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Rhamnosylation of lignans by a *Streptomyces* strain

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Abstract—Screening of 400 *Streptomyces* strains for biotransformation of the natural lignan matairesinol led to the identification of *Streptomyces* sp. LS136, capable of producing a single metabolite in moderate yields. Isolation and purification by preparative HPLC, followed by structural analyses by LC-MS and NMR, established the structure as matairesinol-4-*O*-rhamnoside. This bacterial strain was also used for rhamnosylation of the abundant natural lignans, hydroxymatairesinol and secoisolariciresinol.

1. Introduction

Lignans are secondary plant metabolites, widely distributed in the plant kingdom.[1] These phenolic natural compounds, which are defined by the β–β-linkage of two phenylpropane units, can be found in a variety of structures and, as their glycosides, in different parts of plant species.[1] Coniferous trees, especially their knots (branch bases), have been shown to contain large amounts of lignans. Norway spruce (*Picea abies*) knots contain up to 20% lignans (of the dry weight), with the two diastereomers of the guaiacyl lignan, hydroxymatairesinol (HMR) as the main components.[2] Methods for the separation of knots and the isolation of HMR have recently been developed making it readily available, even on a large scale.[3] In addition to hydroxymatairesinol, (−)-secoisolariciresinol (SECO) and (−)-matairesinol (MR) are commonly found guaiacyl lignans, which can be isolated from wood species.[4,5] MR is more advantageously prepared from HMR by catalytic hydrogenolysis.[6] SECO can be isolated as its diglucoside (SDG) from flax seed in relatively large amounts.[7] HMR, MR, SECO and SDG have been shown to be effectively metabolized by intestinal bacteria to the mammalian lignans, enterolactone and enterodiol, and to be biologically active.[8] Moreover, many lignans have been shown to have antitumour, antiviral, antioxidative as well as other biological properties and may be useful as therapeutic agents.[4,9–13] In this letter, we present a method for producing lignan-glycosides of HMR, MR and SECO using biotransformation by a *Streptomyces* strain.

Screening of 400 *Streptomyces* strains for the biotransformation of MR led to the identification of *Streptomyces* sp. LS136, capable of producing a single metabolite **1** in good yield. This lignan metabolite was identified as a deoxyglycopyranosyl derivative by HPLC-MS. In a scale-up experiment, by feeding 200 mg MR, this compound was produced in sufficient amounts for isolation, purification and for structural analyses. The bacterial strain was also used for the glycosylation of two related lignans: hydroxymatairesinol (HMR), consisting of two different isomers, and secoisolariciresinol (SECO). Both of these were converted by the strain to the corresponding glycosylated derivatives **2** and **3** (Fig. 1). This indicates a rather broad specificity of the enzyme making possible the use of this system to create a library of glycosylated lignans from a selection of different precursors. After isolation and purification of the products by preparative HPLC, the products were analyzed by HPLC-ESI-MS, HREIMS and by NMR spectroscopy. The ^{1}H NMR spectrum of compound **1** showed typical signals for the lignan moiety (MR) with some shift differences, especially for the protons of one of the aromatic rings. Additionally, the presence of an α-rhamnapyranosyl group was shown in the ^{1}H and ^{13}C NMR spectra. The chemical shifts and the coupling constants of the sugar moiety were in accordance with the

