content of gum from *Agathis borneensis*

Parameter	*Agathis borneensis*
Moisture (%)	1.76 ± 0.04
Ash (%)	2.20 ± 0.01
Nitrogen (%)	0.23 ± 0.05
Protein (%)	1.31 ± 0.04

The moisture content of the *A. borneensis* gum is 1.76 ± 0.04. The main factor affected the moisture content of the gum is the condition and time storage. Generally, most of the gums are useful as an agent for moisture-control and to provide the consistency required for the polymer (Whistler, 1993). Thus, the small percentages of moisture in gum from *A. borneensis* may have a potential for moisture-control agents.

The nitrogen and protein contents are useful in distinguishing gums from different species. Joint Expert Committee on Food Additives (JECFA/FAC) has specified 0.26-0.39% of the nitrogen content in Arabic gum as a standard for quality and purity of the gum (Mhinzi, 2003). The immune responses, which are important in providing evidence for the safety of food additives, are accredited based on the proteinaceous component of the food (Mhinzi, 2003). He also stated that film forming, emulsifying and stabilizing properties of a gum arise from the protein fraction. The nitrogen content of *A. borneensis* is 0.23 ± 0.05 and this is comparable to standard value for Arabic gum as recommended by JECFA/FAC. Since only a little amount of protein in this gum, the molecular weight is considered to be lower with 279000 Dalton (Wiley, 1992). The protein percentage in

A. borneensis gum is only 1.31%. This value is low compared to protein content in Arabic gum and Tragacanth gum (1.63-2.5%). The low protein content in *A. borneensis* has a potential to produce hydrolysis product such as syrup. This is due to the low presence of protein does not give unwanted flavour and colours. It may also use as thickening agents in cream, lotions and ointment; same as application in Arabic gum (Wiley, 1992). The low protein content in the polymer will not affect the charge surface and the monosaccharides component in the gum.

The percentage of ash content for *A. borneensis* is 2.2%. This value is comparable to the standard ash content of Arabic gum (3%). Therefore, *A. borneensis* is thought to be related to Arabic gum. The ash content in the polymer is depending on non-organic compositions such as P, Ca, Mg, Na and K (Balmaceda *et al.*, 2003).

Gum from *A. borneensis* is insoluble in most oil and organic solvent but it shows good in solubility in ethanol. The solubility properties of the gum can be referred to Tragacanth gum. Tragacanth gum is soluble in water and the soluble part is called tragacantic acid which is comprise of 60-70% of the original weight of the gum (Wiley, 1992). The acid consists of a backbone of 1,4-liked α-D-galactopyranosyluronic acid residues. The insoluble part of the gum is called basorine. Sugar moieties present in the basorine (arabinogalactan) are 75% L-arabinose, 12% D-galactose, 3% D-galactoronic acid methyl ester and L-rhamnose (Wiley, 1992). *A. borneensis* gum also produced two layers that are soluble and insoluble. Thus, the solubility properties of *A. borneensis* and Tragacanth gum are closely similar when they dissolved in water.

5.2 Structure identification

5.2.1 Thin Layer Chromatography (TLC)

TLC analysis on ethanol extract for gum using solvent system AcOH-EtOAc-HCOOH-H_2O (3:10:1:2) has revealed 2 components. The R_f values for two compounds are 1.1 and 0.96, respectively. The gum was then analyzed using preparative TLC.

5.2.2 GC-MS Data

The interpretation mass spectrum was carried out by studying the fragmentation pattern of each mass spectrum. Chromatographic data of GC-MS for samples from the gum extract reveals six fractions. A gas chromatogram from GC-MS analysis of gum from *A. borneensis* is shown in Figure 1.

