JOURNAL OF NATURAL PRODUCTS

Volume 67, Number 6 June 2004

Full Papers

A New Lariciresinol-Type Butyrolactone Lignan Derived from Hydroxymatairesinol and Its Identification in Spruce Wood

Patrik C. Eklund,*,† Stefan M. Willför,‡ Annika I. Smeds,† Fredrik J. Sundell,† Rainer E. Sjöholm,† and Bjarne R. Holmbom‡

Department of Organic Chemistry, Process Chemistry Centre, Åbo Akademi University, Biskopsgatan 8, FIN-20500, Turku/Åbo, Finland, and Laboratory of Wood and Paper Chemistry, Process Chemistry Centre, Åbo Akademi University, Porthansgatan 3, FIN-20500, Turku/Åbo, Finland

Received December 15, 2003

When the natural lignan hydroxymatairesinol (**1**) was treated with an alkaline aqueous solution, it partially rearranged to isomeric forms of a lariciresinol-type butyrolactone lignan. The two major diastereomers formed (**2** and **3**) were isolated by column and medium-pressure chromatography, and their structures were elucidated by MS and NMR techniques. These previously unknown butyrolactone lignans were identified as naturally occurring in spruce knotwood by GC, GC−MS, and HPLC-ESI MS/MS analyses. The formation of isohydroxymatairesinol (**2**) and epi-isohydroxymatairesinol (**3**) from hydroxymatairesinol (**1**), and their detection in rat urine after administration of **1**, is discussed.

Lignans have attracted much interest due to their widespread occurrence in various plant species and their broad range of biological activity.[1] The chemistry and the occurrence of lignans in tree species and analyses of lignans in human plasma and rat urine have been studied for many years in our laboratories.[2−4] The knots, i.e., the branch bases of softwood tree species, are the richest source of lignans in nature.[5] Knots of Norway spruce (*Picea abies*) contain exceptionally large amounts, i.e., up to 17%, of the butyrolactone lignan hydroxymatairesinol (**1**).[2] Hydroxymatairesinol can be isolated as a mixture of two diastereomers, (7*R*,8*R*,8′*R*)-(−)-7-*allo*-hydroxymatairesinol (**1a**) (minor isomer) and (7*S*,8*R*,8′*R*)-(−)-7-hydroxymatairesinol (**1b**) (major isomer), in large quantities from wood chips and must for the moment be regarded as the most abundant lignan in nature.[6,7] We have also shown that **1** can be converted to several new and previously known lignans and norlignans by synthetic transformations.[8−11]

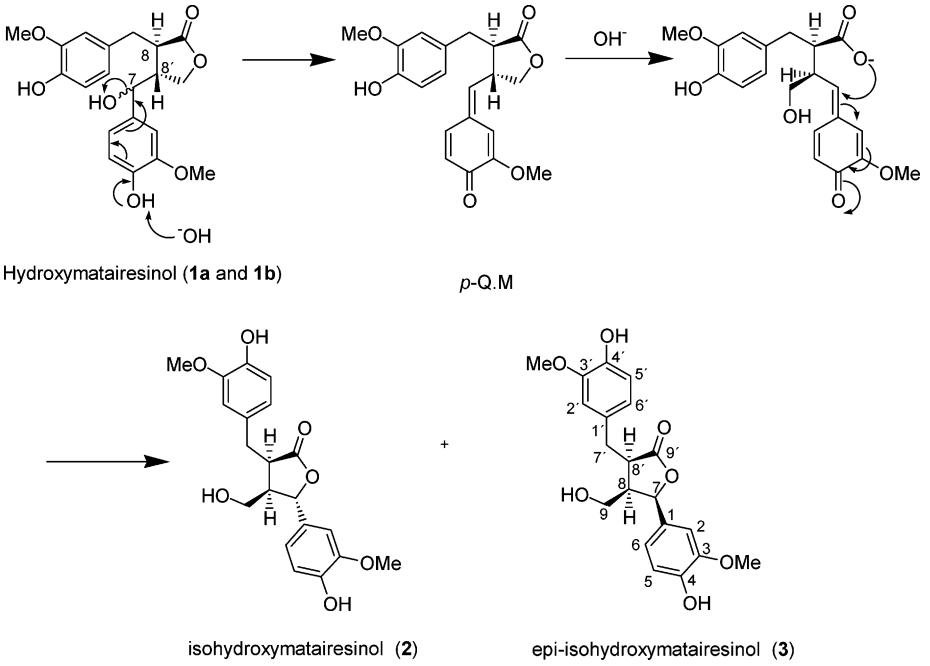
The biological activity of **1** has been widely studied. It has been shown that **1** is metabolized to (−)-enterolactone by intestinal bacteria in rats and in humans and that it has chemopreventive effects on the development of DMBA-induced mammary carcinoma in rats.[12,13] Hydroxymatairesinol has also been shown to have a chemopreventive effect in the Apc^{Min} mice model of human familial adenomatous polyopsis and to be a strong antioxidant able to reduce the oxidation of LDL particles in vitro.[14,15]

