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SEPARATION AND CHARACTERIZATION OF LIPOPHILIC EXTRACTS FROM BARLEY STRAW

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ABSTRACT

Barley straw was extracted with toluene-ethanol (2:1, vol/vol), chloroform, methyl *tert*-butyl ether (MTBE), hexane-acetone (2:1, vol/vol), and dichloromethane for 12 hours in a Soxhlet. The 51 individual compounds in 5 lipophilic extracts were separated by gas chromatography. Free fatty acids (15.18–38.20%), sterols (1.41–10.42%), waxes (3.45–11.87%), steryl esters (5.04–24.70%), and triglycerides (1.16–10.03%) were identified as the major 5 classes of lipids in the barley straw extracts. Minor components such as diglycerides (0.07–0.89%) and resin acids (0.12–0.57%) were also verified from the straw lipophilic extracts. Nonlipids detected from the barley straw extracts were composed

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of minor amounts of phenolic compounds (0.17–2.05%), noticeable amounts of azelaic and maleic acids (0.48–15.06%), and some quantities of co-extracted polysaccharides, ash, or salts. Of the individual compounds in each group, 16 free fatty acids, 5 steryl esters, 3 sterols, 3 triglycerides, and 2 waxes were quantitatively determined. Extraction with MTBE gave the lowest yield of total extracts (1.19%) but contained the highest amounts of lipophilic extracts (81.04%), which were enriched with steryl esters (24.70%), free fatty acids (23.60%), waxes (11.22%), sterols (10.42%), and triglycerides (10.03%). In contrast, extraction with 2:1 toluene-ethanol produced the highest quantities of total extracts (3.92%) but of the lowest purity of the lipid substances (49.69%) and were comprised mainly of free fatty acids (38.20%) and nonlipid substances such as maleic and azelaic acids (15.06%), phenolic compounds (1.89%), and noticeable amounts of released polysaccharides, ash, or salts.

Key Words: Barley straw; Lipophilic extracts; Separation; Fatty acids; Sterols; Waxes; Steryl esters; Triglycerides

INTRODUCTION

Lipophilic extracts, also known as wood resin or pitch, are composed mainly of free fatty acids, typically linoleic acid; resin acids, such as abietic acid; waxes, such as, palmitic acid oleyl ester; sterols, such as β-sitosterol; sterol esters, such as, sitosteryl oleate; and triglycerides, such as triolein (1,2). The amount and composition of the extracts are dependent on the wood species, tree age, and the environmental conditions (3). However, during the pulping process, the resin canals and parenchyma cells are broken down releasing a complex mixture of organic components, mainly lipophilic substances, which do not dissolve fully in the pulping water but form colloidal suspensions. The colloidal suspensions affect the operability of the paper machines and the quality of the final paper by producing pitch deposits, increased drainage time, decreased paper strength, and impaired sheet brightness (4,5).

Conventional approaches used to reduce wood extract deposits include the use of wood species low in extracts and the debarking or seasoning of logs. During seasoning, extracts undergo volatilization, enzymatic hydrolysis, and air oxidation. Unfortunately, these reactions are slow, particularly in cold weather. Furthermore, a long period of seasoning inflates costs and promotes microbial deterioration (3). Recently, new biological methods using fungal pretreatment have been developed to reduce extract levels with a minimal period of storage.

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However, the fungi are poorly adapted to low temperatures and to some of the wood species used, and some fungi appear effective only on certain wood types and under specific pulping conditions (6). Efforts to find a solution to this problem must begin with the development of a rapid, simple, and quantitative method for separating and identifying the complex lipid extracts in wood samples. Such a method would be useful in understanding pitch formation and optimizing reactions to remove the extracts prior to pulping.

Extracts are removed from both wood and pulp by organic solvents such as acetone, ethanol, ether, or dichloromethane. Traditionally, this has been carried out in a Soxhlet extractor (7). The mass of substances extracted from fresh wood samples decreased as the polarity of solvents decreased in the order, methanol > acetone > dichloromethane > hexane. However, the relative amounts of individual components in the lipophilic extracts were not substantially different from various extraction solvents (8). Additionally, no single solvent is capable of removing all the lipophilic substances, and different solvents removed different combinations of lipids (9).

Classical methods for quantitatively analyzing extracts are time consuming. Such methods involve separating free fatty and resin acids from the neutral materials by ion-exchange chromatography, saponifying the neutral fraction, and finally methylating the acid fractions prior to analysis by gas chromatography (GC). Since 1970, the ease and speed of techniques have simplified the analyses of extracts. Thin layer chromatography (TLC) has been widely used for the separation of various wood extracts, but no TLC separation has been reported which allows the simultaneous separation and quantification of the fatty acids, resin acids, and triglycerides in a single analysis (10). High-performance liquid chromatography (HPLC) has been adapted to the analysis of total extracts in softwoods (10) and to resin acids in aqueous samples (11). The related technique, high-performance size-exclusion chromatography, has allowed separation of extracts from effluents of softwood processing (12), while high-performance TLC has provided a convenient method for analysis of resin in wood, pulp, and paper (13). Gel permeation chromatography has also been employed for the separation and semiquantitative analysis of wood extracts (14). Other approaches to the analysis involve Fourier transform infrared (FTIR) (15) and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy studies from wood resin and pitch samples (2,16,17). Particularly in the last 10 years, with the use of modern gas chromatographs with short capillary columns, free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides can be separated in a single chromatographic run (1,3,6,8,18,19). However, separation of the individual components of waxes, steryl esters, and triglycerides are still limited by co-elution problems frequently encountered in GC operations.

Barley straw is an agricultural by-product that is not used as a significant industrial raw material in developed regions like Europe and North America. However, in many developing countries, wood is a limited resource, so nonwood

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fibers, such as cereal straws, for the pulp and paper industry are of growing importance because they contain comparative amounts of the major constituents common to wood species. In particular, wheat and barley straws are extensively used as raw materials for pulp and paper production in China. As we attempt to reduce the pitch deposits and find solutions to product-quality problems, the development of effective technologies for identification of the straw extracts is considered both important and significant.

In this study, a rapid separation method has been developed that enables convenient quantitative determination of individual components in all 5 classes of free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides from barley straw lipophilic extracts. The method is comprised of solvent extraction, silylation, and gas chromatography with a medium-length high-temperature capillary column with thin films. Minor amounts of phenolic compounds in the extracts are quantitatively verified by HPLC. In addition, spectroscopic analyses, such as FTIR and ^{1}H and ^{13}C NMR, and thermal analysis of the extracts were also performed for a comparative study.

