Synthesis of (-)-Matairesinol, (-)-Enterolactone and (-)-Enterodiol from the Natural Lignan Hydroxymatairesinol.

Patrik Eklund,* Anna Lindholm, J-P Mikkola, Annika Smeds, Reko Lehtilä and Rainer Sjöholm.

Department of Organic Chemistry , Åbo Akademi University, Biskopsgatan 8, 20500-FIN, Åbo, Finland. Tel: +358 2 215 4502, Fax: +358 2 215 4866. E-mail: paeklund@abo.fi

Supporting information

General Experimental:

All commercially available chemicals were used as supplied by the manufacturers. Hydroxymatairesinol was isolated from Norway spruce knots. Knots 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 petroleum ether, was purified by flash chromatography (eluent $CHCl_2$: EtOH 98:2 v/v) to yield hydroxymatairesinol. Alternatively, knots were extracted with ethanol, K-acetate was added and the HMR-K-acetate adduct was separated by precipitation and filtration to yield the adduct in > 95 % purity (large-scale). The structure of the adduct has not been established. Hovewer, the adduct is easily destroyed by extraction in dichloromethane/water and free HMR is obtained, which indicates a non-covalent complex.

GC analyses were performed on a HP-5890 standard gas chromatograph equipped with a HP-5 column and a FI detector. The samples were silylated using hexamethyldisilazane-chlorotrimethylsilane in pyridine, prior to analyses. HRMS were recorded on a ZabSpecETOF system.

^1H and ^{13}C spectra were recorded on a JEOL JNM-A500 spectrometer at 500 and 125 MHz, respectively. 2D experiments were recorded using JEOL standard pulse sequences and chemical shifts are reported downfield from tetramethylsilane. The assignments of the ^1H and ^{13}C signals were based on homonuclear and heteronuclear direct and long-range correlation spectroscopy (COSY, HMQC, HMBC, COLOC).

Optical rotations were measured with a Perkin Elmer 241 digital polarimeter, using a 1 dm, 1ml cell. Analytical TLC was carried out on pre-coated aluminium based sheets (Merk 60 F_{254}). Chiral HPLC-MS was performed on a PE-Sciex API 3000 instrument equipped with a CHIRALCEL OD-R analytical column (0.46 x 25 cm) using multiple reaction monitoring techniques (MRM).

Experimental procedures:

Hydrogenolysis of hydroxymatairesinol (1) to matairesinol (2) at athmospheric pressure.

The hydrogenolysis was performed in a 50 mL double necked round bottom flask with vigorous magnetic stirring. 515 mg of Pd-C 10 % (0.48 mmol) as a slurry in 4 mL of 1,2-dichloroethane was activated with H_2-gas (1 bar) at rt. for 1 h 10 min. Hydroxymatairesinol (**1**) (1.5 g as an app. 3:1 mixture of the diastereomers) was added to the slurry in 8 ml of 1,2-dichloroethane. The mixture was heated on an oil bath, and the temperature was kept between 40 and 60 °C for 17 h under hydrogen. Then 10 mg of Pd-C 10% (0.9 mmol) was added and the reaction was continued for 1 h 45 min at 50 °C. The reaction mixture was filtered through two filter papers, S&S 589 and Whatman 1-PS with CH_2Cl_2. The product was purified by column chromatography (silica gel) using toluene/EtOAc from 100:0 to 170:40 as eluent. After evaporation of the solvents and drying *in vacuo*, 327 mg (69%) of **2** was obtained.

$[\alpha]^{24}_D$ = –36.9° (c=3.33 in THF, Lab Scan 99.8%), in literature $[\alpha]^{20}_D$ = –42.2° (c=1 in acetone) Barton, G. M.; Gardner, J. A. F *J. Org. Chem.* **1962**, 27, 322-323.
All other spectroscopic and spectrometric data in accordance with previously reported, Fonseca, S. F.; Campello, J. P.; Barata, L. E. S.; Rúveda E. A. *Phytochemistry* **1978**, *17*, 499-502. Lin, R. C.; Skaltsounis, A-L.; Seguin, E.; Tillequin, F.; Koch, M. *Planta Med*. **1994**, 60, 2, 168.

