JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2008; **43**: 97–107
Published online 30 August 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jms.1276

JMS

Identification of lignans by liquid chromatography-electrospray ionization ion-trap mass spectrometry

Patrik C. Eklund,* M. Josefin Backman, Leif Å. Kronberg, Annika I. Smeds and Rainer E. Sjöholm

Laboratory of Organic Chemistry, Process Chemistry Centre, Åbo Akademi University, Biskopsgatan 8, FIN-20500 Åbo, Finland

Received 22 December 2006; Accepted 2 July 2007

The fragmentation pattern of 30 compounds belonging to different classes of the lignan family was studied by liquid chromatography-electrospray ionization ion-trap mass spectrometry. On the basis of the observed fragmentation patterns, identification of different types of lignans was achieved. For example, dibenzylbutyrolactone lignans showed a characteristic fragmentation pathway by the loss of 44 Da (CO_2) from the lactone moiety, whereas dibenzylbutanediols showed a loss of 48 Da by a combined loss of formaldehyde and water from the 1,4-butanediol moiety. Lignan glycosides readily lost the sugar residue to give the parent lignan as their primary product ion. In addition, several compound-specific fragmentations were observed and used for identification of individual compounds.

A versatile method for analyses of lignans was developed using LC separation on a C8 column followed by fragmentation and detection of ions produced in the ion trap. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: lignan; HPLC; ESI; MS; ion trap; fragmentation

INTRODUCTION

The combination of liquid chromatography and electrospray ionization mass spectrometry (LC/ESI/MS) has become a routine method in many areas of analytical organic chemistry. The provided mass spectrometric data as well as the attainable high sensitivity has made these methods well suited for the identification of specific compounds in mixtures of natural products. For example, successful qualitative and quantitative analyses of lignans, flavonoids, alkaloids, and lignin-based polyphenolic mixtures by LC/ESI/MS have been reported previously.[1–8] For qualitative purposes, the use of electrospray ionization in combination with an ion trap is superior, as the isolation of molecular ion species, followed by fragmentation in the ion trap, provides useful structural information on the analytes. Moreover, an unambiguous identification of compounds can be achieved by comparison of known fragmentation patterns or by using authentic reference compounds.

Lignans are plant constituents, which are defined by a β-β-linkage of two phenyl propane units. These natural products have attracted much interest over the years owing to their widespread occurrence in the plant kingdom and their broad range of biological activity.[9] A dietary intake of lignans has been shown to have beneficial effects on human health by their protective effects against hormone-related cancers and due to their antioxidant effects.[10–12] Therefore, new and improved methods for analyses of lignans in different matrices are needed and, in fact, regularly reported.[13,14]

The analysis of lignans in plant material as well as in mammalian body fluids has traditionally employed GC and GC/MS techniques of derivatized samples. The use of LC/ESI/MS offers an alternative and sensitive method for the analyses of underivatized lignans and lignans in glycosidic and oligomeric forms. However, compared to traditional electron ionization (EI), the fragmentation behaviors of organic compounds in collision induced dissociation (CID) employed in MS/MS techniques are still not fully understood. The use of fragmentation patterns for structural analysis may therefore not always be straightforward, especially when unexpected fragmentations are observed. In order to allow the identification of lignans by examining their fragmentation behavior, we here report the fragmentation data of different types of lignans using ESI-ion-trap mass spectrometry.

EXPERIMENTAL

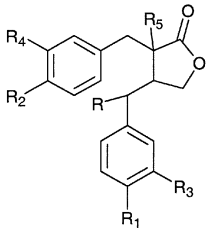
Reference compounds and chemicals

The structures of the analyzed compounds are presented in Fig. 1.

Hydroxymatairesinol (**5**) was isolated from knotwood of Norway spruce (*Pica abies*) as previously described.[15]

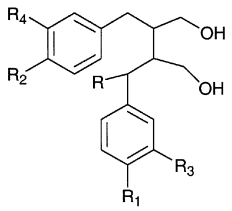
*Correspondence to: Patrik C. Eklund, Laboratory of Organic Chemistry, Process Chemistry Centre, Åbo Akademi University, Biskopsgatan 8, FIN-20500 Åbo, Finland. E-mail: paeklund@abo.fi

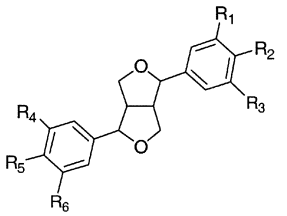
JMS



Compound	R	R_1	R_2	R_3	R_4	R_5
Matairesinol (**1**)	H	OH	OH	OCH_3	OCH_3	H
7-Oxomatairesinol (**2**)	O	OH	OH	OCH_3	OCH_3	H
Enterolactone (**3**)	H	H	H	OH	OH	H
Nortrachelogenin (**4**)	H	OH	OH	OCH_3	OCH_3	OH
7-Hydroxy-matairesinol (**5**)	OH	OH	OH	OCH_3	OCH_3	H
Trachelogenin (**6**)	H	OCH_3	OH	OCH_3	OCH_3	OH
Arctigenin (**7**)	H	OH	OCH_3	OCH_3	OCH_3	H
Hinokinin (**8**)	H	$-OCH_2O-$	$-OCH_2O-$	$-OCH_2O-$	$-OCH_2O-$	H
Dimethyl-Matairesinol (**9**)	H	OCH_3	OCH_3	OCH_3	OCH_3	H
4,4′-Dihydroxy-enterolactone (**10**)	H	OH	OH	OH	OH	H
7-Hydroxy-enterolactone (**11**)	OH	H	H	OH	OH	H
7-Oxo-enterolactone (**12**)	O	H	H	OH	OH	H

Compound	R	R_1	R_2	R_3	R_4
Secoisolariciresinol (**13**)	H	OH	OH	OCH_3	OCH_3
7-Hydroxy-secoisolariciresinol (**14**)	OH	OH	OH	OCH_3	OCH_3
Enterodiol (**15**)	H	H	H	OH	OH
Dimethyl-Secoisolariciresinol (**16**)	H	OCH_3	OCH_3	OCH_3	OCH_3





Compound	R_1	R_2	R_3	R_4	R_5	R_6
Pinoresinol (**17**)	OCH_3	OH	H	OCH_3	OH	H
Phillygenin (**18**)	OCH_3	OH	H	OCH_3	OCH_3	H
Medioresinol (**19**)	OCH_3	OH	H	OCH_3	OH	OCH_3
Syringaresinol (**20**)	OCH_3	OH	OCH_3	OCH_3	OH	OCH_3
Sesamin (**21**)	$-OCH_2O-$	$-OCH_2O-$	H	$-OCH_2O-$	$-OCH_2O-$	H

Figure 1. Chemical structures of the analyzed lignans.

Compounds **1–3, 14, 15,** and **22–25** were synthetically prepared from hydroxymatairesinol according to our previously published methods.[16–19] Compound **13** was isolated from *Araucaria angustifolia* knotwood and compound **4** from *Pinus sylvestris* knotwood as previously reported.[15] Compound **27** was isolated from *Picea pungens*.[20] Compounds **29** and **30** were obtained by rhamnosylation of **1** and **13** by a *streptomyces* strain[21] and compound **26** was obtained by cyclization of **13** under acidic conditions. The methylated derivatives **9** and **16** were prepared in our laboratory by methylation of the parent lignans (**1** and **13**) by MeI/K_2CO_3 in dry acetone. Compound **10** was prepared by demethylation of **1** in BBr_3/CH_2Cl_2, and a subsequent etherification of the product with diiodomethane afforded compound **8**. Compounds **11** and **12** were prepared by total synthesis essentially as described by Mäkelä *et al.*[22]; however, the desulfurization of the intermediate was performed with $AgNO_3$ and *N*-chlorosuccinimide[23] to afford the oxo-functionalized product (**12**), which was reduced by $NaBH_4$ to the corresponding alcohol (**11**). Compound **17** was isolated from *Picea abies* according to Erdtman.[24]

Compounds **6**, **7** and **18–21** were provided by Prof. S. Nishibe (University of Hokkaido, Japan). Podophyllotoxin (**28**) was purchased from Fluka. The identity and purity of the reference compounds was determined by GC, GC-MS and NMR spectroscopy (and in this work by LC-MS).