EXPERIMENTAL METHODS

Materials and Reagents

Barley straw was obtained from the experimental farm of the North-Western University of Agricultural and Forest Sciences and Technology (Yangling, P. R. China). It was dried in sunlight and then cut into small pieces. The cut straw was ground to pass through a 1.0-mm screen. The extract yield was calculated on oven-dried (60°C, 16 hours) samples. All standard compounds used were obtained from Sigma (Xian, P.R. China). The solvents used were all HPLC or analytical grade. The fatty acids are abbreviated with a code indicating the number of carbon atoms: number of double bonds. For example, C18:2 indicates *cis*-9,12-octadecadienoic acid (linoleic acid).

Extraction and Silylation

Oven dried samples (approximately 50 g) were weighed into cellulose thimbles and extracted with 1800 mL of toluene-ethanol (2:1, vol/vol), chloroform, methyl *tert*-butyl ether (MTBE), hexane-acetone (2:1, vol/vol), and dichloromethane for 12 hours in a Soxhlet. The solvents were removed at 35°C by rotary evaporation, and the mixture was taken to further dryness in a nitrogen steam and then weighed to determine the extract yield. Note that the extract released in toluene-ethanol (2:1, vol/vol) was labeled fraction 1 (F1), and those dissolved in chloroform, MTBE, hexane-acetone (2:1, vol/vol), and dichloromethane

were named extract fractions 2 (F2), 3 (F3), 4 (F4), and 5 (F5), respectively. The dried lipophilic extracts (approximately 3 mg) were then silylated with 120 μL *bis*-trimethylsilyltrifluoroacetamide and 60 μL trimethylchlorosilane. The reactions were completed by keeping the glass-stoppered test tubes in an oven at 75°C for 20 minutes. When cooled, 180 μL toluene was added. The solution was shaken and thereafter ready for separation by GC.

GC Separation of Lipophilic Extracts

The derivatized lipophilic extracts were separated on an Rtx-1 capillary column (15 m, 0.53 mm i.d., 0.10-μm film thickness, Hewlett-Packard Co, Beijing, China) with a flame ionization detector (FID). The injector and detector temperatures were set at 340°C. The oven was temperature-programmed from 70°C to 340°C (2 minutes) at 8°C/min. Sample volumes of 1 μL were injected in splitless mode. Helium was used as the carrier gas. The integration baseline was checked for each analysis and corrected manually if necessary.

Individual compounds of free fatty and resin acids and sterols were identified both by comparison of their GC retention times and by total ion detection of mass spectra (MS) with those in authentic compounds. Each component of the waxes, steryl esters, and triglycerides was verified only by GC retention times because GC-MS detected only the fragments arising from the moiety affected by electron-impact MS and rarely gave detectable molecular ion spectra. A similar phenomenon was observed by Gutierrez et al.(19) in the detection of very high-mass lipids from wood extracts. In addition, based on the determination of wood, pulp and wheat straw extracts, Chen et al. (3) and Chaves das Neves and Gaspar (20) independently revealed that the major components of steryl esters in the wood and wheat straw were β-sitosterol esters. However, these compounds were not available commercially; therefore, they were replaced by the closely related cholesteryl esters as standards in this study. A mixture of standard compounds (palmitic acid, azelaic acid, abietic acid, β-sitosterol, palmitic acid palmitic ester (palmityl ester), cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol) was used to calibrate the quantitation of free fatty, azelaic and maleic, and resin acids; sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides, respectively. All of the fatty acids were assumed to have the same response factor with a flame ionization detector. Similar estimations were made for all of the resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides. In other words, free fatty acids were determined relative to the palmitic acid standard, azelaic and maleic acids relative to azelaic acid, resin acids relative to abietic acid, sterols relative to β-sitosterol, waxes relative to palmitic acid palmitic ester, steryl esters relative to cholesteryl palmitate, monoglycerides relative to

1-monopalmitoyl-rac-glycerol, diglycerides relative to 1,2-dipalmitoyl-sn-glycerol, and triglycerides relative to the 1,2-dipalmitoyl-3-oleoyl-rac-glycerol standard compound.

HPLC Separation of Phenolics

Both qualitative identification and quantitative determination of released minor amounts of phenolic compounds in the extracts were carried out on a Hichrom H5ODS HPLC column of dimensions 250 × 4.6 mm (Phenomenex Co, Beijing, China). Samples (approximately 10 mg) were dissolved in 1 mL methanol and separation was obtained using a linear gradient of 2 solvent systems: solvent A (water–methanol–acetic acid, 84:15:1) and solvent B (methanol–water–acetic acid, 90:9:1). A linear gradient was run for over 30 minutes from 0% to 40% B at a flow rate of 1 mL/min. The compounds were detected at 280 nm and the retention times and peak areas were compared with the authentic phenolics by computer. Note that 2,2′-dihydroxybiphenyl was used as a standard compound to calibrate the content of lignans (phenylpropane dimers) in the extracts because of the commercial lack of the authentic compound.

FTIR and 1H and 13C NMR Spectroscopy Studies

FTIR spectra were obtained on an FTIR spectrophotometer (Nicolet 510) using a KBr disc containing 1% finely ground samples. The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker MSL-300 spectrometer at 300 and 74.5 MHz in deuterochloroform, respectively. The ^{1}H NMR spectrum was recorded at 25°C from 15 mg of sample dissolved in 1.0 mL deuterochloroform for a total of 32 scans using an 10 μs (approximately 90°C) pulse and a 4 second delay time between scans. Solution ^{13}C NMR spectrum was recorded for 300 scans at 25°C from 80 mg of sample dissolved in 1.0 mL chloroform-d. A 70° pulse flipping angle, a 10 μs pulse width, and a 15 second delay time between scans were used.

Thermal Analysis

Thermal analysis of lipophilic extracts was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409). The apparatus was continually flushed with nitrogen. The sample weighed between 8 and 15 mg. Each sample was heated from room temperature to 600°C at a rate of 10°C per minute.