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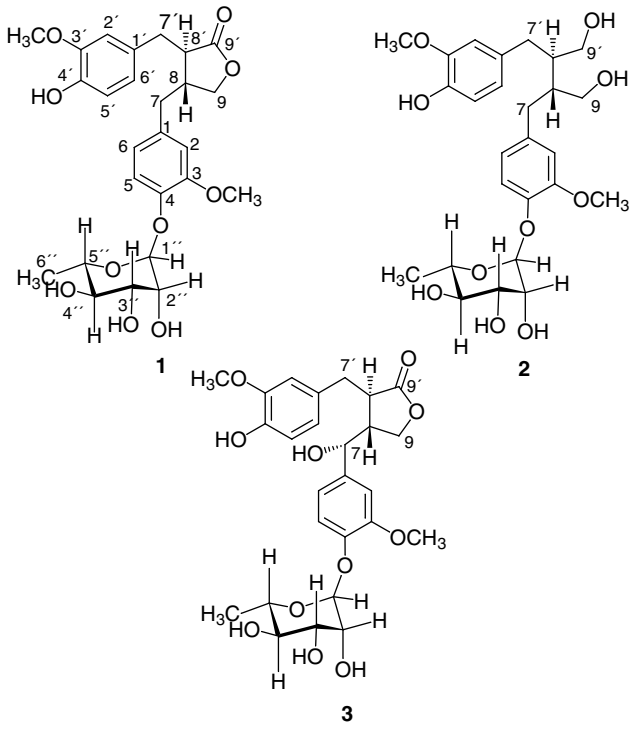

Figure 1. Lignan rhamnosides.

previously reported NMR data on *O*-α-L-rhamnosides.[14] Although the absolute stereochemistry of the sugar moiety was not determined, the identity of the sugar was assumed to be L-rhamnose as the L-form is commonly found in plants and bacteria. Also, natural rhamnosides are almost exclusively L-rhamnosides. The position of the sugar moiety was determined by the cross-peak between the anomeric proton signal (5.19 ppm) and the signal of C-4 (143.6 ppm) in the HMBC spectrum, which allowed us to conclude that the structure of **1** was the phenyl rhamnoside, 8*R*,8′*R*-(−)-matairesinol-4-*O*-α-L-rhamnoside (Fig. 1). In addition to compound **1**, one regioisomer, probably the 4′-*O*-α-L-rhamnoside, was detectable in the NMR spectra. HPLC-MS showed a molecular ion peak at *m*/*z* 503 $[M-H]^-$, which, after isolation and fragmentation in the ion trap, gave *m*/*z* 357 $[M-\text{rhamnose}]^-$, that is, loss of the sugar unit. Compound **2** showed NMR signals for both the lignan moiety (SECO) and for the glycoside moiety in the ^1H and ^{13}C NMR spectra. The ^1H NMR signals of the lignan moiety were severely overlapped and the data could not be completely interpreted. However, the signals from the sugar unit were well separated and showed both chemical shifts and coupling constants similar to those of **1**. As in the spectrum of **1**, a correlation between the anomeric proton of the sugar (5.16 ppm) and C-4 (143.1 ppm) of the lignan was found in the HMBC spectrum. As a consequence, the structure **2**, that is, 8*R*,8′*R*-(−)-secoisolariciresinol-4-*O*-α-L-rhamnoside, was assigned to this product. HPLC-MS analyses showed a deprotonated molecular ion at *m*/*z* 507 $[M-H]^-$, which gave *m*/*z* 361 (lignan moiety) after isolation and fragmentation, nicely in accordance with the proposed structure (Tables 1 and 2).

Table 1. ^1H NMR spectral data of compounds **1**, **2** and **3** (600 MHz, DMSO-d_6)