In the early 1980s there was much discussion about the detection and the role of the mammalian lignans enterolactone (ENL) and enterodiol (END) in human and vervet monkey urine.[16,17] It was soon shown that these metabolites originated from plant lignan precursors, which were metabolized by intestinal microflora in the lower gut.[18,19] The biological activity and the formation of these lignans in vivo have been thoroughly studied over the years.[20] We have analyzed the excretion of lignans in rat urine after administration of different plant lignans. Many lignans are metabolized not only to the mammalian lignans but also to other plant lignans during the oxidative metabolism. Most plant lignans are also excreted unmetabolized in the urine.

* Corresponding author. Tel: +358 2 2154502. Fax: +358 2 2154866. E-mail: paeklund@abo.fi.
† Department of Organic Chemistry.
‡ Laboratory of Wood and Paper Chemistry.

10.1021/np0340706 CCC: $27.50
Published on Web 05/13/2004

Scheme 1. Formation of Isohydroxymatairesinol (**2**) and Epi-isohydroxymatairesinol (**3**) from Hydroxymatairesinol (**1a** and **1b**) via a *para*-Quinone Methide Intermediate in Aqueous Alkaline Conditions



Recently, we discovered that hydroxymatairesinol could be converted to isomers of a new type of lignan that does not belong to any of the previously known groups of naturally occurring lignans. Isolation and structural determination led to the identification of two lariciresinol-type butyrolactone lignan diastereomers (**2** and **3**, Scheme 1). We here report the formation of **2** and **3** from hydroxymatairesinol, as well as the occurrence of these new lignans in spruce wood. We also report the excretion of **2** and **3** and briefly discuss the excretion of hydroxymatairesinol and mammalian lignans in rat urine.

Results and Discussion

When hydroxymatairesinol (**1**) was dissolved in alkaline aqueous/THF solution at pH 8−9, epimerization at C-7 and the formation of conidendric acid were the main transformations detected. However, **1** was also partially (4−7%) converted into two previously unknown lignans (compounds **2** (5%) and **3** (1%)) and to detectable amounts of their stereoisomeric forms, tentatively identified as the 8′-epimers by the similar mass spectra (~0.5%). Variation of the reaction parameters (concentration, reaction time, temperature, and pH) did not significantly alter the amounts of compounds **2** and **3**. Analysis of compounds **2** and **3** by LC-MS/MS ESI showed a molecular ion peak equal to that of **1** (*m*/*z* 373, $M - H^-$), but differences were observed both in retention times and in the fragmentation patterns. Similar results were obtained by GC−MS analyses of the trimethylsilyl ethers (*m*/*z* 590, M^+). Instead of the fragment ion *m*/*z* 297 $[Ar - CHOTMS]^+$ resulting from the benzylic cleavage of **1**, *m*/*z* 324 $[Ar - C_3H_4OTMS]^+$ was the most abundant ion peak in the mass spectra (of the trimethylsilyl ether derivatives) of **2** and **3**. Compounds **1**, **2**, and **3** all showed the fragment *m*/*z* 209 $[ArCH_2]^+$; however, the lack of *m*/*z* 297 in **2** and **3** indicated that no free benzylic alcohol group was present in these compounds. Also, *m*/*z* 324 is a typical fragment for lariciresinol-type lignans.[27] High-resolution mass spectrometry gave the mass 374.136 and the elemental composition of $C_{20}H_{22}O_7$ for **1**, **2**, and **3**, which indicated that these compounds were isomeric.