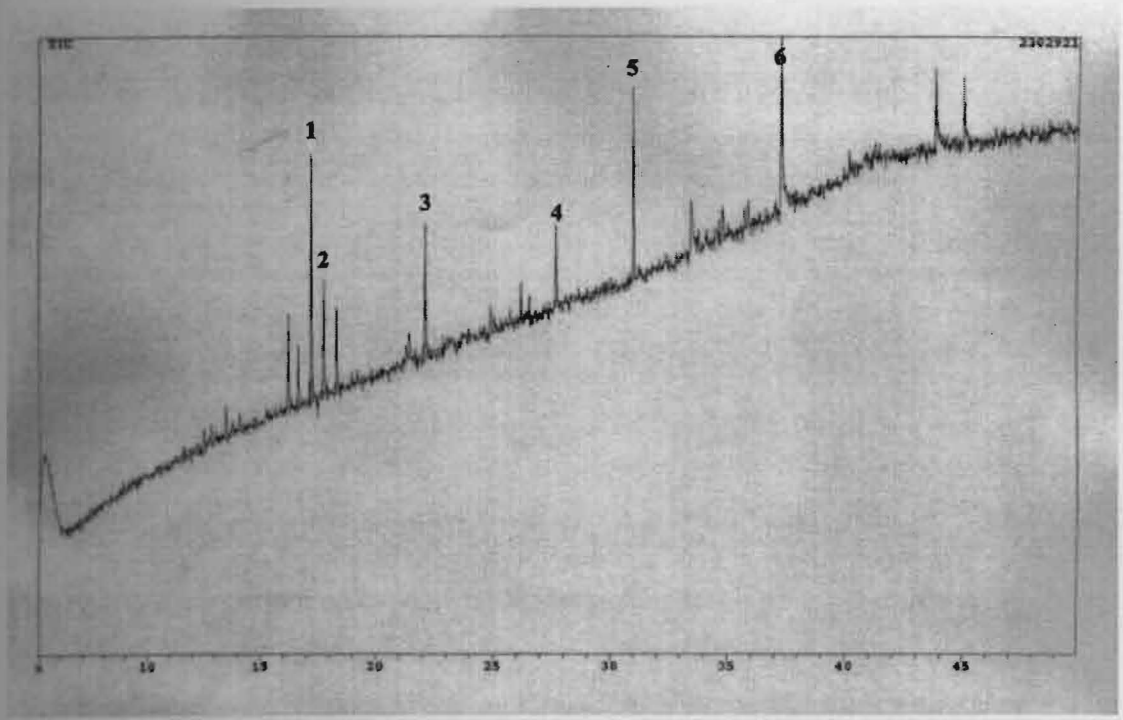

Figure 1: GC-MS chromatogram of extract from gum from *Agathis borneensis*

Identification on individual component of the gum has been carried out by comparing the mass spectrum with the Wiley Library Database. The organic components identified are arabinose, rhamnose, glucuronic acid, 2,6-dimethyltridecanenitrile, N-isopropyl-3-phenylpropanamide and 6-azaspiro-2,5-octa-4,7-diens-6-carboxylic acid. The mass spectra for those compounds are shown in Figure 2-5.