Stock solutions (1mg/ml) of the reference samples were prepared in MeOH (HPLC grade, Rathburn). The dilutions for both LC and direct infusion analyses were done with equal amounts of MeOH/ACN (1:1, v/v) and 0.1% acetic acid. Water was purified with a Simplicity 185 purifying system (Millipore, Bedford, MA, USA). The final concentrations of analytes were 10–50 µg/ml. All solutions were stored at −20 °C and protected from light.

Sesame seeds were purchased from a local store. The seeds were milled using a Polymix analytical mill A 10 (Kinematica AG, Switzerland). Approximately 0.5 g of the milled seeds was weighed, thoroughly mixed with

DOI: 10.1002/jms

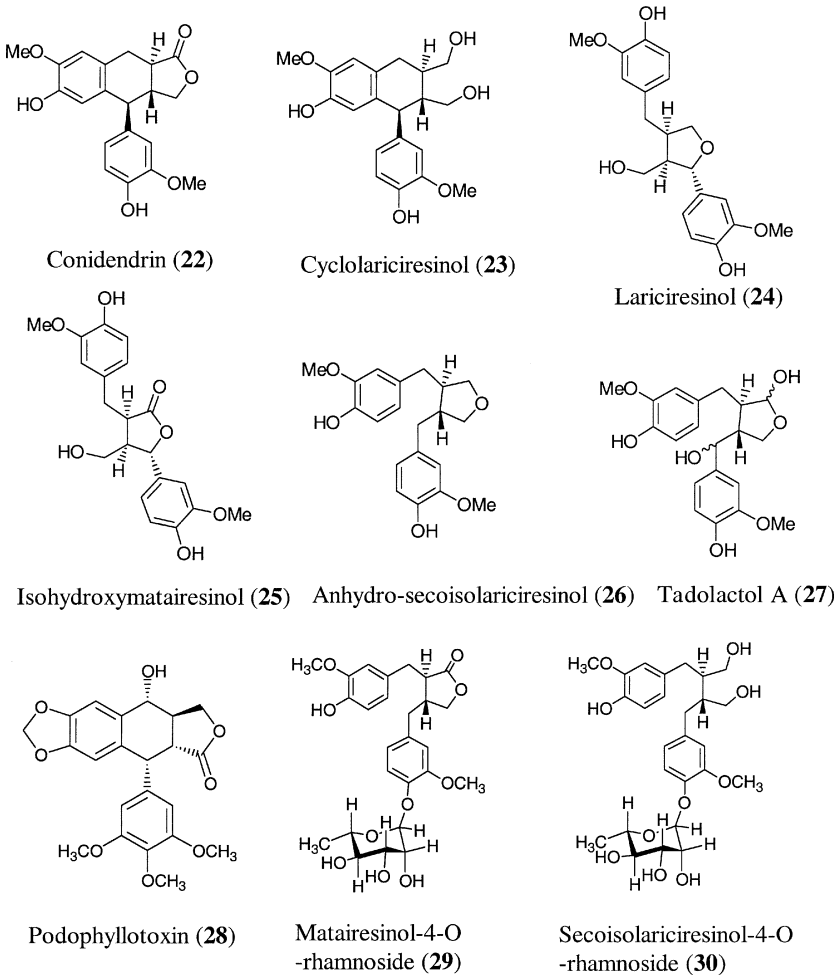


Figure 1. (*Continued*).

quartz sand (granularity 1–2 mm, Mallinckrodt Baker B.V.) and poured into extraction tubes for accelerated solvent extraction (ASE). The ASE extraction was performed using an apparatus from Dionex Corp. (Sunnyvale, CA, USA) as described previously[25] with slight modifications. The material was extracted first with hexane, then with acetone and finally with acetone–water (70 : 30, v/v). The three fractions were collected in separate glass tubes. The acetone and acetone–water fractions were combined, and the solvent was evaporated to dryness using a rotary evaporator. The extract was redissolved in 9 ml of acetone–water (70 : 30, v/v). An aliquot of 3 ml was transferred into a test tube and the solvent was evaporated using a stream of nitrogen gas. Enzymatic hydrolysis was performed by adding 1 mg of an enzyme preparation containing 492 units of β-glucuronidase and 10 units of sulfatase (β-glucuronidase/sulfatase, type H-1, from *Helix pomatia* (Sigma-Aldrich Co., St Louis, MO, USA)) dissolved in 1 ml of 0.01 M acetate buffer pH 5.0 and keeping at 37 °C for 19 h, i.e. according to a slight modification of the method optimized by Milder *et al.*[13] The enzymatically hydrolyzed solution was liquid–liquid extracted with 2 × 0.75 ml of ethyl acetate. The ethyl acetate phase was evaporated to dryness under nitrogen gas and 0.5 ml of MeOH/ACN/0.1% HAc (15 : 15 : 70 v/v/v) was added. The sample was placed in an ultrasonic bath for 5 min and then centrifuged for 10 min at 4000 rpm.

Liquid chromatography

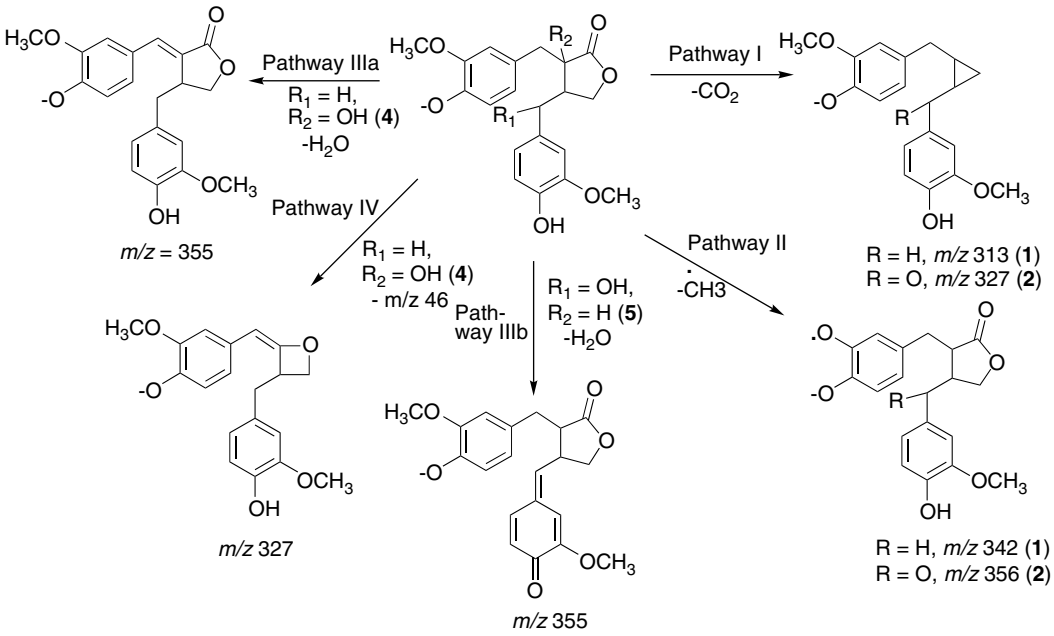
The liquid chromatography/UV absorbance/electrospray ionization ion-trap mass spectrometry (LC/UV/ESI/MS^n) analyses were performed on an Agilent 1100 Series HPLC instrument (Agilent Technologies) consisting of a binary pump, a vacuum degasser, an autosampler, a thermostatted column compartment and a variable wavelength detector (VWD-UV). The analyzed compounds were chromatographed on a 3.5 µm, 2.1 × 150 mm reversed-phase C8 analytical column (XTerra MS C8, Waters). Eluent A was 0.1% acetic acid with 1% 2-propanol (v/v) and eluent B was acetonitrile–methanol (50 : 50). The gradient was as follows: 10–30% B from 0 to 20 min, 30–50% from 20 to 45 min, 50–95% from 45 to 46 min, 95% from 46 to 50 min, 95–10% from 50 to 51 min and isocratic at 10% to equilibrate the column from 51–55 min. The sample injection volume was 20 µl and the column flow rate was 0.3 ml/min. UV detection was set at 280 nm.