Compounds **2** and **3** were separated from the reaction mixture by normal-phase silica column chromatography followed by medium-pressure liquid chromatography (MPLC). Both compounds showed ^{13}C and ^1H NMR signals characteristic of the guaiacyl (4-hydroxy-3-methoxyphenyl) substitution pattern in the aromatic region. In the aliphatic region, they showed signals closely related to those of **1**. However, chemical shifts and coupling constants of **2** and **3** were different from those of **1**. Compared to **1**, the doublets of H-7 were shifted approximately 0.5 ppm downfield and showed coupling constants of 2.6 Hz ($J_{7,8}$) for **2** and 9.3 Hz ($J_{7,8}$) for **3**. Correspondingly, the ^1H signals of H-9a and H-9b were shifted upfield, which indicated a rearrangement of the butyrolactone ring. Correlation spectroscopy (COSY, long-range COSY, NOESY, HETCOR, and COLOC) experiments confirmed that the two compounds were diastereomers of 7,8′,8-substituted butyrolactone lignans (3,4,5-substituted-dihydro furanones) as depicted in Scheme 1 (compounds **2** and **3**). The configurations at positions 8 and 8′ were predetermined by the known configuration 8*R* and 8′*R* for compound **1**.[8] The coupling constants between H-8 and H-8′ were in accordance with the 8*R*,8′*R cis* configurations ($J_{8,8'} = 8.1$ Hz for compound **2** and 10.2 Hz for compound **3**), which in both cases showed a dihedral angle close to 0° by molecular modeling.[21] Both compounds showed NOESY correlation between H-8 and H-8′. The stereochemistry at C-7 was deduced from the coupling constant and NOESY experiments. Compound **3** showed NOESY correlation between H-7 and H-8 and a coupling constant of 9.3 Hz, indicating a *cis* configuration. Compound **2** showed no NOESY correlation between H-7 and H-8 and a coupling constant of 2.6 Hz, indicating a *trans* configuration, which by molecular modeling showed a dihedral angle close to 90°. The structure of (3*R*,4*R*,5*S*)-(+)-3-(4′-hydroxy-3′-methoxyphenylmethyl)-4-(hydroxymethyl)-5-(4-hydroxy-3-methoxyphenyl)dihydro-2-furanone was therefore assigned to compound **2**. Correspondingly, the structure of compound **3** was (3*R*,4*R*,5*R*)-(+)-3-(4′-hydroxy-3′-methoxyphenylmethyl)-4-(hydroxymethyl)-5-(4-hydroxy-3-methoxyphenyl)dihydro-2-furanone. According to the nomenclature for lignans and neolignans,[22] these com-

pounds may be semisystematically named as (8*βR*,8′*βR*,-7α*S*)-(+)-4,4′-dihydroxy-3,3′-dimethoxy-8-hydroxymethyl-lign-7−9′-lactone (**2**) and (8*βR*,8′*βR*,7*βR*)-(+)-4,4′-dihydroxy-3,3′-dimethoxy-8-hydroxymethyl-lign-7−9′-lactone (**3**). As a trivial name we suggest isohydroxymatairesinol (**2**) and epi-isohydroxymatairesinol (**3**), on the basis that these compounds are epimers and constitutional isomers of hydroxymatairesinol.

To the best of our knowledge, this type of butyrolactone lignans has not previously been reported among naturally occurring lignans. However, similar structures have been used as intermediates in lignan synthesis,[23,24] and a similar base-catalyzed conversion of acetylparabenzlactone has been reported.[25]

In a more detailed study of the transformation of **1** in alkaline aqueous solutions, we speculated that **1** formed a *para*-quinone methide intermediate (*p*-QM), which was easily attacked by nucleophiles (unpublished results). It is therefore probable that, under alkaline conditions, the lactone ring is opened and a nucleophilic attack by the carboxylic acid anion to the position 7 of the *para*-quinone methide occurs, leading to the rearranged products (Scheme 1). The 7*S*-diastereomer (**2**) is predominantly formed, which is in accordance with the reasonable proposition that the 7−8-*trans* location of the bulky substituents should be favorable on stereochemical grounds. Moritani et al. used similar structures as intermediates in the synthesis of α-hydroxy-α,*β*-dibenzylbutyrolactone lignans.[23] The formation of the 7−9′-butyrolactone ring was accomplished by a rearrangement of the 7-hydroxydibenzylbutyrolactone precursors, using a translactonization reaction in NaH/DMF. The rearrangement proceeded with complete epimerization at the α-carbon to yield the thermodynamically most stable all-*trans* compound. In our experiment, only detectable amounts of what were apparently the α-isomers were formed (8′α-epi-isohydroxymatairesinol and 8′α-isohydroxymatairesinol). In the slightly alkaline aqueous solution the enolization (α-deprotonization) of the lactone is not expected. Niwa et al. reported the transformation of acetylparabenzlactone to the lariciresinol-type lignan during deacetylation in 2% methanolic KOH. They obtained only one diastereomer, and no α-epimerization during the rearrangement was reported.[25] Under the same conditions, hydroxymatairesinol yielded the 7-methoxy-substituted (7-methoxymatairesinol) product by the nucleophilic attack of methanol to the quinone methide intermediate.