^1H	**1**	**2**	**3**
Lignan moiety			
2	6.70 d (1.9)	6.71 d (1.7)	6.81 d (1.8)
5	6.94 d (8.0)	6.91 d (8.3)	6.99 d (8.3)
6	6.59 dd (1.9, 8.0)	6.61 dd (1.8, 8.3)	6.74 dd (1.8, 8.3)
7a	2.47 m	2.45–2.6 m	4.30 d (6.1)
7b	2.47 m	2.45–2.6 m	—
8	2.43 m	1.83 m	2.44 m
2′	6.72 d (1.9)	6.62 d (1.8)	6.55 d (1.8)
5′	6.68 d (8.0)	6.63 d (8.2)	6.63 d (8.0)
6′	6.55 dd (1.9, 8.0)	6.49 dd (1.8, 8.2)	6.41 dd (1.8, 8.0)
7′a	2.79 dd (5.4, 13.7)	2.45–2.6 m	2.63 dd (6.1, 13.9)
7′b	2.74 dd (7.0, 13.7)	2.45–2.6 m	2.41 dd (5.7, 13.9)
8′	2.70 m	1.83 m	2.78 m
9a	4.08 dd (7.6, 8.9)	3.34–3.45 m	4.11 dd (7.8, 8.8)
9b	3.68 t (8.9)	3.34–3.35 m	3.98 t (8.8)
9′a and 9′b	—	3.34–3.45 m	—
OMe	3.72 s	3.68 s	3.71 s
OMe′	3.71 s	3.70 s	3.67 s
Rhamnose moiety			
1″	5.20 d (1.7)	5.16 d (1.1)	5.22 d (1.7)
2″	3.83 dd (1.7, 3.4)	3.83 dd (1.1, 3.3)	3.82 dd (1.7, 3.3)
3″	3.64 dd (3.4, 9.6)	3.64 dd (3.3, 9.5)	3.62 dd (3.3, 9.4)
4″	3.27 t (9.6)	3.27 dd (9.2, 9.5)	3.24 t (9.4)
5″	3.58 dq (9.6, 6.2)	3.62 dq (6.2, 9.2)	3.54 dq (6.3, 9.4)
6″	1.08 d (6.2)	1.10 d (6.2)	1.04 d (6.3)

Chemical shifts (ppm), multiplicity and coupling constants (in Hz in parentheses).

When the natural mixture of the hydroxymatairesinol diastereomers (7*S*,8*R*,8′*R*-(−)-hydroxymatairesinol and 7*R*,8*R*,8′*R*-(−)-*allo*-hydroxymatairesinol) was biotransformed by *Streptomyces* sp. LS136, one product was isolated by HPLC. HPLC-MS analysis showed a molecular ion at *m*/*z* 519 $[M-H]^-$, which after fragmentation gave *m*/*z* 373 (MS^2) [aglycone] and *m*/*z* 355 (MS^2) $[HMR-H_2O]^-$, typical for HMR. However, NMR analysis showed a complex mixture of products of the two diastereomers of HMR. Evidently, the diastereomeric derivatives were not separated by HPLC. Due to the severe overlapping of both the signals of the lignan and the rhamnose moiety of the diastereomers, NMR data could not be extracted from the spectra. Therefore, pure *allo*-hydroxymatairesinol was biotransformed to yield compound **3** after isolation and purification.

This compound showed clear NMR signals for both the lignan and the rhamnose moiety. As above, the position of the rhamnose was determined by HMBC data and the structure **3**, that is, 7*R*,8*R*,8′*R*-*allo*-hydroxymatairesinol-4-*O*-α-L-rhamnoside, was assigned to the product.

Interestingly, only phenolic monoglycosides of the lignans were encountered although there are two possible

Table 2. ^{13}C NMR spectral data of compounds **1**, **2** and **3** (150.9 MHz, DMSO-d_6)

^{13}C	1	2	3
Lignan Moiety			
1	133.7	136.4	138.2
2	113.0	113.3	110.3
3	149.9	149.8	149.9
4	143.6	143.1	144.0
5	117.9	117.8	117.6
6	120.5	120.9	117.9
7	36.9	34.0[a]	72.1
8	40.6	42.4[a]	45.5
9	70.7	60.2[a]	67.6
1′	128.8	132.1	128.6
2′	113.3	112.8	113.2
3′	147.3	147.2	143.2
4′	144.3	144.3	144.8
5′	115.2	115.0	115.0
6′	121.6	121.1	121.5
7′	33.6	33.8[a]	33.6
8′	45.6	42.8[a]	42.8
9′	178.5	60.2[a]	178.8
OMe	55.5	55.4	55.6
OMe′	55.5	55.5	55.4
Rhamnose moiety			
1″	99.6	99.8	99.4
2″	70.1	70.3	70.0
3″	70.2	70.4	70.1
4″	70.6	71.7	71.5
5″	69.4	69.5	69.4
6″	17.8	17.8	17.7

[a] The assignments of the carbon signals may be interchanged.