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RESULTS AND DISCUSSION

Yield and Purity

Results in Table 1 show that the yield of total extracts was lowest for the chloroform and MTBE extracts (1.21%, 1.19%) and highest for the toluene-ethanol extract (3.92%), whereas the hexane-acetone and dichloromethane extracts gave similar medium values (2.10%, 1.65%). This result is reflected in the

Table 1. The Yield (% Dry Straw) and Chemical Composition (% Dry) of Barley Straw Extracts

Yield/Composition	F1	F2	F3	F4	F5
Yield	3.92	1.21	1.19	2.10	1.65
Chemical composition					
Free fatty/resin/other acids (P 1–24)[a]	53.62	15.78	24.77	33.73	21.09
Compounds analyzed	52.03	12.02	19.86	23.71	16.01
Decanoic acid, C10:0 (P 1)	0.23	0.15	0.16	0.14	0.11
Dodecanoic acid, C12:0 (P 3)	0.80	0.20	0.18	0.42	0.60
Azelaic acid (P 5)	7.98	0.48	0.75	3.08	1.48
Tetradecanoic acid, C14:0 (P 6)	13.84	3.31	7.50	4.86	4.65
Pentadecanoic acid, C15:0 (P 7)	9.43	1.26	1.99	2.95	1.62
Maleic acid	7.08	N	N	N	N
Hexadecenoic acid, C16:1 (P 8)	1.56	0.82	0.93	0.86	0.78
Hexadecanoic acid, C16:0 (P 9)	7.96	2.55	3.31	4.25	1.88
Heptadecanoic acid, C17:0 (P 10)	0.11	0.22	0.73	0.55	0.51
Linoleic acid (C18:2) + oleic acid (C18:1) (P 11)	0.16	1.78	2.60	3.22	2.53
Octadecanoic acid, C18:0 (P 12)	0.58	0.11	0.28	0.42	0.18
Abietic acid (P 14)	0.36	0.12	0.42	0.57	0.24
Nonadecanoic acid, C19:0	0.12	N	N	0.30	0.11
Gondoic acid, C20:1 (P 15)	0.13	0.15	0.07	0.30	0.06
Eicosanoic acid, C20:0 (P 16)	0.29	0.40	0.30	0.45	0.32
Heneicosanoic acid, C21:0	0.21	0.06	N	0.10	0.10
Docosanoic acid, C22:0 (P 18)	0.51	0.18	0.38	0.46	0.43
Tetracosanoic acid, C24:0 (P 22)	0.68	0.23	0.26	0.78	0.41
Sterols (P 25–28)	1.41	7.11	10.42	6.20	8.40
Compounds analyzed	1.41	7.11	10.42	6.20	8.40
Cholesterol (P 25)	0.48	0.18	0.52	0.35	0.47
Stigmasterol +β-sitosterol (main compound) (P 26–28)	0.93	6.93	9.90	5.85	7.93
Waxes (P 29–32)	3.45	11.18	11.22	8.54	11.87
Compounds analyzed	3.11	7.71	8.41	6.13	7.53
Palmitic acid palmitic ester (P 29)	0.62	4.69	6.36	3.95	4.55
Palmitic acid oleyl ester (P 31)	2.49	3.02	2.05	2.18	2.98

(continued)

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Table 1. Continued

Yield/Composition	F1	F2	F3	F4	F5
Diglycerides (P33, 34)	0.07	0.57	0.65	0.38	0.89
Compound analyzed	0.05	0.29	0.34	0.24	0.51
Dipalmitin (P 33)	0.05	0.29	0.34	0.24	0.51
Sterol (mainly sitosterol) esters (P 35–44)	5.04	23.26	24.70	11.40	23.28
Compounds analyzed	3.69	16.87	18.25	8.54	15.92
Steryl laurate (P 37)	1.45	4.02	4.25	1.87	3.26
Steryl myristate (P 39)	1.08	7.02	7.24	3.38	6.90
Steryl palmitate (P 41)	0.46	2.64	2.70	1.38	2.30
Steryl heptadecanoate (P 42)	0.38	1.03	1.60	0.62	1.36
Steryl oleate (P 43)	0.32	2.16	2.46	1.29	2.10
Triglycerides (P 45–51)	1.16	8.51	10.03	5.87	8.26
Compounds analyzed	0.84	5.58	6.65	4.13	6.48
Tripalmitin (P 46)	0.18	2.01	2.64	1.46	2.75
Dipalmitoyl-oleoylglycrol (P 47)	0.14	1.49	1.68	0.93	1.28
Triolein (P 48)	0.52	2.08	2.33	1.74	2.45
Total lipophilic substances	49.69	66.03	81.04	63.04	72.31
Total substances	64.75	66.50	81.79	66.12	73.79

Barley-straw fractions obtained by extraction for 12 hours in a Soxhlet are denoted as follows: toluene-ethanol (2:1, vol/vol), F1; chloroform, F2; MTBE, F3; hexane-acetone (2:1, vol/vol), F4; and dichloromethane, F5.

[a] P value represents peak number in Fig. 2.

N Not detectable.

high amounts of free fatty and resin acids, 38.56% in toluene-ethanol extract and 30.65% in hexane-acetone extract, and steryl esters, ranging between 23.26–24.70% in chloroform, MTBE, and dichloromethane extracts. However, as the table shows, the total extract amounts detected by GC were highest in MTBE extract (81.79%) and lowest in the toluene-ethanol, chloroform, and hexane-acetone extracts (64.75–66.50%).

The purity of extract, the percentage of the total lipophilic extracts, was highest in the MTBE extract (81.04%) and lowest in the toluene-ethanol extract (49.69%), which contained noticeable amounts of azelaic and maleic acids (15.06%). The portion of the total lipophilic extracts in dichloromethane extract (72.31%) was higher than that in both the chloroform and hexane-acetone extracts (63.04–66.03%). This indicated that extraction with 2:1 toluene-ethanol, chloroform, or 2:1 hexane-acetone released not only substantial amounts of lipophilic substances but also noticeable quantities of nonlipid components, such as low molecular weight carbohydrates, ash, salt, and other water-soluble substances (9).

MTBE appeared to be an effective solvent for extraction of lipophilic extracts from barley straw. Similar observations were made during the extraction of fatty and resin acids with MTBE in wood pulp mill effluents (1).

The results suggested that MTBE could be a useful alternative solvent for extraction of lipophilic materials from barley straw. However, care must be taken when interpreting results of total extracts. If both the total extracts and purity of the extract are considered, dichloromethane could be used as a selective solvent for extraction of barley straw.

Chemical Composition

Separation of barley straw extracts by GC on a medium-length capillary column gave chromatograms in which all of the individual components in 5 classes of lipids (free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides) were generally well separated, although the linoleic acid (C18:2) and oleic acid (C18:1) co-eluted. Among these, free fatty and resin acids were well separated as their trimethylsilyl (TMS) esters, while the sterols were separately eluted as their TMS ethers, which gave better GC peak shapes than did the sterols themselves. Waxes, steryl esters, and di- and triglycerides were also well eluted without derivations. As mentioned above, the identification of components is obtained by comparing the GC retention times or MS spectra of the components with those of authentic compounds run under the same conditions. For example, a typical chromatogram for 5 authentic compounds (palmitic acid, β-sitosterol, palmitic acid palmitic ester, cholesteryl heptadecanonate, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol) in 5 classes of lipids is shown in Fig. 1, in which the high-molecular-

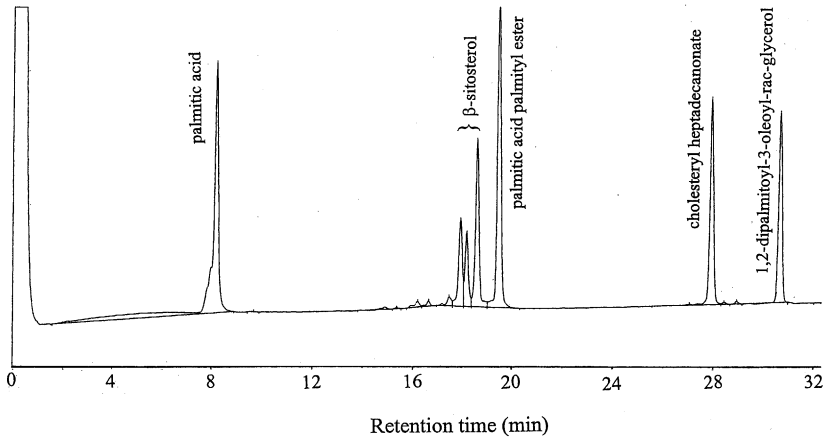

Figure 1. Typical chromatogram for a standard mixture.