Mass spectrometry

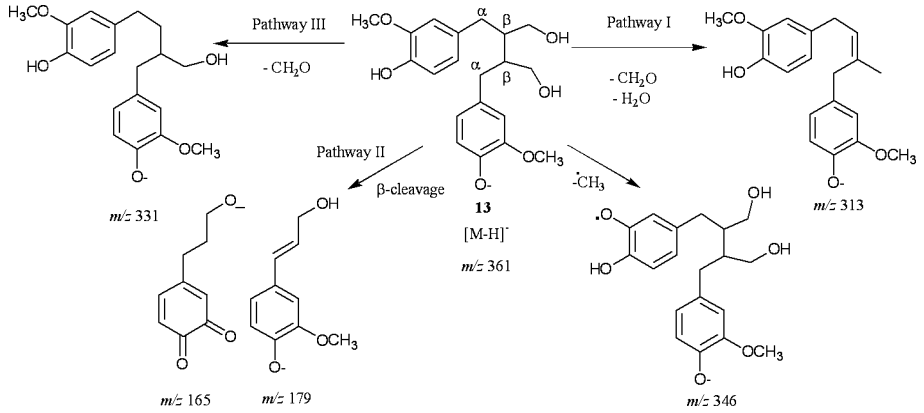
For mass spectral analyses an Agilent 1100 Series LC/MSD SL Trap ion-trap mass spectrometer was coupled to the

DOI: 10.1002/jms

JMS



Scheme 1. Some commonly observed fragmentation patterns of dibenzylbutyrolactone lignans of the guaiacyl family.



Scheme 2. Fragmentation of dibenzylbutanediol lignans, exemplified by compound **13**.

Agilent 1100 Series LC system via an ESI interface. The operation parameters of the ESI ion source were as follows: (1) drying gas (N_2) temperature, 350 °C, (2) drying gas flow, 12 l/min, (3) nebulizer gas (N_2) pressure, 40 psi, (4) end plate voltage, 3.5 kV, (5) end plate offset, −500 V, (6) capillary exit, *ca* −116 V, and (7) skim 1 set at −40 V. Ion-trap parameters were as follows: (1) accumulation time, 20 ms, (2) averages 5 and rolling averaging on and ion charge control on, target set at 20 000. CID experiments coupled with multiple mass spectrometry (MS^n) employed helium as the collision gas. For full-scan MS analysis, the spectra were recorded in the mass range m/z 100–600 and target mass was set at m/z 350. The fragmentation amplitude was 1.5 V and compound stability 100%. The data acquisition (Auto MS^n) was set to subject the two most abundant ions to multiple mass spectrometry (MS^n, $n = 3$).

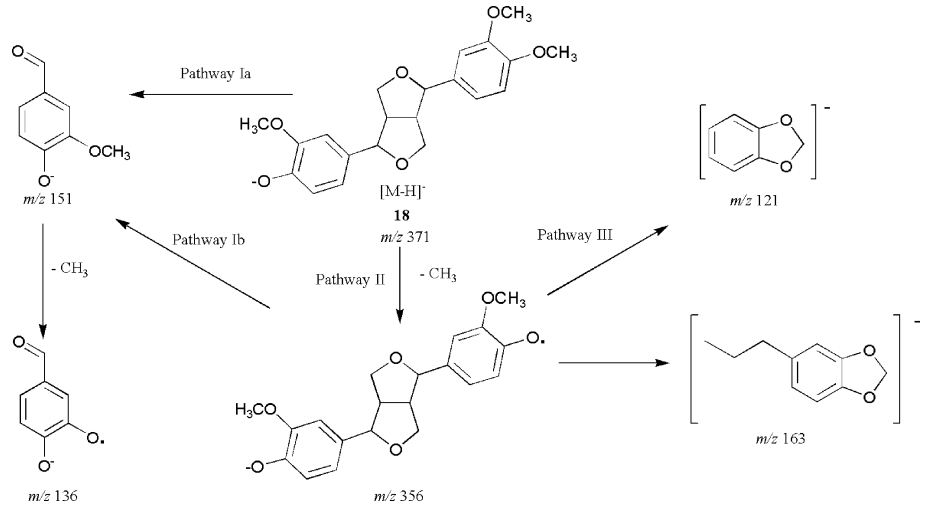
In additional studies of fragmentation patterns, the pure compounds were directly infused into the ESI source by a syringe pump at a rate of 5 µl/min and at a concentration of 200 µg/ml. The solvent was a 50/50 v/v mixture of 0.1% acetic acid and acetonitrile. The gas flows to the source were as follows: (1) drying gas temperature: 325 °C, (2) drying gas flow: 5 l/min, and (3) nebulizer gas pressure: 15 psi.

RESULTS AND DISCUSSION

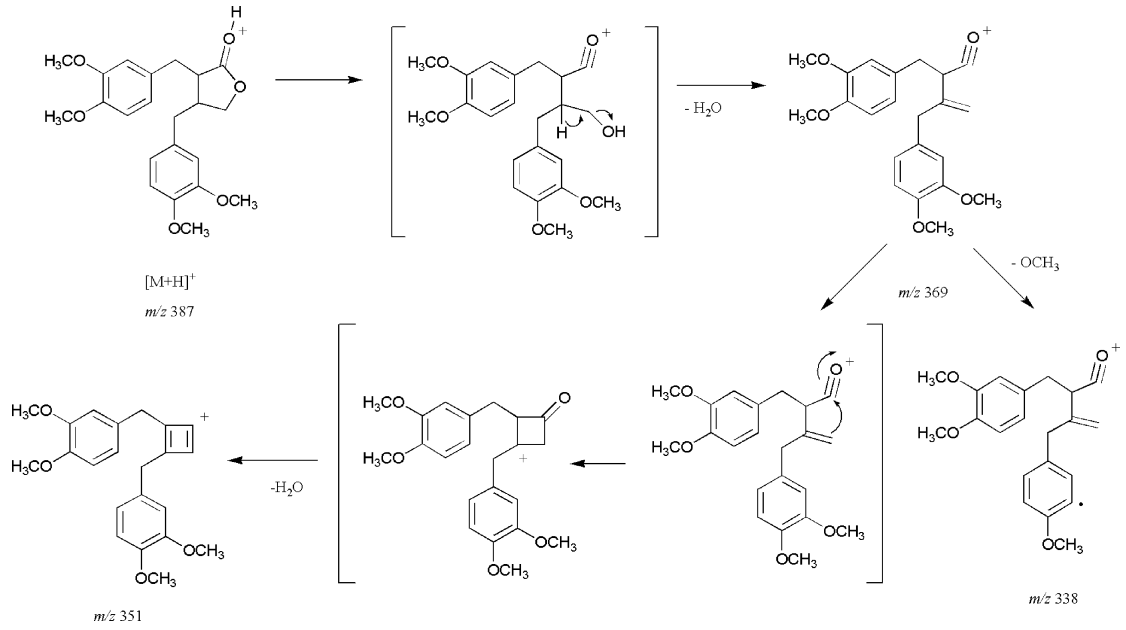
Although lignans have previously been analyzed by LC-ESI-ion-trap mass spectrometry, no systematic study of characteristic fragmentation patterns of different types of lignans has been reported. Recently, the fragmentation patterns of furofuran-type lignans and their glucosides were reported by Ye *et al*.[5] In accordance with this study, we have noticed that lignan glycosides initially lose the sugar unit. In our case, where lignan rhamnosides were used as reference compounds (**29** and **30**), the corresponding fragmentation was the loss of the deoxyhexose residue to give an ion at $[M - H - 146]^-$. Upon further fragmentation, the aglycones behaved as the parent lignans.