The free *para*-hydroxyl group of **1** significantly enhances the formation of the *para*-quinone methide at alkaline conditions. In fact the reaction mixture turned yellow on addition of base, and such a color change has been ascribed to quinone methide intermediates and stable quinone methides.[27] We therefore suggest that the formation of **2** and **3** proceeds as outlined in Scheme 1, although the direct attack of the deprotonated benzylic alcohol on the carbonyl carbon cannot be excluded.

Guaiacyl-type lignans are the most abundant group of lignans in nature, and lignans found in spruce wood almost entirely belong to this particular group. In a comprehensive study by Ekman, 19 lignans were detected in spruce wood.[26] However, several minor components were not identified, and knots were not analyzed. In our study of lignans in Norway spruce, we have shown that the knots contain as much as 6−24% of lignans, with hydroxymatairesinol (**1**) as the predominant lignan (70−80% of the total lignan content).[2] In a typical sample of the hydrophilic extractives of Norway spruce knots, both isomers of **1** are by far the most dominating components. Secoisolariciresinol, liovil, α-conidendrin, and the previously unknown lignan isohydroxymatairesinol (**2**) are other lignans occurring in notable amounts (as shown in Figure 1 and Table 1, Supporting Information). Isohydroxymatairesinol (**2**) was also detected (1−2%) in the hydroxymatairesinol fraction after flash-chromatographic isolation and purification of hydroxymatairesinol from knotwood. Normal-phase silica column chromatography and flash chromatography were unable to separate the totally coeluting compounds **1b** and **2**.

Table 1. Amount of Lignans Excreted in the Urine of Female Rats ($n = 5$) within 48 h after Administration of Hydroxymatairesinol in a Dose of 50 mg/kg of Body Weight Expressed as % Excretion (average excreted molar amount/administered molar amount of **1a**, **1b**, or **2**) or % Conversion (average excreted molar amount/total administered molar amount of **1**) as nmol (mean ± SD)

compound	% excretion	% conversion	nmol
1a	14.3		1395 ± 417
1b	6.0		1100 ± 359
2	5.9		39 ± 13
3		0.01	3.1 ± 1
END		1.3	372 ± 186
ENL		16.2	4650 ± 1549
HENL		11.5	3317 ± 1592

Preliminary analyses of knotwood of other spruce species indicated that **2** is a minor component in most of the species containing **1** as a major component. Also an isomeric compound tentatively identified as 8′α-isohydroxymatairesinol can be detected in small amounts. Compound **2** was indicated to occur also in the stemwood of several spruce species. The fact that the occurrence of **1** correlates with the occurrence of **2** indicates that **2** is formed from **1** by a rearrangement in vivo. Thus, it is also possible that **2** is formed in minor amounts during an earlier step in the biosynthesis of **1**.

In the animal experiments, 5 female rats were orally administered a dose of 50 mg/kg of bodyweight of **1**, and the urine was collected during the following 48 h. Both prior to and during the experiment the rats were fed with an RM1 diet. This diet resulted in a background concentration of some lignans, and therefore the background concentrations (prior to administration) were subtracted from the analyzed amounts. In Table 1 the excretion of **1a**, **1b**, **2**, **3**, enterodiol (END), 7-hydroxyenterolactone (HENL), and enterolactone (ENL) is shown, after administration of the preparation containing **1a** and **1b** in a ratio of approximately 37:61 and 2.5% of **2**. The relative amount of **2** compared to the total amount of both isomers of **1** was smaller in the urine extracts (1.5%) than in the hydroxymatairesinol preparation (2.5%). This indicated that **2** is not absorbed and excreted in the same way as **1** or, alternatively, it is metabolized to a higher degree than **1**. Compound **3** was not present in the preparation, indicating that this isomer is either a metabolite of **1** or alternatively formed by isomerization of **2** in vivo. However, in comparison with other lignans **3** was present only in small amounts in the urine samples.