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mass lipids, such as sterol esters and triglycerides, were well separated. In the quantitative study, no significant differences in the peak areas were found between the equal amounts of authentic compound standards among lipids of the same class. However, the peak areas among different classes of lipids showed noticeable variations. Thus, a mixture of equal amounts of the standard compounds with palmitic acid, azelaic acid, abietic acid, β-sitosterol, palmitic acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol, which gave peak areas in ratios of 1.0:0.88:0.86:0.92:0.85:0.74:0.96 :0.79:0.61, was used to calibrate the peak areas before quantitation of free fatty acids, azelaic and maleic acids, resin acids, sterols, waxes, sterol esters, monoglycerides, diglycerides, and triglycerides, respectively. These ratios were easily reproducible and were not notably affected by small variations in injection technique, injection volume, injection rate, and carrier gas flow. Similar observations were made for the analyses of wood extracts in papermaking process waters (1). The use of these 9 appropriate standards compensated for the FID detector differences between classes of lipids. The FID response was assumed to be the same for the component group and results corresponded to the 9 standards. The peak areas of free fatty acids in the extracts were quantitated against the standard. For example, palmitic acid was divided by 1.0 before calculation of its concentration. Likewise, resin acids, azelaic and maleic acids, sterols, waxes, sterol esters, monoglycerides, diglycerides, and triglycerides were quantitated against abietic acid, azelaic acid, β-sitosterol, palmitic acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol were divided by 0.86, 0.88, 0.92, 0.85, 0.74, 0.96, 0.79, and 0.61, respectively, prior to calculation of their concentrations.

Fifty-one individual compounds in the MTBE extract were separated by GC, under the described conditions, as illustrated by the chromatogram in Fig. 2.

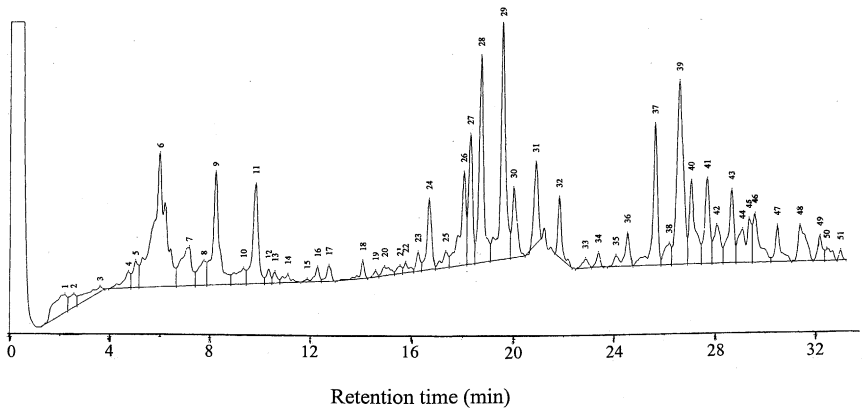

Figure 2. The chromatogram of MTBE extracts (F3) from barley straw.

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The designation of the peak numbers is given in Table 1. Free fatty acids (15.18–38.20%), sterol esters (5.04–24.70%), waxes (3.45–11.87%), sterols (1.41–10.42%), and triglycerides (1.16–10.03%) were the dominant 5 lipid classes present in the barley straw lipophilic extracts. Diglycerides were detected in minor amounts (0.07–0.89%). No monoglycerides were found in the extracts. Our results are compatible with the conclusions of Gutierrez et al.(19) and Cooper et al. (21): Diglycerides are present in trace amounts in wood extracts and presumed to be intermediates in the synthesis of triglycerides.

As can be seen from Table 1, the major saturated fatty acids were myristic acid (C14:0), palmitic acid (C16:0), and pentadecanoic acid (C15:0), which together comprised 85.30, 62.35, 68.49, 60.12, and 57.03% of the total free fatty acids identified in the toluene-ethanol (F1), chloroform (F2), MTBE (F3), hexane-acetone (F4), and dichloromethane (F5) extracts, respectively. Linoleic acid (C18:2) and/or oleic acid (C18:1) and palmitoleic acid (C16:1) were the main components of unsaturated free fatty acids and together accounted for 4.70, 22.77, 18.89, 20.34, and 29.04% of the total free fatty acids found in the extracts F1, F2, F3, F4, and F5, respectively. In addition, minor quantities of decanoic acid (C10:0, 0.11–0.23%), dodecanoic acid (C12:0, 0.18–0.80%), heptadecanoic acid (C17:0, 0.11–0.73%), octadecanoic acid (C18:0, 0.11–0.58%), eicosanoic acid (C20:0, 0.29–0.45%), docosanoic acid (C22:0, 0.18–0.51%), and tetracosanoic acid (C24:0, 0.23–0.78%) were also identified in all of the 5 extracts. Nonadecanoic acid (C19:0), gondoic acid (C20:1), and heneicosanoic acid (C21:0) were verified in trace amounts. In general, the results were consistent with the free fatty acid compositions reported by Gutierrez et al. (19) from *Eucalyptus globulus* labill wood extract. The authors reported that free fatty acids accounted for 27.69 mg/100 g wood and were found with chains from C14 to C26. The dominant component was palmitic acid (C16:0), followed by linoleic (C18:2), oleic (C18:1), and stearic (C18:0) acids.

Seven resin acids such as pimaric, sandaracopimaric, isopimaric, palustric, dehydroabietic, abietic, and neoabietic acids present in slash pine wood were identified by comparing their GC-retention times with those of model compounds and by GC-MS spectra (5). Analogously, dehydroabietic, abietic, and pimaric acids were found to be the major resin acids in radiata pine woods (8). We were surprised that the present findings showed that abietic acid was the only resin acid identified in the barley straw extracts, and it was found in minor amounts, ranging from 0.12% in F2 to 0.57% in F4. The dearth of nonabietic acids in the extracts is probably due to the thermal isomerization reactions of resin acids, such as neoabietic and palustric acids, during the Soxhlet extractions.