All lignans with a free phenolic group could be analyzed in the negative ion mode, and were detected as deprotonated molecules, although the eluent was acidic (Table 1). This 'wrong-way-round' deprotonation mechanism is probably

DOI: 10.1002/jms



Scheme 3. Fragmentation of furofurano lignans, exemplified by compound **18**.



Scheme 4. Proposed fragmentation pattern of non-phenolic butyrolactone lignans demonstrated by compound **9**.

due to the fact that the acetate anion is able to abstract the proton from the phenol in gas-phase reactions, i.e. the following ionization mechanism is plausible: $M + CH_3COO^- \rightarrow [M - H]^- + CH_3COOH$. This mechanism is supported by the results of Znamenskiy *et al.*[26] from their studies on the mechanism of ion formation in ESI. They found that evaporation of hydronium ions from the charged droplets could result in drastic increase in the pH followed by the deprotonation of the analyte. Lignans lacking phenolic groups cannot be deprotonated and need to be analyzed in positive ion mode either by protonation or by formation of adducts (Table 2). All lignans formed protonated species $[M + H]^+$ in positive ion mode, but Na^+ or K^+ adducts were also detected at almost equal intensities. Some of the non-phenolic lignans could also be analyzed as their acetate adducts in negative ion mode. Comparison of negative and positive ion mode detection, however, showed that adduct formation was detrimental to further fragmentation. The first fragmentation of adducts usually gave $[M + H]^+$ of low abundance, and further fragmentation to MS^3 was in most cases possible but with a serious decrease in sensitivity. In negative ion mode, the deprotonated molecule $[M - H]^-$ was the only ion detected. Compared with positive ion mode, in which one fragmentation step was needed to give the parent lignan, the lignan moiety could directly be further fragmented in negative ion mode by $MS^2 - MS^4$ experiments. In general, clearer and more useful information was obtained in negative ion mode detection.

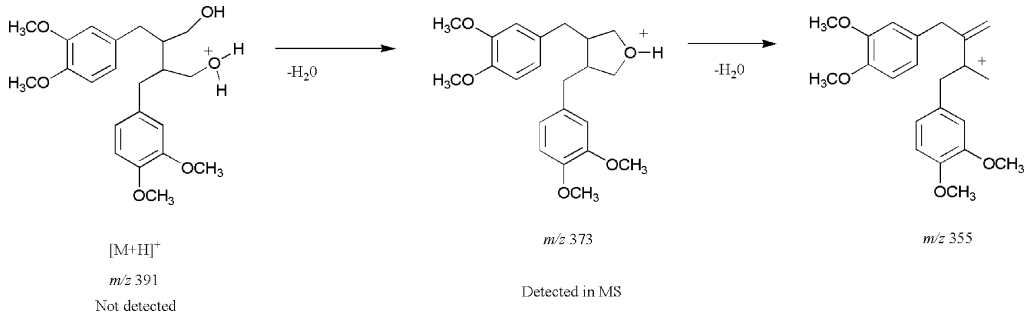
When different classes of lignans: dibenzylbutyrolactones, dibenzylbutanediols, aryltetralines, furano- and furofuranolignans (Fig. 1), were compared, some characteristic fragmentations of the different classes, as well as of the individual lignans, could be observed.

Dibenzylbutyrolactone lignans

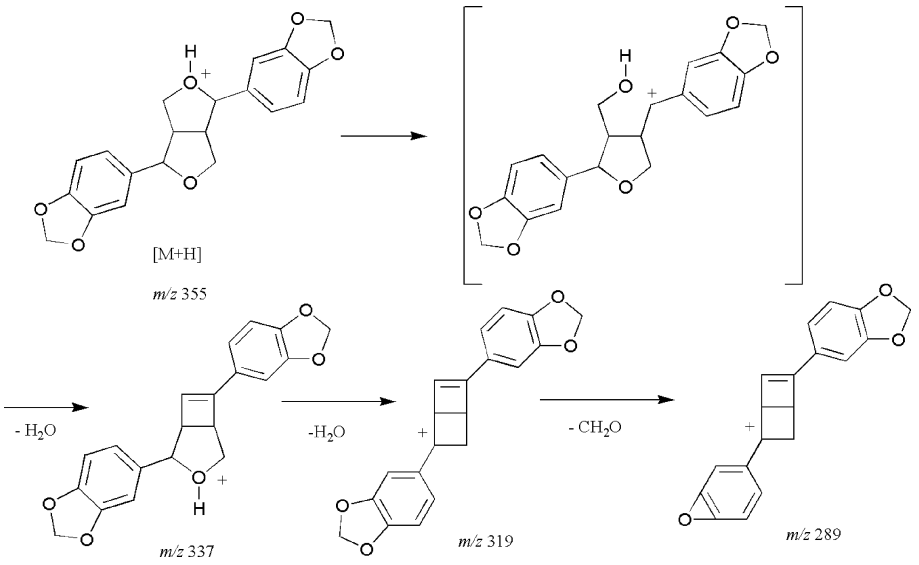
Most of the dibenzylbutyrolactones (**1–5, 7, 10**, and **12**) gave the characteristic primary product ion $[M - H - 44]^-$ presumably by the loss of CO_2 from the lactone ring

DOI: 10.1002/jms

JMS



Scheme 5. Possible positive ion mode fragmentation of non-phenolic dibenzylbutanediol lignans demonstrated by compound **16**.



Scheme 6. Positive ion mode fragmentation of compound **21**.

(Scheme 1, Pathway I). The lactone lignans **2** and **7** gave $[M-H-15]^-$ as the major product ion (Pathway II), but it was accompanied by the loss of CO_2 at almost the same intensity. Compounds **4, 5**, and **25** gave $[M-H-18]^-$ as their primary product ion (MS^2), which can be explained by the loss of water from the aliphatic alcohol groups (Pathway IIIa and IIIb). Compound **4** also lost 46 Da almost to the same extent as it lost water. This may be explained by a concerted loss of CO_2 and H_2 or alternatively CO and H_2O (Scheme 1, Pathway IV). The loss of CO (28 Da) was, however, not a generally observed fragmentation reaction for the analyzed lignans.

The aryltetralin lactone lignan **22** did not lose CO_2 as a primary fragmentation; instead the primary fragmentation was found to be the loss of a methyl radical. It therefore seemed that the more flexible structure of the dibenzylbutyrolactones (compared with aryltetralines) facilitated and favored the cleavage of CO_2. The loss of a methyl radical was the most commonly observed fragmentation behavior (MS^1–MS^4) of all lignans containing methoxyl groups. This result has also been obtained from methoxylated flavonoids.[27] It is worth mentioning that **22** and **25** also gave the lactone characteristic loss of 44 Da (CO_2) upon further fragmentations.