Interestingly, the relative amount of **1b** was significantly lower in the urine extracts of the female rats than in the extracted hydroxymatairesinol preparation and was kinetically excreted similarly as **2**. The reason for this phenomenon remains to be investigated. However, one possibility may be different receptor interactions of the hydroxymatairesinol isomers in body tissues. It has been shown that lignans, especially mammalian lignans, can, due to their weak estrogenic effects, be bound to estrogen receptors, sex-hormone-binding globulins, sex-steroid-binding

proteins, and steroid-metabolizing enzymes.[20] Also, the excretion of lignans has been correlated to the menstrual cycle, and several aspects regarding the estrogenic effects of lignans have been discussed (see ref 20 and refs therein). Compared to our previous study, performed with male rats,[4] the female rats excreted much larger amounts of both **1** and the mammalian lignans. In our ongoing studies we have found exceptionally large differences in the excreted amounts of lignans between male and female rats. When administered plant lignans, female rats excrete up to 30-fold more of the mammalian lignans compared to male rats (unpublished results).

Although **1** can theoretically undergo isomerization and rearrangement to **2** and **3** under physiological conditions, it is unlikely that it occurs in vivo, and from the results it seems that **1** is not metabolized to **2** in vivo. However, the detection of **3** indicates that, to a small extent, either isomerization or metabolization occurs.

The experiment also demonstrated that **1** is well absorbed and metabolized in rats, which was in accordance with earlier studies.[4,13] In 48 h the total excretion of analyzed lignans was over 56% of the administered molar amounts.

We have shown that hydroxymatairesinol, under alkaline conditions, rearranges to a new lariciresinol-type butyrolactone via a possible quinone methide intermediate. This lignan skeleton has not previously been reported to occur in nature. However, we showed that Norway spruce knots can contain up to 5 mg/g (0.5%) of two diastereomers of this lignan. When administered to rats, this lignan is (partially) absorbed and excreted in the urine similarly to hydroxymatairesinol. The biological properties and the possible metabolization to enterolactone or other lignans remain to be explored.

Experimental Section

General Experimental Procedures. All commercially available chemicals were purchased and used as supplied by the manufacturers. Hydroxymatairesinol (**1**) was isolated from Norway spruce knots by the methods described previously.[2,27] Knots of Norway spruce were separated, ground, and freeze-dried prior to extraction in a Soxhlet apparatus. The raw extract obtained with acetone/water (9:1 v/v) after the removal of lipophilic extractives with hexane was purified using flash chromatography to yield **1**. GC−MS analyses of the reaction mixures, fractions, and pure components were performed on a HP-5890 Series II gas chromatograph equipped with a 5971A mass selective detector and an HP-1 column. Prior to analyses, the samples were silylated with hexamethyldisilazane and chlorotrimethylsilane in pyridine. Elemental compositions and mass were determined with a Fisons ZAB-Spec high-resolution MS EI instrument. HPLC-MS/MS analyses were performed with a Micromass Quattro Micro equipped with an electrospray source. HPLC conditions were the following: column XTerra MS C8 (2.1 × 150 mm, 3.5 μm), linear gradient elution from MeOH/0.1% HAc 20:80 to 66:34, flow rate 0.20 mL/min, column oven 30 °C. MPLC purification was performed with a Buchi 684 MPLC system, using normal-phase silica (silica gel 40, Fluka) and isocratic elution with ethyl acetate/petroleum ether (55:45 v/v), pressure 5 bar, flow rate 9 mL/min. Column chromatography was performed with normal-phase silica (silica gel 60) using isocratic elution with $CHCl_3$/MeOH (98:2 v/v). ^{1}H and ^{13}C NMR spectra were recorded with a JEOL JNM-A500 instrument at 500 and 125 MHz, respectively. 2D experiments (COSY, HMQC, HMBC, COLOC) were recorded using standard pulse sequences, and chemical shifts are reported downfield from TMS. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter, using a 1 dm, 1 mL cell.

Base-Catalyzed Formation of Isohydroxymatairesinol (2) and Epi-isohydroxymatairesinol (3). A mixture of the hydroxymatairesinol isomers **1a** and **1b** (**1a**:**1b** 4:96, 11 g) was dissolved in THF (80 mL) and H_2O (80 mL). The pH was adjusted to 8 with diluted NaOH solution. The mixture was heated to 50 °C and stirred for 6 h and then allowed to cool to room temperature. During the reaction the pH was kept around 8 by continuous addition of NaOH solution. The cooled reaction mixture was extracted with CH_2Cl_2 (3 × 120 mL), and the organic phases were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was submitted to fractionation and purification by column chromatography. The fractions containing **2** and **3** were further purified by MPLC to yield pure compounds for NMR, HRMS, and optical rotation analyses.