In addition to the chemical composition of lipophilic components listed in Table 1, noticeable amounts of nonlipids, such as azelaic and maleic acids (15.06%), were found in the toluene-ethanol extract, and small quantities of azelaic acid (0.48–3.08%) were also identified in the other 4 extracts by GC and GC-MS; however, they were substantially lower in the chloroform (0.48%) and

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MTBE extracts (0.75%). The current results were in good agreement with the studies of wheat straw extracts reported by Chaves das Neves and Gaspar (22). The authors stated that azelaic acid was the dominant nonlipid component and maleic acid occurred in a noticeable amount in the wheat straw extracts.

The compounds giving the peaks between 25 and 28 were identified to be sterols, as TMS ethers, and were highest in the MTBE extract (10.42%) and lowest in toluene-ethanol fraction (1.41%). Stigmasterol and β-sitosterol were co-eluted. However, Fig. 1 shows that β-sitosterol was the predominant component of the total sterols. Stigmasterol was found in a small amount but co-eluted with β-sitosterol at peak 27. Cholesterol was detected in a minor amount (0.18–0.52%). This is very similar to the value (64.50 mg sterols/100g wood) reported previously from *Eucalyptus globulus* labill wood (19) in which β-sitosterol was the most abundant component and accounted for 76.60% of the total sterols.

In the present study, the range of analyses temperature were extended by using a high-temperature medium-length capillary column with thin films, which were preferred for the separation of high molecular-mass lipids, such as waxes, steryl esters, and triglycerides. As shown in Fig. 2, 4 components of waxes were well separated at peaks 25–28. Palmitic acid palmityl ester and palmitic acid oleyl ester were detected as the major compounds verified by GC, which together accounted for 90.15% of the total waxes in toluene-ethanol extract, 68.96% in chloroform extract, 74.96% in MTBE extract, 71.78% in hexane-acetone extract, and 63.44% in dichloromethane extracts, respectively.

Sterol esters were the second most abundant class of lipids in barley straw lipophilic extracts. They were found in particularly high amounts in chloroform, MTBE, and dichloromethane extracts, ranging from 23.26 to 24.70% of the total extracts. This implied that these 3 solvents favored isolation of steryl esters from barley straw. As Fig. 2 illustrates, 10 constituents of steryl esters were well eluted in the chromatogram. Steryl laurate, myristate, steryl palmitate, steryl margarate, and steryl oleate were revealed as the major components, which together comprised 68.38–74.91% of the total steryl esters. Among these identified, steryl laurate and steryl myristate were dominant, accounting for 68.56% in F1, 65.44% in F2, 62.96% in F3, 61.48% in F4, and 63.82% in F5, respectively. The distribution of esterified fatty acids was the same as that of the free fatty acids as described above.

Finally, triglycerides were also identified as an important group of lipids in the barley straw extracts; however, each of the triglycerides eluted in a relatively small peak area compared to equal amounts of free fatty acids, sterols, waxes, and steryl esters. To calibrate the quantitative analyses, the individual peak area of the triglycerides was divided by 0.61 before calculation of their concentrations. As can be seen from the chromatogram in Fig. 2, 7 different components of the triglycerides were well resolved. Glyceryl tripalmitate, 1,2-dipalmitoyl-3-oleoyl-rac-glycerol, and triolein (*cis*-9) were quantitatively verified and together comprised 72.41% of the total triglycerides in the toluene-ethanol extract, 65.57% in

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the chloroform extract, 66.30% in the MTBE extract, 70.36% in the hexane-acetone extract, and 78.45% in the dichloromethane extracts, respectively. Other minor components in the barley straw extracts included diglycerides, which comprised 0.07–0.89% of the total extracts. 1,2-Dipalmitoyl-rac-glycerol was the only compound identified in this group and occurred in a trace amount in the F1 fraction (0.05%) and in minor quantities in the F2–F5 fractions (0.24–0.51%). Monoglyceride was not detected in any of the 5 barley straw extracts.

Phenolic substances are widely distributed in plants such as wheat, oat, and barley. Particularly, cinnamic acids have been found in various combined forms, such as with glycerides and polysaccharides in cell walls (23). Because phenolic acids and aldehydes released during the Soxhlet extractions are nonvolatile compounds, they were analyzed by HPLC at 280 nm. The results are given in Table 2,

Table 2. The Content (% Dry Extractives, wt/wt) of Phenolic Acids and Aldehydes, Lignan, and Other Compounds Identified in the Barley Straw Extractives by HPLC

Phenolic Acids and Aldehydes, Lignan, and Other Compounds	F1	F2	F3	F4	F5
Gallic acid	0.050	N	N	0.055	0.025
Protocatechuic acid	0.078	0.008	0.011	0.12	0.045
p-Hydroxybenzoic acid	0.065	0.012	0.010	0.086	0.047
p-Hydroxybenzaldehyde	0.059	0.004	0.006	0.041	0.037
Vanillic acid	0.098	0.009	0.023	0.067	0.043
Syringic acid	0.034	0.036	0.003	0.043	0.032
Vanillin	0.078	0.12	0.028	0.11	0.084
Syringaldehyde	0.22	0.67	0.012	0.16	0.11
Acetovanillone	0.043	0.003	N	0.005	0.005
Acetosyringone	0.065	0.005	N	0.008	0.006
p-Coumaric acid	0.22	0.026	0.008	0.20	0.12
Ferulic acid	0.11	0.013	0.004	0.066	0.031
Sinapic acid	0.19	0.003	0.002	0.074	0.051
Cinnamic acid	0.030	0.024	N	0.050	0.022
Fumaric acid	0.033	0.006	N	0.32	0.078
Benzoic acid	0.19	0.028	0.006	0.32	0.19
m-Toluic acid	0.14	0.21	0.035	0.24	0.11
1-Naphthoic acid	0.094	0.042	0.018	0.065	0.023
Lignan	0.090	0.27	0.008	0.020	0.034
Total (%)	1.89	1.49	0.17	2.05	1.09

Barley-straw fractions obtained by extraction for 12 hours in a Soxhlet are denoted as follows: toluene-ethanol (2:1, vol/vol), F1; chloroform, F2; MTBE, F3; hexane-acetone (2:1, vol/vol), F4; and dichloromethane, F5.
N Not detectable.