Butanediol lignans

The most generally observed fragmentation pattern (in negative ion mode) for the butanediol lignans (**13–15**) was the loss of 48 Da. This loss could be explained by the combined loss of formaldehyde and water from the diol structure (Scheme 2, Pathway I). Compared to the dibenzylbutyrolactone lignans, the dibenzylbutanediol lignans were also more readily cleaved at the β-position (Pathway II). On the basis of our observations, it can be concluded that most lignans containing hydroxymethyl groups (**13–15, 24**) lose formaldehyde easily in the negative ion mode (Scheme 2, Pathway III). Lariciresinol (**24**), a tetrahydrofuranolignan, which like the butanediol lignans contains a hydroxymethyl group, also gave $[M-H-30]^-$ as its primary product ion. This fragmentation was observed already in MS^1 and seemed to take place in the ionization source. The lactol lignan **27** gave, as most butanediol lignans, a primary product ion $[M-H-48]^-$. In this case the product ion is most probably formed by the loss of formaldehyde from the lactol moiety and water from the benzylic alcohol group. The loss of formaldehyde from lactol lignans has been suggested previously.[5] For the aryltetralin diol lignan **23**, the loss of 48 Da was not observed, but $[M-H-46]^-$ was detected as a minor ion in addition to the base peak $[M-H-15]^-$ in MS^2. The loss of 46 in the case of lignan diols could be attributed to a combined loss of a methoxyl (31 Da) and a methyl radical (15 Da) or by the loss of CH_2OH and a methyl radical. In accordance with the fragmentation patterns of the corresponding butyrolactone lignan **22,** the aryltetralin

DOI: 10.1002/jms

Table 1. Negative ion mode fragmentations (phenolic lignans)

Compound	Retention time (min)	$[M-H]^-$ m/z	ESI-MS^n m/z (Relative intensity %)[a]
Matairesinol (**1**)	29.6	357	MS^2 [357]: 342 (60), **313** (100), **298** (30) 209 (80)
			MS^3 [357 → 313]: **298** (100), 161 (40)
			MS^3 [357 → 342]: 313 (85), **298** (100), 178 (70), 122 (80)
			MS^3 [357 → 209]: 191 (100), 165 (40)
7-Oxomatairesinol (**2**)	25.3	371	MS^2 [371]: **356** (100), 327 (50), 205 (60)
			MS^3 [371 → 356]: **327** (100), 136 (25)
			MS^3 [371 → 327]: 312 (100), 175 (50)
			MS^4 [371 → 356 → 327]: 297 (25), 283 (100), 191 (40), 136 (40)
Enterolactone (**3**)	32.7	297	MS^2 [297]: **253** (100), 189 (17)
			MS^3 [297 → 253]: 235 (20), 195 (100), 143 (47)
Nortrachelogenin (**4**)	23.8	373	MS^2 [373]: **355** (97), **327** (100), 235 (28), 223 (40)
			MS^3 [373 → 355]: 340 (69), 311 (100)
			MS^3 [373 → 327]: 312 (100), 147 (41)
7-Hydroxymatairesinol (**5**)	18.4	373	MS^2 [373]: **355** (100), 311 (13)
			MS^3 [373 → 355]: 340 (30), **311** (100), 296 (59), 231 (37), 160 (58)
			MS^4 [373 → 355 → 311]: 296 (100), 281 (17), 160 (30)
Trachelogenin (**6**)	29.7	387	MS^2 [387]: 357 (17), **339** (48), 329 (100), 249 (15) 193 (13)
			MS^3 [387 → 329]: **314** (100), 193 (8), 135 (3)
Arctigenin (**7**)	34.7	371	MS^2 [371]: **356** (100), **312** (21), 295 (12), 209 (5)
			MS^3 [371 → 356]: 341 (15), 327 (33), 297 (29) **205** (29), 147 (37), 121 (100)
4, 4′-Dihydroxyenterolactone (**10**)	20.1	329	MS^2 [329]: 285 (60), **207** (100), 189 (20)
			MS^3 [329 → 207]: 161 (100)
7-Hydroxyenterolactone (**11**)	22.7	313	MS^2 [313]: 251 (18), **239** (100), 189 (35), 147 (22)
			MS^3 [313 → 239]: 131 (100)
7-Oxoenterolactone (**12**)	27.9	311	MS^2 [311]: **267** (100), 265 (12), 249 (10), 159 (75)
			MS^3 [311 → 267]: 249 (7), **159** (100)
Secoisolariciresinol (**13**)	21.3	361	MS^2 [361]: 346 (90), 331 (14), 313 (30), **165** (100), 179 (30)
			MS^3 [361 → 346]: 223 (30), 179 (80), 165 (100), 122 (45)
			MS^3 [361 → 165]: 147 (100), 129 (8)
7-Hydroxysecoisolariciresinol (**14**)	13.2	377	MS^2 [377]: **329**(100)
			MS^3 [377 → 329]: 299 (49), 284 (24), 193 (31), **178**(100)
Enterodiol (**15**)	27.3	301	MS^2 [301]: 271 (15), **253**(100)
			MS^3 [301 → 253]: 146(100)
Pinoresinol (**17**)	26.3	357	MS^2 [357]: 342 (7), 311 (6), **151** (100), 136 (30)
			MS^3 [357 → 151]: 136 (100)
Phillygenin (**18**)	34.6	371	MS^2 [371]: **356** (100), 326 (2)
			MS^3 [371 → 356]: 341(9), 163 (26), **151** (18), 136(13), 121 (100)
Medioresinol (**19**)	25.9	387	MS^2 [387]: 181 (100), **372** (40), 166 (34), 151 (95) 136 (37)
			MS^3 [387 → 181]: 136 (100), 123 (35)
Syringaresinol (**20**)	25.7	417	MS^2 [417]: 402 (33), **181** (100) 166 (33), 151 (21)
			MS^3 [417 → 181]: 166 (100), 151 (66)
Conidendrin (**22**)	26.7	355	MS^2 [355]: **340**(100)
			MS^3 [355 → 340]: 325(100), 296 (70), 278 (30)
Cyclolariciresinol (**23**)	17.2	359	MS^2 [359]: **344**(100), 313 (10)
			MS^3 [359 → 344]: 329 (30), 313(100), 159 (60)
Lariciresinol (**24**)	22.3	359/**329**	MS^2 [329]: 299 (35), 284 (20), 192 (30), **178** (100), 160 (30)
			MS^3 [329 → 178]: 160 (100), 147 (45), 122 (20)
Iso-hydroxymatairesinol (**25**)	19.1	373	MS^2 [373]: 355 (100), 311 (10)
			MS^3 [373 → 355]: 311(100), **281** (50), 216 (30), **159** (65)
Anhydro-secoisolariciresinol (**26**)	35.1	343	MS^2 [343]: **328** (100), 313 (2)
			MS^3 [343 → 328]: 313 (100), 297 (26), **203** (43), 163 (32), 161(24)
Todolactol A (**27**)	17.3	375	MS^2 [375]: 327(100), 191 (30)
			MS^3 [375 → 327]: **191** (100), 176 (10)

DOI: 10.1002/jms

Table 1. (*Continued*)

Compound	Retention time (min)	$[M-H]^-$ m/z	ESI-MS^n m/z (Relative intensity %)[a]
Matairesinol-rhamnoside (**29**)	26.7	503	MS^2 [503]: **357** (100) MS^3 [503 → 357]: 313 (100), 298 (30), 209 (60), 147 (35)
Secoisolariciresinol-rhamnoside (**30**)	19.5	507	MS^2 [507]: **361** (100) MS^3 [507 → 361]: 346 (90), 165 (100)

[a] Compound-specific fragment ions are in boldface.