HRMS (EI) *m/z* calculated for $C_{20}H_{22}O_6$ (M^+) 358.1417 found 358.1419; **EIMS** m/z 358 (62 %, M^+), 221 (7), 164 (6), 137 (100), 122 (8) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.39 (1H, m, H-8´), 2.43 (1H, dd, overlapping, H-7´a) 2.49 (1H, m, H-8), 2.52 (1H, dd, J = 13.5, 6.6 Hz, H-7´b), 2.80 (1H, dd, J = 14.1, 7.1 Hz, H-7a), 2.88 (1H, dd, J = 14.1, 5.2 Hz, H-7b), 3.73 (3H, s, OMe´), 3.74 (3H, s, OMe), 3.81 (1H, dd, J = 9.2, 7.2 Hz, H-9´a), 4.07 (1H, dd, J = 9.2, 7.3 Hz, H-9´b), 5.55 (1H, s, OH´), 5.56 (1H, s, OH), 6.34 (1H, d, J = 2.0 Hz, H-2´), 6.44 (1H, dd, J = 2.0, 8.0 Hz, H-6´), 6.53 (1H, dd, J = 2.0, 7.8 Hz, H- H-6), 6.55 (1H, d, J = 2.0 Hz, H-2), 6.73 (1H, d, J = 8.0 Hz, H-5´), 6.75 (1H, d, J = 7.8 Hz, H-5); **^{13}C NMR** (500 MHz, $CDCl_3$) δ 34.62 (C-7), 38.32 (C-7´), 41.04 (C-8´), 46.59 (C-8), 55.85 (2 x OMe), 71.37 (C-9´), 111.07 (C-2´), 111.61 (C-2), 114.17 (C-5´), 114.47 (C-5), 121.35 (C-6´), 122.11 (C-6), 129.59 (C-1´), 129.83 (C-1), 144.46 (C-4´), 145.59 (C-4), 146.68 (C-3´), 146.78 (C-3), 178.85 (C-9).

Pressurized hydrogenolysis of hydroxymatairesinol.

An isothermal, laboratory scale, stainless steel pressure autoclave (with baffles) having an internal diameter of 64 mm and a length of 103 mm was filled with 150 ml of 1,2-dichloroethane. Hydroxymatairesinol (1.5 g) and Pd-C 10 % (0.3 g) catalyst were added to the reaction vessel. The reaction mixture was warmed with an electrical coil to 50 °C while it was flushed with N_2 (99,999 % pure, AGA OYj) to remove oxygen from the vessel (equipped with a cooling coil and temperature controller). The reaction mixture was stirred first at 500 rpm and after the temperature had reached 36 °C, at 1000 rpm. When the reaction mixture reached a temperature of 50 °C, H_2 (99,999 % pure, AGA OYj) was introduced to the reaction mixture followed by N_2 to make sure that all oxygen had been removed from the reaction mixture. After this the hydrogenolysis was performed by introducing again H_2 to the vessel. The pressure was adjusted to 8 bar. The reaction was allowed to proceed for 240 minutes after which the reaction mixture was filtered through a filter paper. According to GC-MS (HP-5890 Series II Gas chromatograph equipped with a HP-5971A-mass selective detector; column 30 m x 0.25 mm x 0.25 µm HP-1MS) the conversion of **1** to **2** was quantitative.

High pressure hydrogenolysis of HMR-KAc adduct.