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which shows that extraction with mixtures of 2:1 toluene-ethanol or with 2:1 hexane-acetone released higher amounts of phenolic compounds (1.89%, 2.05%) than did single solvents such as chloroform (1.49%), MTBE (0.17%) or dichloromethane (1.09%). MTBE extracted the least amount of phenolic compounds, indicating that MTBE was a selective solvent for extraction of lipophilic extracts from straw samples. *p*-Coumaric acid (0.008–0.22%), ferulic acid (0.004–0.11%), syringaldehyde (0.012–0.67%), vanillin (0.028–0.12%), sinapic acid (0.002–0.19%), benzoic acid (0.006–0.32%), and *m*-toluic acid (0.035–0.24%) were the major compounds in the straw extracts. Protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, 1-naphthoic acid, and lignan were quantified in relatively small amounts. In addition, trace quantities of gallic acid, acetovanillone, acetosyringone, cinnamic acid, and fumaric acid were also found to be present in the lipophilic extracts. Chaves das Neves and Gaspar (22), using capillary gas chromatography coupled with mass spectrometry (HRGC-MS) and HRGC-FTIR, qualitatively verified the existence of another 20 phenolic compounds in wheat straw extracts.

Lignans, characterized by two phenylpropane units bound together at their β-carbon, are found in the heartwood of trees. The lignan concentrations vary considerably between trees and also within each tree. The concentration in heartwood ranges from trace amounts to approximately 0.5% of dry wood weight. Hydroxymatairesinol and α-conidendric acid are the most abundant lignans in heartwood and effluents (24). In this study, the release of barley-straw lignans during the different solvent extractions in a Soxhlet was quantitatively determined using 2,2′-dihydroxybiphenyl as a standard. Note that extraction with chloroform led to 0.27% lignan in the extract, whereas extractions with toluene-ethanol, MTBE, hexane-acetone, or dichloromethane released only trace amounts of lignan (0.008–0.09%).

FTIR Spectra

Figure 3 illustrates FTIR spectra of chloroform extract (spectrum 1), MTBE extract (spectrum 2), and dichloromethane extract (spectrum 3). Despite some small differences in the peak intensities between the spectra, all 3 spectra exhibit similar absorption bands characteristic of the main functional groups, indicating that each was comprised of a similar mixture of the extracts. A prominent broad band at 3434 cm^{-1} is attributed to the hydroxyl group stretching vibration in sterols, mono- and diglycerides, co-extracted polysaccharides, or water in samples (25). The very strong methylene and methyl stretching frequencies occur at 2928 and 2863 cm^{-1}, respectively. A strong band at 1740 cm^{-1} corresponds to absorption by carbonyl bonds in esters (waxes, sterol esters, triglycerides). The carbonyl bonds in free fatty and resin acids give a band at 1712 cm^{-1} in the spectrum of

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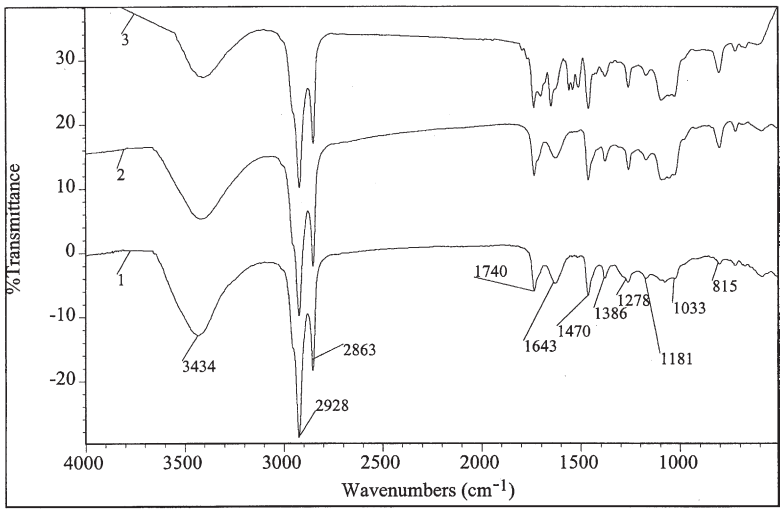

Figure 3. FTIR spectra of barley straw extracts obtained from chloroform (spectrum 1), MTBE (spectrum 2), and dichloromethane (spectrum 3). Extraction lasted 12 hours in a Soxhlet.

dichloromethane extract (spectrum 3), whereas it is strongly overlapped with the previous one in the spectra of chloroform and MTBE extracts (Fig. 3, spectra 1 and 2). The carbon double-bond stretching (C═C in unsaturated fatty acids and their esters or in sterols and steryl esters) exhibits an absorption band at 1643 cm^{-1}. Two sharp bands at 1470 and 1386 cm^{-1} arise from the methylene bending vibration and methyl symmetrical bending, respectively. A much stronger intensity at 1470 cm^{-1} than at 1386 cm^{-1} is typical of long-chain fatty acids or their esters. The carbon single-bonded oxygen (C—O) or bonded hydroxyl (C—OH), bending or stretching vibration gives an absorption band at 1278 cm^{-1}. A weak band at 1181 cm^{-1} is assigned to the C—O stretching in the aliphatic esters (O═C—O—$CHCH_2$—) (25). The broad band between 1110 and 1033 cm^{-1}, particularly in spectra 2 and 3, is attributed to the C—C stretching and ether bond (C—OC) symmetrical stretching in co-extracted polysaccharides, respectively. This finding indicated that the 3 lipophilic extracts also contained some released polysaccharides, typical of xylans (26). The peaks at 815 and 728 cm^{-1}, particularly at high frequency in spectra 2 and 3 represent the ring (in sterol or steryl esters), out-of-plane, carbon single-bonded hydrogen (C—H) bending vibrations associated with 2 adjacent hydrogen atoms and in-plane methylene (CH_2) rocking absorptions in straight chain paraffin, respectively (15). The peak at 728 cm^{-1} has been associated with straight-chain methylene at least 4 units long, implying the existence of long, straight methylene chains in the straw extracts (27).

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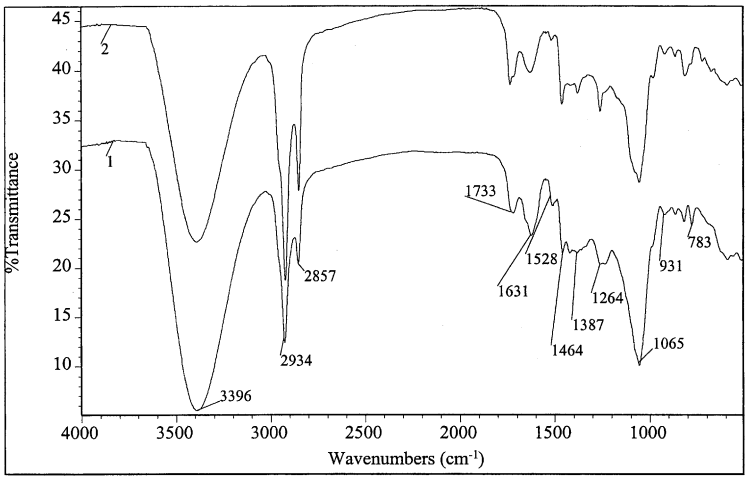

Figure 4. FTIR spectra of barley straw extracts from toluene-ethanol (2:1, vol/vol, spectrum 1) and hexane-acetone (2:1, vol/vol, spectrum 2). Extraction time was 12 hours.