Table 2. Positive ion mode fragmentations

Compound	Retention time (min)	$[M+H]^+$ m/z	$[M+K^+]^+$	ESI-MS^n m/z (Relative intensity %)[a]
Hinokinin (**8**)	49.3	355	393	MS^2 [355]: 337 (100), 319 (30), **261** (12), 135 (24) MS^3 [355 → 337]: 319 (100), 289 (75), 261 (18), 161 (25)
Podophyllotoxin (**28**)	33.3	415	453	MS^2 [415]: **397** (100), 313 (4), 229 (6) MS^3 [415 → 397]: 379 (58), 351 (89), 313 (94), 282 (100)
Dimethylmatairesinol (**9**)	39.3	387	425	MS^2 [387]: **369** (100), 319 (12), 249 (4), 204 (5), 151 (80) MS^3 [387 → 369]: 338 (80), 351 (50), 151 (18)
Dimethylsecoisolariciresinol (**16**)	31.5	373 $[M+H-H_20]^+$	429	MS^2 [373]: 355 (100), 340 (6), **151** (8) MS^3 [373 → 355]: 340 (100), 291 (2), 177 (70), 147 (20)
Sesamin (**21**)	49.4	337 $[M+H-H_2O]^+$	–	MS^2 [337]: 319 (100), **289** (89), 279 (18), 267 (34) 173 (41) MS^3 [337 → 319]: 289 (100), 261 (36)

[a] Compound specific fragment ions are in boldface.

structure seemed to stabilize the aliphatic part of the molecule and the loss of a methyl radical was the major fragmentation pathway observed.

Furofuranolignans

Phenolic lignans belonging to the furofurano class (**17, 19–20**) were generally cleaved between the α- and β-position in the side chain to give the product ion m/z 151 (guaiacyl) or m/z 181 (syringyl) (negative ion mode). Compound **17** gave m/z 151 as the major primary product ion (MS^2), which in turn gave the base peak m/z 136 in MS^3 (Scheme 3, Pathway Ia) in accordance with results obtained for the corresponding glucoside by Ye *et al*.[5] This fragmentation pattern can certainly be considered as the most characteristic fragmentation for this class of lignans (in negative ion mode). However, compound **18** gave $[M-H-15]^-$ as the primary product ion (Scheme 3, Pathway II), and product ions from the α,β-cleavage were in fact observed only in MS^3. Fragmentation of $[M-H-CH_3]^-$ gave m/z 121 and 163 as the most abundant ions. These product ions are most probably formed from the dimethoxyphenyl moiety as proposed in Scheme 3, Pathway III. Product ions from the guaiacyl moiety (m/z 151 and 136) were observed only in low abundances (Scheme 3, Pathway Ib), which indicated that the dimethoxyphenyl unit is more readily cleaved from the lignan skeleton and rearranged to form m/z 121 and 163. In addition to the α,β-cleavage, the loss of a methyl radical was the second most abundant fragmentation pathway observed for the furofurano lignans **17–20**.

Non-phenolic lignans

In positive ion mode, the most commonly observed fragmentation of the non-phenolic lignans was the loss of water to give the ion $[M+H-18]^+$ (Table 2). The butyrolactone lignans **8, 9**, and **28** could lose water from the protonated lactone to yield an acylium ion (Scheme 4) as described in detail for butyrolactones previously.[28] Further fragmentation of $[M+H-H_2O]^+$ resulted in a second loss of water. For compounds **8** and **9** this fragmentation must involve the acylium intermediate, whereas compound **28** could eliminate water also from the aliphatic alcohol group. A possible mechanism for the elimination of water from the acylium ion is presented in Scheme 4. The diol lignan **16** also readily loses two molecules of water; in fact, the first water molecule is lost already in the ionization source and $[M+H-H_2O]^+$ was detected as the base peak in MS^1. A proposed fragmentation pattern for compound **16** is shown in Scheme 5. The corresponding phenolic compound **13** showed a similar fragmentation pattern in positive ion mode.

Also, the non-phenolic furofurano lignan **21** lost water from the substituted tetrahydrofuran ring in positive ion mode detection (Scheme 6).

DOI: 10.1002/jms

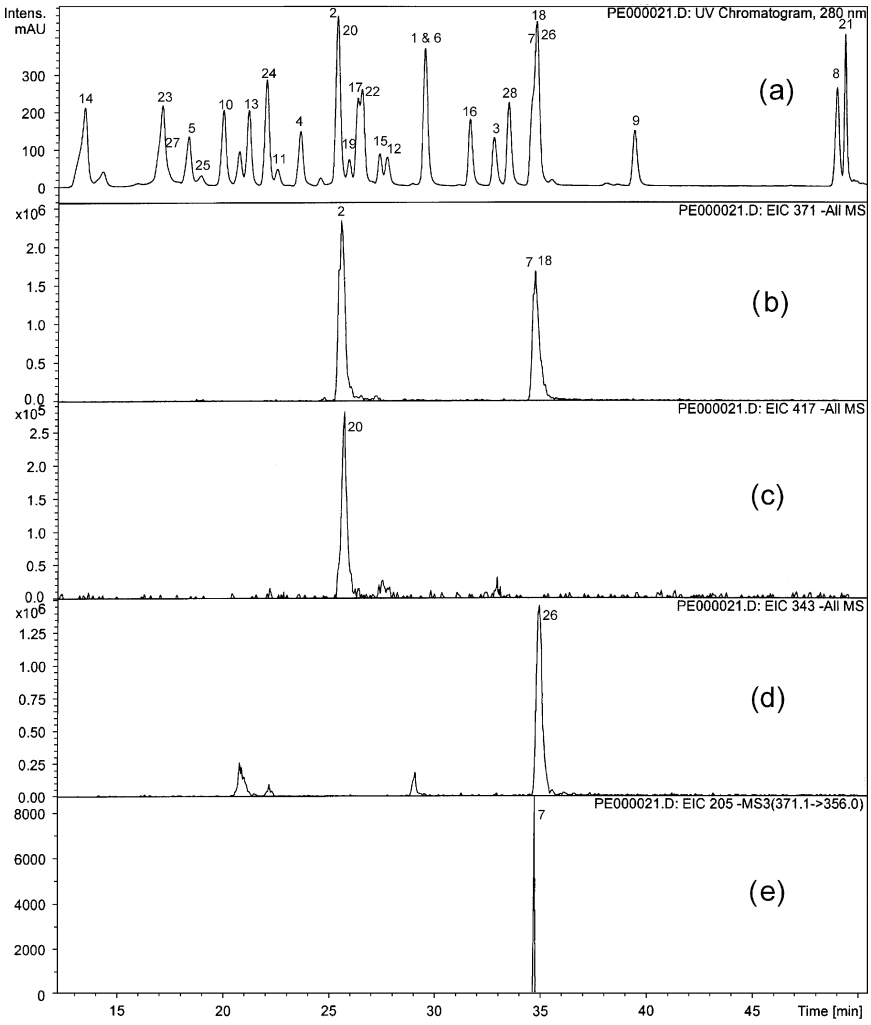


Figure 2. HPLC chromatograms of 28 lignan reference compounds. From top to bottom: (a) UV chromatogram. Extracted ion chromatograms: selected ions (b) 371 (MS) (c) 417 (MS) and (d) 343 (MS) (e) 205 of 371 → 356 (MS^3) showing the identification of the overlapping compounds **7**, **18** and **26**.