glycosylation sites in MR, three in HMR, and four in SECO. This suggests that the glycosylated lignan is not a substrate for the glycosyltransferase responsible for glycosylation, may be because of steric hindrance. Another explanation might be that the monoglycosylated compound is rapidly exported from the cell, out of reach of the intracellular enzyme. *Streptomyces* bacteria are known to produce several rhamnosylated secondary metabolites, including steffimycin.[15]

There are several previous reports on glycosylated lignans from plants.[16–18] However, only a few papers reporting rhamnosylated lignans have appeared. The bis-*O*-rhamnosides of lariciresinol, 5,5′-dimethoxylariciresinol and secoisolariciresinol have been isolated from *Parsonsia laevigata*.[19] One α-L-rhamnosylated nor-lignan derivative has been isolated from liverworts[14a] and recently, 5-methoxyisolariciresinol-α-rhamnoside was isolated from *Vitis thunbergii*.[14b] To our knowledge, no rhamnosides of the abundant lignans, hydroxymatairesinol and matairesinol, have been reported previously.

The pharmacokinetics of lignan glycosides have not been as extensively studied as for the flavonoids. However, secoisolariciresinol diglucoside is known to be efficiently hydrolyzed and metabolized to mammalian lignans.[20] Arctiin and tracheloside, which are lignan glycosides with the sugar moiety attached to the aromatic hydroxyl group, are also hydrolyzed by the intestinal flora of rats and no intact glucoside has been found in the serum.[21]

The new lignan glycosides reported in this letter could be interesting lead molecules for further development of the utility of lignans in chemoprotection as the glycosylation can be used to alter both physicochemical properties and the pharmacokinetic parameters of the compounds. Also, the three lignans used in this study are derived from spruce knots as the starting material. This is an abundant and accessible source for HMR, which can further be converted to MR and SECO through relatively simple chemical conversions. This makes the production of these metabolites economically feasible, and facilitates further development of the compounds. The strain described here may also find use in the glycosylation of other lignans and phenolic natural products.

2. Experimental

Hydroxymatairesinol was isolated from Norway spruce (*Picea abies*) knots as described by Willför et al.[2] Spruce knots were separated, ground, and freeze-dried prior to extraction in a Soxhlet apparatus. The lipophilic extractives were first removed with hexane, and the lignans were then extracted with acetone/water. The lignan fraction was purified by flash chromatography to yield pure hydroxymatairesinol. (−)-Secoisolarisiresinol was isolated from *Araucaria angustifolia* essentially in the same way. (−)-Matairesinol was prepared from hydroxymatairesinol by sodium borohydride reduction, in our laboratory.[22]

Screening phase: The screening was carried out with 400 *Streptomyces* strains (LS001–LS400) from the culture collection of Lividans Ltd. Matairesinol was used as the substrate and new metabolites were detected by HPLC.

2.1. Matairesinol-4-*O*-α-L-rhamnoside 1

Colourless solid (53 mg, yield 19%); EIMS *m*/*z* 504 [M^+] (1%), 494 (7%), 358 (100%), 260 (4%), 221 (6%), 164 (6%), 137 (78%); HREIMS *m*/*z* 504.19940 (calculated for $C_{26}H_{32}O_{10}$, 504.19954).

2.2. Secoisolariciresinol-4-*O*-α-L-rhamnoside 2

Colourless solid (51 mg, yield 18%); EIMS *m*/*z* 508 [M^+] (2%), 362 (20%), 344.1 (12%), 220 (4%), 175 (6%), 137 (100%), 122 (10%); HREIMS *m*/*z* 508.23140 (calculated for $C_{26}H_{36}O_{10}$, 508.23085).

2.3. *allo*-Hydroxymatairesinol-4-*O*-α-L-rhamnoside 3

Colourless solid (47 mg, yield 17%); EIMS *m*/*z* 520 [M^+] (1%), 374 (35%), 356 (21%), 298 (5%), 232 (10%), 180 (24%), 153 (31%), 137 (100%); HREIMS *m*/*z* 520.19360 (calculated for $C_{26}H_{32}O_{11}$, 520.19446).

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