Example 1

An isothermal, laboratory scale, stainless steel pressure autoclave (no baffles, equipped with a standard propeller stirrer) having an internal diameter of 64 mm and a length of 103 mm was filled with 100 g of EtOH (Ethanol Aa, purity 96 %-vol, Primalco Oy) in which 15 g of HMR K-acetate adduct (used as such - not dried) was dissolved on a heat plate. 9.8 g of washed (deionized water and ethanol). (Mo-promoted Raney nickel (Acticat, Catalloy Ltd) catalyst was inserted into the reactor vessel together with the reaction mixture and heating was switched on. The mixture was flushed with hydrogen (99.999 % pure, AGA Oyj) for 2 minutes to remove oxygen from the vessel. During the heating period the stirrer was not engaged. The heating was swiched on and the reactor reached the desired reaction temperature of 130 ºC (403 K) in 9 min. The stirrer was switched on (1100 rpm) and this was considered the initial start of the hydrogenation batch. The pressure was adjusted to 700 PSI (approx. 50 bar).
The reaction was allowed to proceed for approximately 300 min and during the reaction small amounts of samples were withdrawn from the reaction mixture for later analysis by means of GC. The samples (a few milliliters) were obtained through a 5 µm metallic sinter filter by cracking a sample valve, immediately wrapped into an aluminium folio to protect them from light exposure and transferred to a freezer (-20 ºC, 253 K). The optimal yield of matairesinol 95.4 wt-% was reached in 161 min. The initial concentration of 0.61 wt-% (some reaction takes place immediately since active Ra-Ni catalyst contains chemisorbed hydrogen) increased to the earlier mentioned 95.4 wt-%, whereas the initial HMR content of 93.9 wt-% (*allo*-HMR 9.9 wt-%, HMR 84 wt-%) was reduced to 5.5 wt-% (*allo*-HMR 3.1 and HMR 2.4 wt-%, respectively). Minor amounts of the following by-products were detected

during the cause of the reaction: 7-hydroxy secoisolariciresinol, secoisolariciresinol, liovil, conidendric acid, conidendrin and lariciresinol.

Example 2

An isothermal, laboratory scale, stainless steel pressure autoclave (no baffles, equipped with a highly efficient turbine gas disperser coupled to a traditional propeller stirrer) having an internal diameter of 64 mm and a length of 103 mm was filled with 100 g of EtOH (Ethanol Aa, purity 96 %-vol, Primalco Oy) in which 10 g of HMR-Kac adduct (used as such - not dried) was dissolved on a heat plate. 1 g of Pd/C (Palladium on active carbon, Acros Ltd) catalyst that was used in an earlier experiment was inserted into the reactor vessel together with the reaction mixture. The mixture was flushed with hydrogen (99.999 % pure, AGA Oyj) for 2 minutes to remove oxygen from the vessel. During the heating period (10 min.) the stirrer was not engaged. After the reactor reached the desired reaction temperature of 100 ºC the stirrer was switched on (1100 rpm) and this was considered the initial start of the hydrogenation batch. The pressure was adjusted to approx. 30 bar.
The reaction was allowed to proceed for 295 minutes and no samples (except a few ml in the beginning and at the end of the batch) were withdrawn from the reaction mixture.
After 295 minutes the contents of the reaction vessel were emptied, the catalyst was filtrated off by means of vacuum filtration through a filter paper. The solvent was removed under reduced pressure and residue was redissolved in CH_2Cl_2 and washed with water to remove K-acetate. The organic phase was dried over $NaSO_4$, concentrated and chromatographed on a short silica column using $CHCl_3$:MeOH (92:2 v/v) as eluent, yielding 5.55 g (73 %, calculated from the undried HMR-KAc adduct of unknown composition) pure matairesinol after drying in vacuo.

Matairesinyl 4,4´-*bis*triflate (3)

To a solution of 1.1 g (3 mmol) matairesinol (**2**) in dry CH_2Cl_2 (15 ml) lutidine (2.6 g) was added. The reaction mixture was mainatined at 0 °C under argon and 1.2 ml (7.2 mmol) of triflic anhydride was added slowly to the mixture. After 48 hrs, 200 ml of dichloromethane was added and the mixture was extracted (5 x 100ml) with distilled water. The organic phase was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The product was purified by flash column chromatography, using ethyl acetate:petroleum ether as eluent (1:3 v/v) to afford 1.6 g (88 %) of pure matairesinyl 4,4´-*bis* triflate (**3**).