The protonated molecule $[M + H]^+$ was detected only in low abundance for compound **21**, and as for the diol lignans, $[M + H - H_2O]^+$ was the major ion detected in MS^1. Further fragmentation led to a second loss of water (Scheme 6). The loss of water already in the ionization source prior to the detection in the ion trap seems to be specific (under these conditions) for non-phenolic furofurano- and butanediol lignans in positive ion mode. Moreover, compound **21** showed a poor response in positive ion mode, which must be due to a low proton affinity. Therefore there seems to be variation in the proton affinity of structurally related lignans, which leads to variation in mass spectrometric response. When non-phenolic lignans are analyzed under these conditions, attention should be paid to this problem.

In addition to the generally observed fragmentation patterns discussed above, compound-specific fragmentations were observed both in the negative and in the positive ion mode detection. These specific fragmentations in combination with the observation of molecular ion species can be used for the identification of individual compounds. Moreover, generally observed fragmentations and specific fragmentation may provide a means for structural elucidation of hitherto unknown lignans. Retention times, the molecular ion species and the fragmentation patterns of the compounds studied in this work are presented in Table 1. Some specific product ions suitable for identification purposes are displayed in boldface.

LC/ESI/MS analyses of the reference lignans and hydrophilic lignans in a sesame seed extract

An LC/ESI/MS method was established with the aim of analyses of lignans in plant extracts. Initially, the method was optimized by the use of a mixture of 28 reference compounds. Auto-MS detection was performed in such a way that the two most abundant ions were subjected to MS^2–MS^3 experiments.

The chromatographic separation was performed on a C8 reversed-phase column and the column was eluted with a gradient consisting of (1) methanol : acetonitrile (50 : 50) and (2) 0.1% acetic acid (see 'Experimental'). The UV chromatogram in Fig. 2(a) was obtained from the analysis of the mixture of a reference compounds, showing that some of the compounds coeluted. Each compound could easily be identified in the chromatogram by the knowledge of their specific ion-trap fragmentation behavior and according to their retention times. For example, by extracting from the MS^1

DOI: 10.1002/jms

JMS

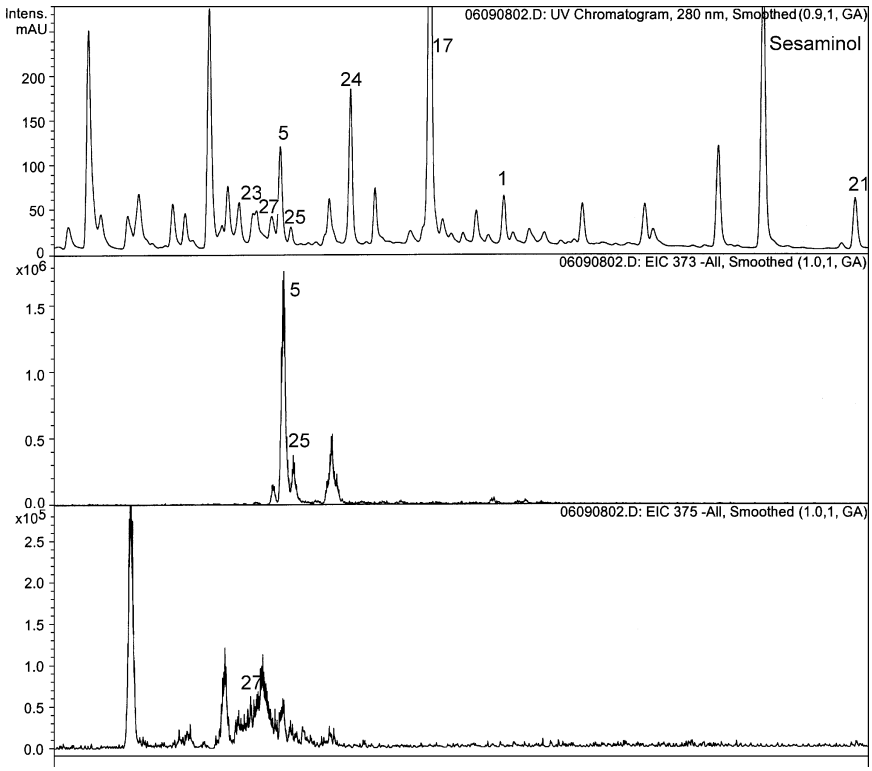


Figure 3. HPLC chromatogram of a sesame seed extract. Extracted ion chromatograms of $[M - H]^-$ at *m/z* 373 and 375.

data the ion *m/z* 371, which corresponds to the deprotonated molecule of lignans **2, 7**, and **18**, it could be shown that at least one lignan detected at this *m/z* eluted with the peaks at approximately 25 and 35 min (Fig. 2(b)). By comparing the retention times and the extracted ion chromatogram with that obtained of the ions *m/z* 417 and 343 (deprotonated molecules of **20** and **26**, respectively), it was evident that the peak at 25 min was due to lignans **2** and **20** and that the lignan **26** was one of the components in the peak at 35 min (Fig. 2(c) and (d)). To be able to separately observe lignan **7** and **18**, the specific product ions generated in MS^3 had to be used since both compounds generate almost exclusively *m/z* 356 in MS^2 (loss of 15 Da from *m/z* 371). In MS^3, lignan **7** produces an abundant product ion at *m/z* 205 (Fig. 2(e)), while lignan **18** forms a product ion at *m/z* 151 (not shown).

The usefulness of this method was further demonstrated by the analysis of an acetone/water extract (i.e. hydrophilic lignans) of sesame seeds, which are known to be rich in dietary lignans.[3,29,30] In Fig. 3 the HPLC chromatogram of the acetone/water extract is shown. The identification of specific lignans in the extract was based on the retention times, the observation of the deprotonated molecules and fragmentation patterns in the ion trap. Several known lignan constituents of sesame seeds[24] (**1, 5, 17, 21, 23, 24**) were identified along with the lignans **25** and **27**, which have been recently reported by Smeds *et al*.[3] Sesaminol, a major lignan in sesame seeds, was tentatively identified by its fragmentation pattern ($[M - H]^-$ *m/z* 369 → 219 $[M - 150]^-$). In addition, isomeric structures of **27** and **25** were detected as shown by the extracted ion chromatograms of *m/z* 373 and 375 (Fig. 3). The chromatogram also contained several compounds with the $[M - H]^-$ at *m/z* 357.

CONCLUSIONS

On the basis of the fragmentation patterns of reference compounds, a method for the identification of different types of lignans was developed. The CID fragmentation patterns showed some general trends for different types of lignans. In negative ion mode all butyrolactone lignans lost CO_2 from the lactone moiety, and the diols or lignans containing aliphatic hydroxyl groups generally lost water. Lignans containing hydroxymethyl groups lost formaldehyde easily. The most generally observed fragmentation for all lignans containing guaiacyl (3-hydroxy-4-methoxyphenyl) moieties was the loss of a methyl radical. In positive ion mode, most lignans lost water. Owing to extensive adduct formation and loss of sensitivity upon further fragmentation, positive ion mode detection was not as informative as negative ion detection.

Although several general fragmentation pathways for different types of lignans were observed, there was large variation in the fragmentation behavior between compounds of the same class. Small changes in the structure, i.e. methylation of a phenolic group or even a different diastereomer, could lead to totally different fragmentation behavior. Therefore, identification of a compound should not be based on the observed fragmentation pattern alone. In combination with retention times, molecular ion species (protonated or deprotonated) and compound-specific product ions produced in the ion trap, the reference compounds used here, can be identified unambiguously.

One should also bear in mind that the fragmentation patterns and results reported here are specific for these conditions. Changes in ionization, fragmentation or detection conditions will certainly affect the results obtained.