The FTIR spectra of toluene-ethanol extract (spectrum 1) and hexane-acetone extract (spectrum 2) are given in Fig. 4. Prominent peaks for lipids include strong absorptions at 3396 cm^{-1} (OH stretching), 2934 and 2857 cm^{-1} (CH_2 and CH_3 stretching), 1733 cm^{-1} (C═O stretching of esters), 1712 cm^{-1} (C═O stretching of acids), and 1631 cm^{-1} (C═C stretching). Weaker absorptions in the region of 1464 and 1387 cm^{-1} are assigned to the CH_2 and CH_3 bending vibrations, respectively. The band at 1264 cm^{-1} is due to the C—O or C—OH bending or stretching vibrations. A much stronger band at 1065 cm^{-1} (C—O—C stretching in polysaccharides) indicated that a substantial amount of co-extracted polysaccharides exist in these 2 extracts. These FTIR results confirmed the lower purity (content of lipophilic substances) of toluene-ethanol extracts (49.69%) and hexane-acetone extracts (63.04%) obtained by GC analysis. In addition, occurrence of a very weak peak at 1528 cm^{-1} in both spectra of Fig. 4 is probably due to minor amounts of released phenolic compounds in the extracts, which corresponded to the results obtained by HPLC analyses.

^{1}H and ^{13}C NMR Spectra

To obtain further information on all the extracts, ^{1}H and ^{13}C NMR spectroscopy was performed. The ^{1}H NMR spectrum of the MTBE extract is given in Fig. 5. The most intense signal, occurring at 1.28 ppm, is assigned to the methylene aliphatic protons, while that centered at 0.88 ppm is attributed to methyl pro-

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tons. Both methine and naphthenic peaks were obscured by the methylene peak. Minor signals from protons on carbons adjacent to the carbonyl group in esters (CH_2—C═O) or adjacent to an acid group ($C\underline{H}_2$—COOH) occur at approximately 2.0 and 2.3 ppm, respectively. A rather small band at approximately 2.8 ppm could have arisen from protons adjacent to a ketone or on a carbon adjacent to an aromatic ring (27). The chemical shifts for protons on carbons adjacent to alcohols (C<u>H</u>OH) or ethers (C<u>H</u>—O—C) give peaks between 3.5 and 4.5 ppm. The spectrum exhibits peaks between 5.1 and 5.5 ppm for protons on carbons adjacent to an alkene. Note that the peak at 7.28 ppm is presumed to represent the residual chloroform present in $CDCl_3$ and should be ignored.

Figure 6 illustrates the solution-state ^{13}C NMR spectrum of the dichloromethane extract. The carbon atoms from unsaturated compounds (110–160 ppm), aliphatic (0–34 ppm) and C-O substituted (40–90 ppm) structures, and functional groups (170–200 ppm) were clearly distinguished from each other (28). The signal at 194.5 ppm is due to the carbonyl groups in lipids. The carbonyl groups in fatty acids and fatty acid esters give signals at 178.7 and 174.1 ppm, respectively. The signals at 156.3, 140.8, 138.6, 135.1, 124.2, and 121.7 ppm originated from unsaturated carbons (>C═) in resin acids or sterols and steryl es-

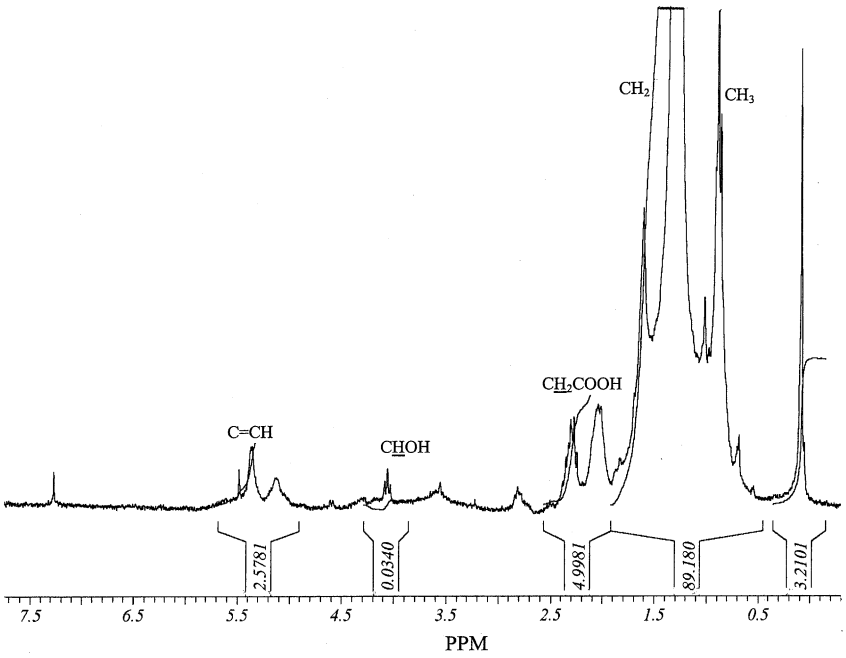

Figure 5. ^{1}H NMR spectrum of MTBE extract.

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ters, while the signals at 130.1 and 128.2 ppm are assigned to unsaturated carbon double-bonds (—CH═CH—) in fatty acids or fatty acid esters. The glycerol carbons (—C—O or >C—O) of triglycerides exhibit a signal at 64.3 ppm. The carbon-bonded oxygen group (>C—O) in sterol or steryl esters gives signals at 71.8, 56.8, 56.2, 50.1, 45.8, and 42.3 ppm. Five signals at 39.7, 39.3, 38.3, 37.4, and 37.2 are presumed to be due to the methine group in resin acids, sterols or steryl esters; however, in general, methine could not be differentiated from methylene peaks. The peak at 14.0 ppm relates to the methyl end of the chain. The largest 3 peaks at 22.6, 29.1–29.6, and 31.9 ppm together with the signals at 15.9, 19.7, 21.0, 22.5, 22.6, 24.7, 25.0, 25.5, 25.7, 25.9, 26.6, 27.1, 27.9, 28.6, 31.4, 32.7, and 34.3 ppm are originated from the methylene units successively further from the methyl group (27).