(+)-Isohydroxymatairesinol (2): colorless powder; $[\alpha]^{25}_D$ +51.0° (*c* 0.005, THF); EIMS *m*/*z* 374 [M$^+$] (43%), 356 (11), 194 (9), 180 (56), 153 (97), 137 (100), 124 (15), 93 (21); EIMS (TMS-ether derivatives) *m*/*z* 590 [M$^+$] (10%), 324 (100), 293 (19), 280 (6), 223 (23), 209 (53), 193 (9), 179 (15), 73 (99); HREIMS *m*/*z* 374.13600 (calculated for $C_{20}H_{22}O_7$, 374.13655); ^{1}H NMR ($CDCl_3$, 500 MHz, 30 °C) δ 6.88 (1H, d, J = 8.1 Hz, H-5), 6.80 (1H, d, J = 8.1 Hz, H-5′), 6.77 (1H, d, J = 2.0 Hz, H-2), 6.75 (1H, ddd, J = 8.1, 2.0, 0.8 Hz, H-6), 6.72 (1H, d, J = 2.1 Hz, H-2′), 6.67 (1H, dd, J = 8.1, 2.1 Hz, H-6′), 5.46 (1H, d, J = 2.6 Hz, H-7), 3.94 (1H, dd, J = 10.7, 4.4 Hz, H-9a) 3.86 (3H, s, O*Me*), 3.83 (3H, s, H−O*Me*′), 3.76 (1H, dd, J = 10.7, 6.9 Hz, H-9b), 3.19 (1H, dd, J = 15.0, 5.0 Hz, H-7′a), 3.05 (1H, ddd, J = 10.2, 8.1, 5.0 Hz, H-8′), 2.76 (1H, dd, J = 15.0, 10.2 Hz, H-7′b), 2.60 (1H, dddd, J = 8.1, 6.9, 4.4, 2.6 Hz, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz, 30 °C) δ 178.10 (CO, C-9′), 146.84 (C, C-3), 146.66 (C, C-3′), 145.56 (C, C-4), 144.34 (C, C-4′), 130.80 (C, C-1), 130.40 (C, C-1′), 120.72 (CH, C-6′), 117.74 (CH, C-6), 114.51 (CH, C-5), 114.47 (CH, C-5′), 111.10 (CH, C-2′), 107.61 (CH, C-2), 81.61 (CHOH, C-7), 60.70 (CH_2OH, C-9), 56.03 (CH_3O, OMe), 55.96 (CH_3O, OMe′), 47.64 (CH, C-8), 41.40 (CH, C-8′), 30.74 (CH_2, C-7′).

(+)-Epi-isohydroxymatairesinol (3): colorless powder; $[\alpha]^{25}_D$ +59.8° (*c* 0.005, THF); EIMS *m*/*z* 374 [M$^+$] (52%), 356 (13), 194 (11), 180 (100), 151 (9), 137 (81), 124 (21); EIMS (TMS-ether derivatives) *m*/*z* 590 [M$^+$] (6%), 324 (37), 293 (7), 280 (4), 223 (14), 209 (36), 193 (6), 179 (15), 73(100); HREIMS *m*/*z* 374.13640 (calcd for $C_{20}H_{22}O_7$, 374.13655); ^{1}H NMR ($CDCl_3$, 500 MHz, 30 °C) δ 6.83 (1H, d, J = 8.1 Hz, H-5), 6.82 (1H, d, J = 8.0 Hz, H-5′), 6.81 (1H, d, J = 2.0 Hz, H-2′), 6.70 (1H, ddd, J = 8.1, 2.0, 0.4 Hz, H-6), 6.69 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.49 (1H, d, J = 2.0 Hz, H-2), 5.12 (1H, d, J = 9.3 Hz, H-7), 3.86 (3H, s, O*Me*′), 3.76 (3H, s, O*Me*), 3.57 (1H, ddd, J = 11.0, 4.0, 3.9 Hz, H-9a), 3.50 (1H, ddd, J = 11.0, 4.1, 3.9 Hz, H-9b), 3.12 (1H, ddd, J = 10.2, 5.8, 5.8 Hz, H-8′), 3.08 (2H, d, J = 5.8 Hz, H-7′a and H-7′b), 2.30 (1H, dddd, J = 10.2, 9.3, 4.0, 4.1 Hz, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz, 30 °C) δ 178.00 (CO, C-9′), 147.00 (C, C-3), 146.78 (C, C-3′), 146.25 (C, C-4), 144.60 (C, C-4′), 130.16 (C, C-1), 129.73 (C, C-1′), 122.37 (CH, C-6′), 120.18 (CH, C-6), 114.48 (CH, C-5′), 114.18 (CH, C-5), 112.03 (CH, C-2′), 108.41 (CH, C-2), 81.34 (CHOH, C-7), 59.63 (CH_2OH, C-9), 56.09 (CH_3O, OMe′), 55.95 (CH_3O, OMe), 50.12 (CH, C-8), 43.87 (CH, C-8′), 34.42 (CH_2, C-7′).