DOI: 10.1002/jms

Acknowledgements

Prof. Sansei Nishibe at the Department of Pharmacognosy, University of Hokkaido, Japan, and Dr. Stefan Willför at the Laboratory of Wood and Paper Chemistry, Åbo Akademi University, are gratefully acknowledged for supplying reference compounds. The authors also thank Ms Tove Slätis, Laboratory of Organic Chemistry, Åbo Akademi University, for running part of the LC-MS experiments. This work was part of the activities at the Process Chemistry Centre at Åbo Akademi University within the Centre of Excellence Programme by the Academy of Finland.

REFERENCES

1. Smeds A, Hakala K. Liquid chromatographic-tandem mass spectrometric method for the plant lignan 7-hydroxymatairesinol and its potential metabolites in human plasma. *Journal of Chromatography B* 2003; **793**: 297.
2. Zhao Y, Chen B, Yao S. Simultaneous determination of abietine-type diterpenes, flavonolignans and phenolic compounds in compound preparations of *Silybum marianum* and *Salvia miltiorrhiza* by HPLC-DAD-ESI MS. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **38**: 564.
3. Smeds AI, Eklund PC, Sjöholm RE, Willför SM, Nishibe S, Deyama T, Holmbom BR. Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. *Journal of Agricultural and Food Chemistry* 2007; **55**: 1337.
4. Wang D, Liu Z, Gou M, Liu S. Structural elucidation and identification of alkaloids in Rhizoma Coptidis by electrospray ionization tandem mass spectrometry. *Journal of Mass Spectrometry* 2004; **39**: 1356.
5. Ye M, Yan Y, Guo D-A. Characterization of phenolic compounds in the chinese herbal drug Tu-Si-Zi by liquid chromatography coupled to electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* 2005; **19**: 1469.
6. Jungbluth G, Ternes W. HPLC separation of flavonols, flavones and oxidized flavonols with UV-DAD-, electrochemical and ESI-Ion trap MS detection. *Fresenius Journal of Analytical Chemistry* 2000; **367**: 661.
7. Syrjänen K, Sipilä J, Björk H, Brunow G. The identification of dilignols from dehydrogenation mixtures of coniferyl alcohol and apocynol [4-(1-Hydroxyethyl)-2-methoxyphenol] by LC-MS/MS. *Journal of Agricultural and Food Chemistry* 2000; **48**: 5211.
8. He X-G, Lian L-Z, Lin L-Z. Analysis of lignan constituents from Schisandra chinensis by liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A* 1997; **756**: 81.
9. Ayres DC, Loike JD. *Lignans: Chemical, Biological and Clinical Properties*. Cambridge University Press: Cambridge, USA; 1990.
10. Saarinen NM, Wärri A, Mäkelä SI, Eckerman C, Reunanen M, Ahotupa M, Salmi SM, Franke AA, Kangas L, Santti R. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutrition and Cancer* 2000; **36**: 207.
11. Kangas L, Saarinen N, Mutanen M, Ahotupa M, Hirsinummi R, Unkila M, Perälä M, Soininen P, Laatikainen R, Korte H, Santti R. Antioxidant and antitumor effect of hydroxymatairesinol (HM-3000, HMR), a lignan isolated from the knots of spruce. *European Journal of Cancer Prevention* 2002; **11**:Suppl. 2 48.
12. Lee K-H, Xiao Z. Lignans in treatment of cancer and other diseases. *Phytochemistry Reviews* 2003; **2**: 341.
13. Milder IEJ, Arts ICW, Venema DP, Lasaroms JJP, Wähälä K, Hollman PCH. Optimization of a liquid chromatography-tandem mass spectrometry method for quantification of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in food. *Journal of Agricultural and Food Chemistry* 2004; **52**: 4643.
14. Peñalvo JL, Haajanen KM, Botting N, Adlercreutz H. Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry* 2005; **53**: 9342.
15. Willför S, Ahotupa M, Hemming J, Reunanen M, Eklund P, Sjöholm R, Eckerman C, Pohjamo S, Holmbom B. Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species. *Journal of Agricultural and Food Chemistry* 2003; **51**: 7600.
16. Eklund P, Sillanpää R, Sjöholm R. Synthetic transformation of hydroxymatairesinol from Norway pruce (*Picea abies*) to 7-hydroxysecoisolariciresinol, (+)-lariciresinol and (+)-cyclolariciresinol. *Journal of the Chemical Society, Perkin Transactions 1* 2002; **19**: 1906.
17. Eklund P, Lindholm A, Mikkola J-P, Smeds A, Lehtilä R, Sjöholm R. Synthesis of (−)-matairesinol, (−)-enterolactone, and (−)-enterodiol from the natural lignan hydroxymatairesinol. *Organic Letters* 2003; **5**: 491.
18. Eklund P, Sjöholm R. Oxidative transformation of the natural lignan hydroxymatairesinol with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. *Tetrahedron* 2003; **59**: 4515.
19. Eklund P, Willför S, Smeds A, Sundell F, Sjöholm R. A new lariciresinol-type butyrolactone lignan derived from hydroxymatairesinol and its identification in spruce wood. *Journal of Natural Products* 2004; **67**: 927.
20. Willför S, Eklund P, Sjöholm R, Reunanen M, Sillanpää R, von Shoultz S, Hemming J, Nisula L, Holmbom B. Bioactive phenolic substances in industrially important tree species. Part 4: identification of two new 7-hydroxy divanillyl bytyrolactol lignans in some spruce, fir and pine species. *Holzforschung* 2005; **59**: 413.
21. Eklund P, Holmström T, Al-Ubaydy L, Sjöholm R, Hakala J. Rhamnosylation of lignans by a *Streptomyces* strain. *Tetrahedron Letters* 2006; **47**: 1645.
22. Mäkelä TH, Wähälä KT, Hase TA. α,β-Dibenzyl-γ-butyrolactone lignan alcohols: total synthesis of (±)-7′-hydroxyenterolactone, (±)-7′-hydroxymatairesinol and (±)-8-hydroxyenterolactone. *Steroids* 2000; **65**: 437.
23. Asano Y, Kamikawa T, Tororoyama T. A simple method for the synthesis of lignan skeletons. Syntheses of (±) -parabenzlactone and (±) -hinokinin. *Bulletin of the Chemical Society of Japan* 1976; **49**: 3232.
24. Erdtman H. Constitution of resin phenols and their biogenetic connections. I. Pinoresinol. Preliminary communication. *Svensk Kemisk Tidskrift* 1934; **46**: 229.
25. Willför S, Hemming J, Reunanen M, Eckerman C, Holmbom B. Lignans and lipophilic extractives in Norway spruce knots and stemwood. *Holzforschung* 2003; **57**: 27.
26. Znamenskiy V, Mariginean I, Vertes A. Solvated ion evaporation from charged water nanodroplets. *Journal of Physical Chemistry A* 2003; **107**: 7406.
27. Cuyckens F, Claeys M. Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry* 2004; **39**: 1.
28. Crotti AEM, Fonseca T, Hong H, Staunton J, Galembeck SE, Lopes NP, Gates PJ. The fragmentation mechanism of five-membered lactones by electrospray ionization tandem mass spectrometry. *International Journal of Mass Spectrometry* 2004; **232**: 271.
29. Peñalvo JL, Heinonen SM, Aura A-M, Adlercreutz H. Dietary sesamin is converted to enterolactone in humans. *The Journal of Nutrition* 2005; **135**: 1056.
30. Moazzami A, Kamal-Eldin A. Sesame seed is a rich source of dietary lignans. *Journal of American Oil Chemist Society* 2006; **83**: 719.