HRMS (EI) *m/z* calculated for $C_{22}H_{20}F_6O_{10}S_2$ (M^+) 622.0402 found 622.0403; **EIMS** m/z 622 (63 %, M^+), 489 (100), 269 (22), 219 (6), 205 (13), 178 (22), 163 (11), 137 (44); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.48 (1H, m, H –8´), 2.62 (1H, m, H -8), 2.69 (1H, dd, *J* =13.5, 7.3 Hz, H –7´a), 2.70 (1H, dd, *J* =13.5, 7.0 Hz, H –7´b), 2.99 (1H, d, *J* =6.7 Hz, H -7), 3.85 (1H, s, CH_3-O´), 3.86 (1H, s, CH_3-O), 3.92 (1H, dd, *J* =9.1, 7.9 Hz, H –9´a), 4.23 (1H, dd, *J* =9.1, 7.5 Hz, H-9´b), 6.60 (2H, 2 x dd, *J* =8.2, 2.1 Hz, H –6, H-6´), 6.64 (1H, d, *J* =2.0 Hz, H –2´), 6.85 (1H, d *J* =2.0 Hz, H -2), 7.11 (1H, d *J* = 8.2 Hz, H –5´), 7.13 (1H, d *J* =8.2 Hz, H -5). **^{13}C NMR** (500 MHz, $CDCl_3$) δ 34.53(C-7), 38.49 (C-7´), 40.87(C-8´), 46.40 (C-8), 55.18 (CH_3-O´), 55.26 (CH_3-O), 70.96 (C-9´), 113.37 (C-2´), 114.04 (C-2), 118.75 (two d, *J* =332.1 Hz, 2 x CF_3), 120.64 (C-6´), 121.39 (C-6), 122.46 (C-5´), 122.82 (C-5), 139.28 (C-1´), 139.42 (C-1), 151.62 (2C, C-4,4´), 177.81 (C-9).

3,3´-dimethylenterolactone (4)

Matairesinyl 4,4-*bis*triflate (**3**) (0.902 g, 1,45 mmol) was dissolved in DMF (4 ml) and triethylamine (0.9 ml) was added. To the mixture, stirred under argon at 85 °C was added 90 mg (0.22 mmol) of 1,3-*bis*(diphenylphosphino)propane and 54 mg (0.09 mmol) $PdCl_2(PPh_3)_2$. Finally formic acid (9 drops) was added. After 21 hours, 70 ml dichloromethane and 70 ml water were addded. The organic phase was washed with 10 % HCl solution (6 x 45 ml) and then with 45 ml saturated NaCl solution. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography, to yield 0.401 g (85%) of 3,3´-dimethylenterolactone (**4**) in 93% purity (GC-MS).

HRMS (EI) *m/z* calculated for $C_{20}H_{22}O_4$ (M^+) 326.1518 found 326.1521; **EIMS** m/z 326 (75 %, M^+), 205 (17), 159 (9), 147 (17), 122 (100), 91 (13); **^{1}H NMR** (500 MHz, acetone-d_6) δ 2.57 (1H, m, C-8´), 2.58 (1H, m, H-7´a), 2.67 (1H, m, H-7´a), 2.69 (1H, m, C-8), 2.91 (1H, dd, *J* = 13.8, 7.1 Hz, H-7), 3.01