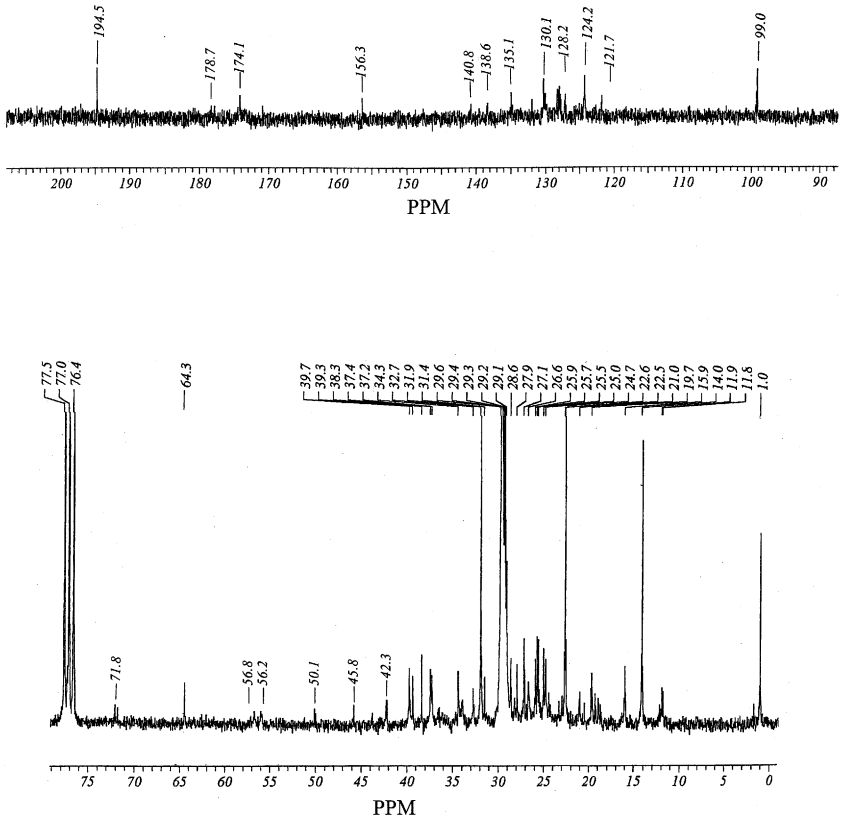

Figure 6. The ^{13}C NMR spectrum of dichloromethane extract.

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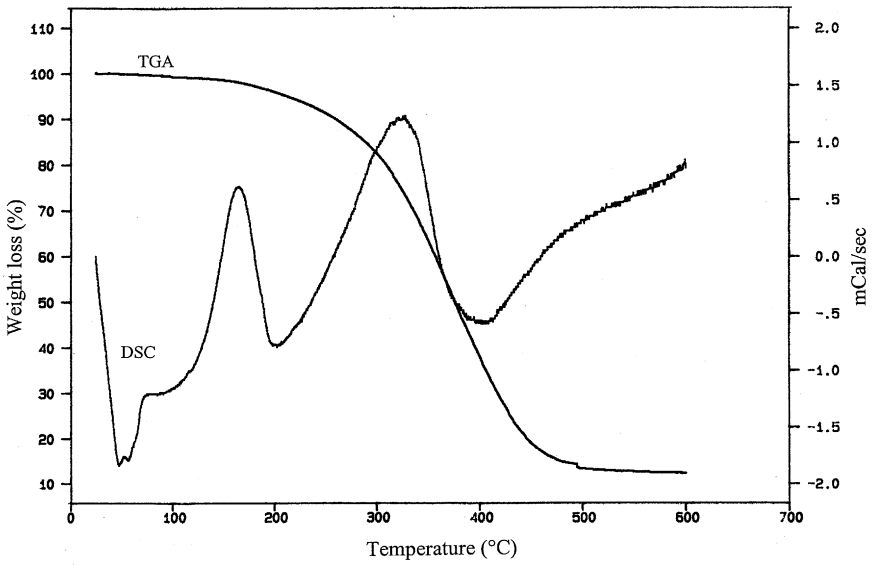

Figure 7. Thermogram of MTBE extract.

Thermal Analysis

Thermal analysis of the extracts was carried out using TGA DSC. Figure 7 shows the TGA and DSC curves of MTBE extract. The extract started decomposition at 167°C, and its maximum rate of weight loss occurred between 333°C and 432°C. The decomposition temperature at 10% weight loss was 257°C. When the temperature was raised to 400°C, the weight loss increased significantly to over 60%. The nonvolatile residues, such as ash or salts, in the extract appeared in a small amount (approximately 12%) as the TGA shows at 600°C. In addition, the melting point, a solid-liquid phase transition from the hexagonal crystal structure to a melt, as shown in the DSC curve, appeared at 45.5–57.2°C. This melting-temperature range of 10°C indicates that the MTBE extract consists of a mixture of lipophilic substances. This result corresponds to the results obtained from GC analysis. The melting temperatures of this MTBE extract were consistent with melting temperatures of lipophilic extracts from wood samples, which had melting temperatures between 30 and 70°C (29).

CONCLUSIONS

Our study revealed that the GC separation of silylated extracts with a medium-length capillary column is an excellent technique for quantitatively de-

termining individual components of lipophilic extracts from barley straw. Free fatty acids (15.18–38.20%), sterols (1.41–10.42%), waxes (3.45–11.87%), steryl esters (5.04–24.70%), and triglycerides (1.16–10.03%) were identified as the major 5 classes of lipids in the barley straw extracts. Sixteen free fatty acids ranging from C10 to C24 were quantitatively verified, in which the most abundant saturated free fatty acids were myristic acid (C14:0, 3.31–13.84%) and palmitic acid (C16:0, 1.88–7.96%), whereas linoleic acid (C18:2) and/or oleic acid (C18:1, 0.16–3.22%) were the most dominant unsaturated free fatty acids. β-Sitosterol was a predominant component identified in a class of sterols. Palmitic acid palmitic ester and palmitic acid oleyl ester were the major components analyzed in a group of waxes, which together comprised 63.44–90.15% of the total waxes. The steryl esters identified were composed mainly of steryl myristate (1.08–7.24%), steryl laurate (1.45–4.25%), steryl palmitate (0.46–2.70%), steryl oleate (0.32–2.46%), and steryl heptadecanoate (0.38–1.60%). Of the triglycerides identified, tripalmitin (0.18–2.75%), dipalmitoyl-oleoylglycrol (0.14–1.68%), and triolein (0.52–2.45%) were the dominant components. Diglycerides amounted to trace or minor quantities (0.07–0.89%) of the total extracts. In addition, minor amounts of phenolic compounds (0.17–2.05%), such as *p*-coumaric and ferulic acids, and noticeable amounts of azelaic and maleic acids (0.48–15.06%) were also quantitatively determined from the barley straw extracts.

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