Analyses of Knotwood Extracts. Lignans in the acetone/H_2O extract were analyzed on a 25 m × 0.20 mm i.d., 0.11 μm HP-1 capillary column coated with cross-linked methyl polysiloxane (Agilent Technologies, Palo Alto, CA). The gas chromatograph was a Perkin-Elmer AutoSystem XL instrument (Perkin-Elmer, Boston, MA). Column oven: 120 °C−6 °C/min−300 °C (10 min); carrier gas H_2 (20 mL/min); split injector (1:20) 260 °C; FID detector 300 °C; injection volume 1 μL. The solvent was evaporated and the extractives were silylated by addition of 80 μL of bis(trimethylsilyl)trifluoroacetamide, 20 μL of trimethylchlorosilane, and 20 μL of pyridine. The reaction was completed by keeping the test tubes in an oven at 70 °C for 1 h. Heneicosanoic acid and betulinol were used as internal standards. The method used was essentially according to Ekman and Holmbom.[28] A correction factor of 1.2 was used for the lignans that were calculated against betulinol. Identification of individual components was performed by GC−MS

analysis of the silylated components with an HP 6890-5973 GC-quadrupole- MSD instrument, using a 25 m HP-1 GC column similar to that above.

HPLC-ESI-MS/MS Analyses of Rat Urine. The animal experiments with Sprague−Dawley rats, 5 females, were done essentially as described by Saarinen et al.[4] The hydroxymatairesinol preparation (prepared by flash chromatographic purification of knotwood extract) contained *allo*-hydroxymatairesinol (**1a**), hydroxymatairesinol (**1b**), and isohydroxymatairesinol (**2**) in a ratio of 36.8:60.6:2.5. The preparation was gavaged orally with a vehicle in a dose or 50 mg/kg of body weight. Individual 48 h urine samples were collected after administration. The urine samples were enzymatically hydrolyzed and solid-phase extracted at pH 4.0 as described previously.[4] The urine extracts were analyzed by HPLC-ESI-MS/MS using multiple-reaction monitoring in the negative ionization mode, monitoring the transitions *m*/*z* 373 → 343 for **2** and **3** and 373 → 355 for **1**, which had been optimized by syringe infusion of the pure compounds. Quantification of the compounds was performed using calibration curves of standard solutions. The standard solutions contained blank urine (collected from adult male rats that had been fed with a semipurified lignan-poor C1000 diet for at least one week) spiked with known amounts of the pure compounds. The standards were treated as the real samples; however, they were not hydrolyzed. Matairesinol-d_6 was used as internal standard.

Acknowledgment. The authors thank the Foundation for Research of Natural Resources in Finland for the financial support of this work; M. Reunanen, C. Eckerman, and N. Granholm, Laboratory of Wood and Paper Chemistry, Åbo Akademi University, Turku, Finland, for the HRMS analyses, for the supply of hydroxymatairesinol, and for GC analyses of knotwood extracts; and S. Mäkelä and N. Saarinen, Department of Anatomy, Intitute of Biomedicine, University of Turku, for the rat urine samples. This work is part of the activities at the Åbo Akademi Process Chemistry Centre within the Finnish Centre of Excellence Programme by the Academy of Finland.

Supporting Information Available: GC−MS spectrum of the TMS-ether of compound **2**: GC chromatogram of lignans in knotwood of Norway spruce, including tabulated data showing the amounts of different lignans. This material is available free of charge via the Internet at http//pubs.acs.org.