(1H, dd, *J* = 13.8, 5.4 Hz, H-7), 3.76 (1H, s, CH_3-O´), 3.77 (1H, s, CH_3-O), 3.90 (1H, dd, *J* = 9.1, 8.0 Hz , H-9´a), 4.10 (1H, dd, *J* = 9.1, 7.3 Hz , H-9´b), 6.63 (1H, dd, *J* = 2.5, 1.6 Hz, H-2´), 6.66 (1H, ddd, *J* = 8.1, 1.6, 1.0 Hz, H-6´), 6.75 (1H, ddd, *J* = 8.1, 2.5, 1.0 Hz, H-4´), 6.76-6.81 (3H, H-2, H-4, H-6, overlapping), 7.17(1H, dd, *J* = 8.4,7.6 Hz, H-5´), 7.20 (1H, dd, *J* = 8.7, 7.5 Hz, H-5). **^{13}C NMR** (500 MHz, acetone-d_6) δ 35.29 (C-7), 38.64 (C-7´), 41.81 (C-8´), 46.56 (C-8), 55.20 (CH_3-O´), 55.24 (CH_3-O), 71.34 (C-9´), 112.44 (C-4´), 112.65 (C-4), 114.80 (C-2´), 115.46 (C-2), 121.36 (C-6´), 122.11 (C-6), 129.92 (C-5), 130.02 (C-5´) 140.28 (C-1), 140.67 (C-1´), 160.43 (C-3 or C-3´), 160.46 (C-3 or C-3´), 178.53 (C-9).

(-)-Enterolactone (5)

3,3´-dimetylenterolactone **4** (400 mg, 1.227 mmol) was dissolved in 20 ml dry CH_2Cl_2. The solution was rapidly stirred and cooled to –10 °C under argon. Then BBr_3 (3.5 eqv., 4.3 mmol, 4.3 ml of 1M BBr_3 in CH_2Cl_2) was added dropwise over a period of 15 min. The mixture was stirred at –10 °C for 6 h and then allowed to warm to room temperature. After totally 18 h , 40 ml water was added and the mixture was extracted with CH_2Cl_2 (2 x 30 ml). The organic phase was washed with 20 ml saturated NaCl solution, dried over Na_2SO_4 and finally the solvent was removed under reduced pressure. The residue was chromatographed on a short silica column using $CHCl_3$: MeOH (99:1 v/v) as eluent to yield 291 mg (79 %) of (-)-enterolactone (**5**) as a colourless powder after drying in vacuo. Purity > 97 % (GC).

$[\alpha]^{24}_D$ = –39.4° (c=0.52 in $CHCl_3$), in literature: $[\alpha]^{24}_D$ = –38.4° (c=1 in $CHCl_3$), Stitch, R. S. *et al.* Nature, **1980**, *287*, 738-740., $[\alpha]^{19}_D$ = –40.3° (c=0.553 in $CHCl_3$) Van Oeveren, A.; Jansen, J. F. G. A.; Feringa, B. L. *J. Org. Chem.* **1994**, *59*, 5999., $[\alpha]^{24}_D$ = –38.3° (c=0.29 in $CHCl_3$), Sibi, M. P.; Liu, P.; Johnson, M. D. *Can. J. Chem.* **2000**, *78*, 133-138.

HRMS (EI) *m/z* calculated for $C_{18}H_{18}O_4$ (M^+) 298.1205 found 298.1200; **EIMS** m/z 298 (37 %, M^+), 191 (20), 145 (14), 133 (17), 108 (100), 77 (15); **^{1}H NMR** (500 MHz, acetone-d_6) δ 2.51 (1H, m, H-7´), 2.55 (1H, m, H-8´), 2.67 (1H, m, H-8), 2.69 (1H, m, H-7´), 2.88 (1H, dd *J* = 13.8, 6.7 Hz, H-7a), 2.97(1H, dd *J* = 13.8, 5.4 Hz, H-7b), 3.88 (1H, t *J* = 8.9 Hz, H-9´a), 4.04 (1H, dd *J* = 8.9, 7.2 Hz, H-9´b), 6.60 (1H, dt *J* = 7.5, 1.6 Hz, complex, H-6´), 6.63 (1H, dd *J* = 1.6, 2.5 Hz, H-2´), 6.68 (1H, ddd *J* = 8.1, 2.5, 1.0 Hz, H-4´), 6.71 (1H, ddd, overlapping, H-4), 6.72 (1H, m, overlapping, H-6), 6.78 (1H, t *J* = 2.1 Hz, H-2), 7.09 (1H, t *J* = 7.7 Hz, H-5´), 7.13 (1H, t *J* = 7.7 Hz, H-5), 8.20 (1H, s, 3´-OH), 8.24 (1H, s, 3-OH). **^{13}C NMR** (500 MHz, acetone-d_6) δ 35.30 (C-7), 38.68 (C-7´), 42.14 (C-8´), 46.79 (C-8), 71.43 (C-9´), 114.26 (C-4´), 114.46 (C-4), 116.15 (C-2´), 117.11(C-2), 120.61 (C-6´), 121.45 (C-6), 130.26 (C-5), 130.36 (C-5´), 140.82 (C-1), 141.30 (C-1´), 158.42 (C-3 and C-3´), 178.66 (C-9).