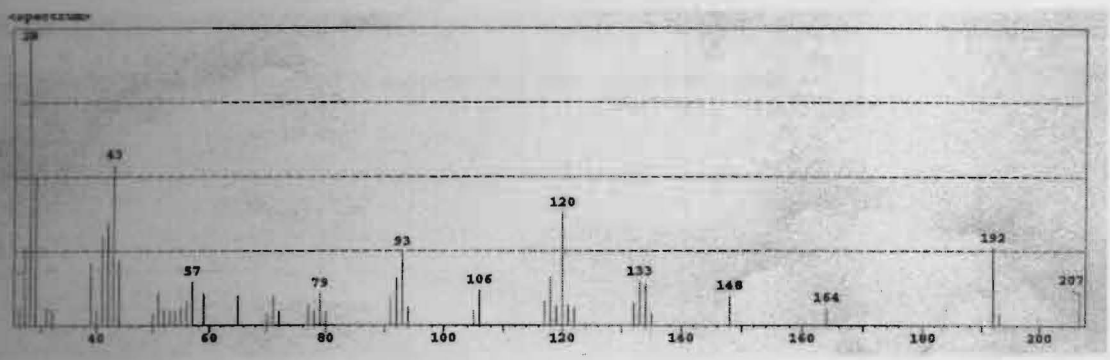

Figure 2: Mass spectrum of 6-azaspiro-2,5-octa-4,7-diens-6-carboxylic acid

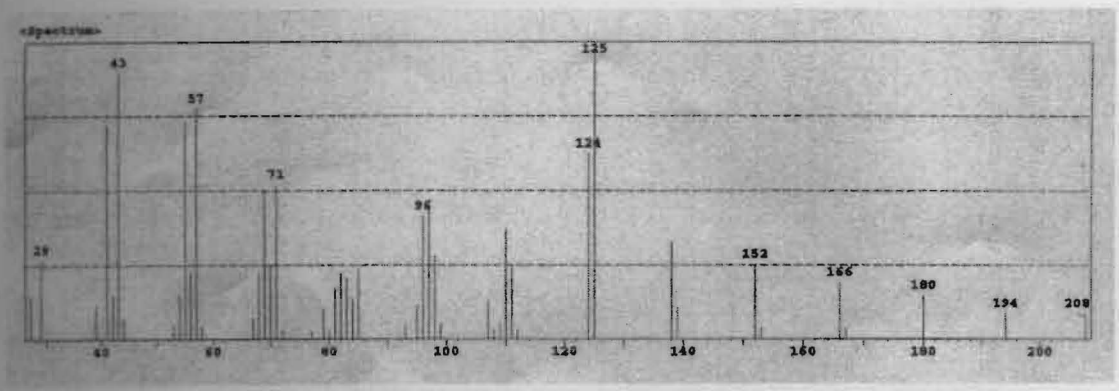

Figure 3: Mass spectrum of 2,6-dimethyltridecanenitrile

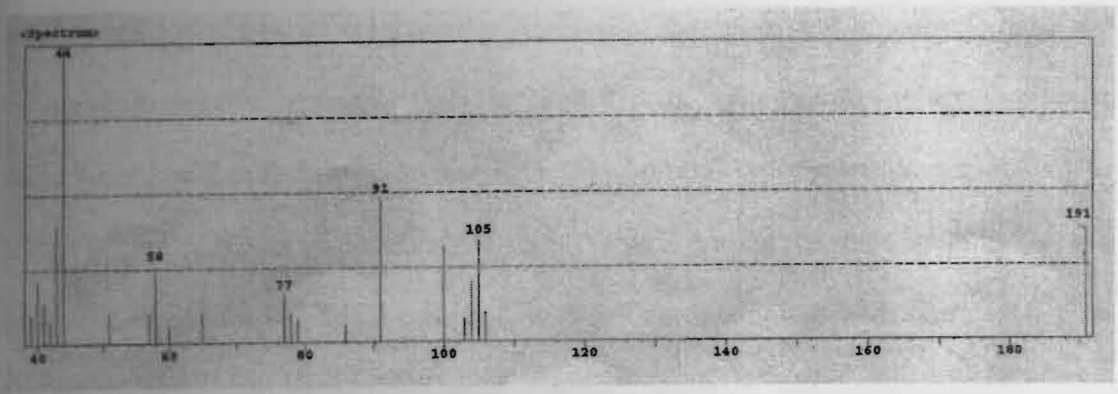

Figure 4: Mass spectrum of N-isopropyl-3-phenylpropanamide

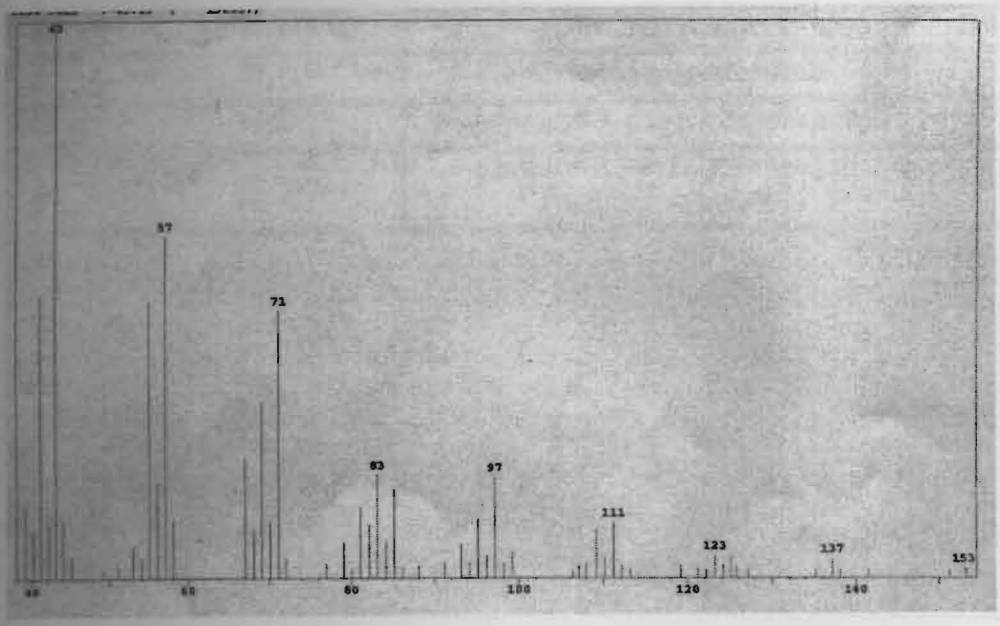

Figure 5: Mass spectrum of arabinose from gum from *Agathis borneensis*