References and Notes

(1) Ayres, D. C.; Loike, J. D. *Lignans, Chemical, Biological and Clinical Properties*; Cambridge University Press, 1990.
(2) Willför, S.; Hemming, J.; Reunanen M.; Eckerman, C.; Holmbom, B. *Holzforschung* **2003**, *57* (1), 27−36.
(3) Smeds, A.; Hakala, K. *J. Chromatogr. B* **2003**, *793*, 297−308.
(4) Saarinen, M. N.; Smeds, A.; Mäkelä, S. I.; Ämmälä, J.; Hakala, K.; Pihlava, J.-M.; Ryhänen, E.-L.; Sjöholm, R.; Santti R. *J. Chromatogr. B* **2002**, *777* (1−2), 311−319.
(5) Holmbom, B.; Eckerman, C.; Eklund, P.; Hemming, J.; Nisula, L.; Reunanen, M.; Sjöholm R.; Sundberg, A.; Willför S. *Phytochem. Rev*, in press.
(6) Eckerman, C.; Holmbom B. PCT WO 0209893 A1, 2001.
(7) Pärhi, S.; Kalapudas, M.; Korte, H.; Hukka, P. PCT WO 2003044004 A1, 2003.
(8) Eklund, P.; Sillanpää, R.; Sjöholm R. *J. Chem. Soc., Perkin Trans. 1* **2002**, *19*, 1906−1910.
(9) Eklund, P.; Lindholm, A.; Mikkola, J.-P.; Smeds, A.; Lehtilä, R.; Sjöholm, R. *Org. Lett.* **2003**, *5*, 491−493.
(10) Eklund, P.; Riska A.; Sjöholm, R. *J. Org. Chem.* **2002**, *67*, 7544−7547.
(11) Eklund, P.; Sjöholm, R. *Tetrahedron* **2003**, *59*, 4515−4523.
(12) Saarinen, N. M.; Wärri, A.; Mäkelä, S. I.; Eckerman, C.; Reunanen, M.; Ahotupa, M.; Salmi, S. M.; Franke, A. A.; Kangas, L.; Santti, R. *Nutr. Cancer* **2000**, *36*, 207−216.
(13) Saarinen, N. M.; Huovinen, R.; Wärri, A.; Mäkelä, S. I.; Valentin-Blasini, L.; Needham, L, Eckerman, C.; Collan, Y. U.; Santti I. R. *Nutr. Cancer* **2001**, *41*, 82−90.
(14) Oikarinen, S. I.; Pajari, A.-M.; Mutanen, M. *Cancer Lett.* **2000**, *161*, 253−258.
(15) Kangas, L.; Saarinen, N.; Mutanen, M.; Ahotupa, M.; Hirsinummi, R.; Unkila, M.; Perälä, M.; Soininen, P.; Laatikainen, R.; Korte, H.; Santti, R. *Eur. J. Cancer Prev.* **2002**, *11* (Suppl. 2), 48−57.
(16) Stitch, S. R.; Toumba, J. K.; Groen, M. B.; Funke, C. W.; Leemhuis, J.; Vink, J.; Woods, G. F. *Nature* **1980**, *287*, 738−740.
(17) Setchell, K. D. R.; Lawson, A. M.; Mitchell, F. L., Adlercreutz, H.; Kirk, D. N., Axelson, M. *Nature* **1980**, *287*, 740−742.
(18) Axelson, M.; Setchell, K. D. R. *FEBS Lett.* **1981**, *123*, 337−342.
(19) Axelson, M.; Sjövall, J.; Gustafsson, B. E.; Setchell, K. D. R. *Nature* **1982**, *298*, 659−660.
(20) Wang, L.-Q *J. Chromatogr. B* **2002**, *777*, 289−309.
(21) Molecular modeling was performed with a CaChe molecular modeling program, Oxford Molecular Ltd, Oxford, UK, and with ball-and-stick models.
(22) Moss, G. P. Nomenclature of lignans and neolignans. *Pure Appl. Chem.* **2000**, *72*, 1493−1523.
(23) Moritani, Y.; Fukushima, C.; Ukita, T.; Miyagishima, T.; Ohmizu, H.; Iwasaki, T. *J. Org. Chem.* **1996**, *61*, 6922−6930.
(24) Yamauchi, S.; Kinoshita, Y. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1669−1672.
(25) Niwa, M.; Iguchi, M.; Yamamura, S.; Nishibe, S. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3359−3360.
(26) (a) Leary, G. *J. Chem. Soc., Perkin Trans. 2* **1972**, *5*, 640−642. (b) Turner, A. B. *Fortschritte der Chemie Organischer Naturstoffe. Prog. Chem. Org. Nat. Prod.* **1966**, *24*, 288−328. (c) Freudenberg, K.; Grion, G.; Harkin J. M. *Angew. Chem.* **1958**, *70*, 743−744.
(27) Ekman, R. *Holzforschung* **1979**, *30*, 79−85.
(28) Ekman, R.; Holmbom, B. *Nord. Pulp Pap. Res. J.* **1989**, *4*, 16−24.

NP0340706