(-)-Enterodiol (6)

(-)-Enterolactone (230 mg, 0.772 mmol) was dissolved in 20 ml dry THF. To the solution $LiAlH_4$ (3 eqv., 2.31mmol, 87.8 mg) was cautiously added in small portions at room temperature. The mixture was stirred at room temperature for 1 h and then heated to 50 °C and stirred for 2 h. The reaction was quenched by pouring the mixture onto 50 ml diluted HCl. The mixture was then extracted first with 30 ml EtOAc and then with 50 ml diethylether and finally with 30 ml CH_2Cl_2. During the extraction the water phase was saturated with NaCl. The organic phase was dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was chromatographed on a silica column using EtOAc:Petroleum ether (2:1 v/v) as eluent to yield (-)-enterodiol (**6**), which upon drying *in vacuo* afforded 165 mg (71 %) of a colourless powder, slowly turning into a colourless gum. Purity > 98 % (GC).

$[\alpha]^{23}_D$ = –15.2° (c=1 in EtOH), in literature: $[\alpha]^{23}_D$ = –13.2° (c=1 in EtOH) Van Oeveren, A.; Jansen, J. F. G. A and Feringa, B. L. *J. Org. Chem.* **1994**, *59*, 5999.

HRMS (EI) *m/z* calculated for $C_{18}H_{22}O_4$ (M^+) 302.15181 found 302.1524; **EIMS** m/z 302 (7 %, M^+), 284 (16), 266 (4), 177 (17), 159 (30), 145 (14), 133 (16), 120 (7), 108 (100); **^{1}H NMR** (500 MHz, acetone-d_6) δ 1.64 (2H, m, H-8), 2.68 (4H, m, H-7), 3.35 (2H, dd *J* =4.4, 12.4 Hz, H-9a), 3.78 (2H, dd, *J* = 1.8, 12.4 Hz, H-9b), 6.63 (2H, ddd, *J* = 7.5, 1.1, 1.5 Hz, H-6), 6.66 (2H, ddd, *J* = 8.0, 1.1, 2.5 Hz, H-4), 6.68 (2H, ddd , complex, *J* = 2.5, 0.5, 1.5 Hz, H-2), 7.07 (2H, ddd, *J* = 8.0, 7.57, 0.5 Hz, H-5);

^{13}C NMR (500 MHz, C_3D_6O) δ 38.12 (C-7), 45.03 (C-8), 61.54 (C-9), 113.66 (C-4), 117.07 (C-2), 121.25 (C-6), 130.02 (C-5), 143.31 (C-1), 158.21 (C-3).

Analyses of human serum and urine samples

1,2 ml human serum samples and a 3.8 ml human urine sample (all from different persons, one being an average sample of 3 perons) were enzymatically hydrolysed and solid-phase extracted (Smeds, A. Hakala, K., Development of a sample cleanup and a HPLC-MS/MS method for the Determination of Lignans in Human Plasma, submitted to *J. of. Chromatography*). The enantiomeric composition was analyzed by chiral HPLC-ESI-MS/MS using a Micromass Quatro Micro, triple quadropole mass spectrometer. The MRM (multiple reaction monitoring) parent and daughter ion combinations, HPLC eluents and column were the same as described previously. Saarinen, N. M.; Smeds A.; Mäkelä, S.; Ämmälä, J.; Hakala, K.; Pihlava J-M., Ryhänen, E-L.; Sjöholm R.; Santti, R. *J. of Chromatography B*, **2002**, *777*, 321-327.