

Influence of Syringyl to Guaiacyl Ratio on the Structure of Natural and Synthetic Lignins

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Several kinds of natural woods and isolated lignins with various syringyl to guaiacyl (S/G) ratios were subjected to thioacidolysis followed by Raney nickel desulfuration to elucidate the relationships between the S/G ratio and the interunit linkage types of lignin. Furthermore, enzymatic dehydrogenation polymers (DHP) were produced by the Zutropf (gradual monolignol addition) method from mixtures of various ratios of coniferyl alcohol and sinapyl alcohol. The analysis of DHPs and natural wood lignins exhibited basically a similar tendency. The existence of both syringyl and guaiacyl units is effective for producing higher amounts of β -O-4 and 4-O-5 structures, but it lowers the total amount of cinnamyl alcohol and aldehyde end groups. The relative frequency of the β - β structure increased, whereas that of β -5 and 5-5 structures decreased with increasing syringyl units.

KEYWORDS: Lignin; thioacidolysis; S/G ratio; interunit linkage

INTRODUCTION

Lignin is one of the main components of woody cell walls, and its structure is very complicated. There are three precursors of lignin: coniferyl alcohol (CA), sinapyl alcohol (SA), and *p*-coumaryl alcohol. These precursors are called monolignols and contribute to guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) propane units in lignin, respectively. Softwood lignin mainly consists of the G unit, whereas hardwood lignin is composed of both G and S units, and grass lignin consists of G, S, and H units. There are several interunit linkages including β -O-4, β -5, β - β , 4-O-5, and 5-5 linkages in macromolecular lignin. The β -O-4 linkage is considered to be an uncondensed structure, and linkages except the β -O-4 linkage are considered to be condensed.

From a practical point of view, plants with syringyl-rich lignin seem to be more advantageous than those with guaiacyl-rich lignin, because hardwood lignin is less condensed and requires lesser quantities of chemicals than softwood lignin during the pulping and bleaching processes (1, 2). Some nonwoody plant lignins such as kenaf (*Hibiscus cannabinus*) bast fibers and leaf fibers of abaca (*Musa textilis*) have extremely high S content (3, 4). Due to a recent modification of lignin biosynthesis by biotechnology it is now possible to alter the lignin syringyl to guaiacyl (S/G) ratio in some kinds of woody and nonwoody plants (5–8). The modified lignin structures have been analyzed by chemical and/or spectroscopic methods. Furthermore, pulping efficiency has been studied in the genetically modified plant species.

Some correlations between lignin structure and the S/G ratio have been reported. The S/G ratio obtained by nitrobenzene oxidation correlates well with the *erythro/threo* ratio of β -O-4 structures in lignin, using 21 wood species (9). Furthermore, the S/G ratio versus lignin content has been investigated in poplars (10). The type and frequency of lignin interunit linkages are affected by the S/G ratio. However, to the best of our knowledge, the influence of the S/G ratio on lignin structures has not been elucidated systematically with experimental estimations.

There are several methods that can provide an estimate of the frequency of lignin interunit linkages, including quantitative ¹³C NMR and 2D NMR measurements and various chemical degradation methods. Among them, thioacidolysis is one of the most frequently used methods to estimate lignin monomer units derived only from a β -O-4 structure using wood samples without lignin isolation (11). Furthermore, by performing the Raney nickel desulfuration procedure after thioacidolysis of polymeric lignin, it is possible to estimate the numbers of dimers originating from several uncondensed structures (12, 13).

In this study, several woods and isolated lignin samples with various S/G ratios were subjected to thioacidolysis and subsequent Raney nickel desulfuration to investigate the influence of the S/G ratio on natural lignin structure. Furthermore, the enzymatic dehydrogenation polymer (DHP) was produced by the Zutropf (gradual monolignol addition) method from mixtures of various ratios of CA and SA. These synthetic DHPs were also subjected to thioacidolysis and subsequent desulfuration.

EXPERIMENTAL PROCEDURES

Materials. Three hardwood samples, Japanese white-bark magnolia (*Magnolia obovata* Thunb.), Japanese beech (*Fagus crenata* Blume), and Japanese white birch (*Betula platyphylla* var. *japonica* Hara), and a

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softwood sample, Japanese fir (*Abies sachalinensis* Mast.), were selected for lignin analysis. Wood meals (40–100 mesh) were produced from the wood samples and extracted with benzene/ethanol (2:1) using a Soxhlet apparatus and dried over P₂O₅ in a vacuum desiccator before use.

Lignin Content in Wood Samples. Klason lignin contents of wood meals were determined according to the standard procedure with a slight modification. Acid-soluble lignin present in the liquor after the removal of solid Klason lignin was estimated by UV spectrometry at 205 nm (TAPPI useful method UM-250).

Preparation of MWL. Milled wood lignins (MWL) were prepared from wood meals (<40 mesh) of Japanese fir and Japanese white birch, according to the standard procedure, with dry milling under nitrogen for 48 h using a vibratory ball mill with stainless steel balls. Isolated lignins were purified according to the method of Lundquist (14). The yields of purified fir and birch MWL were 27.1 and 27.2% for the Klason lignin contents of each wood meal, respectively. Klason lignin contents of the isolated MWL were 95.6 and 82.8%, respectively. Sugar contents, which were measured by the alditol acetate method and gas chromatography, were 0.25 and 4.6%, respectively.

Synthesis of Monolignols. CA was synthesized from vanillin according to the procedure of Freudenberg (15) and SA from ethyl sinapate according to the method of Quideau and Ralph (16).

Preparation of DHP. DHP was produced from a mixture of monolignols in the dark according to the Zutropf (gradual monolignol addition) method. A solution containing various molar ratios (total, 1.5 mmol) of CA and/or SA in 200 mL of phosphate buffer (0.1 M, pH 6.5) was pumped into 50 mL of phosphate buffer containing 1 mg of horseradish peroxidase (HRP) (Wako, Japan) for 48 h. At the same time, 200 mL of H₂O₂ (0.04%) was added to the solution for 48 h. Additional peroxidase (1 mg) was added at 24 h. After 72 h, the precipitate was centrifuged, washed with distilled water, and lyophilized. The obtained DHPs were dissolved in 10 mL of dioxane/water (9:1) and lyophilized to yield powdered DHPs. The low molecular weight products, which were not precipitated, were extracted with ethyl acetate from the supernatant of the phosphate buffer solution.

Size Exclusion Chromatography of Natural and Synthetic Lignins. Lignin samples were acetylated with acetic anhydride and pyridine at room temperature overnight. The acetylated lignin samples were dissolved in tetrahydrofuran, and average molecular weights were analyzed by size exclusion chromatography. A Hitachi L-6200 liquid chromatograph with an L-4000 UV detector (280 nm) was used. Shodex GPC packed columns KF-802 and 803 (30 cm × 8.0 mm) were connected in a series, and molecular weight was calibrated with standard polystyrene (tetrahydrofuran; flow rate = 0.5 mL/min, 50 °C).

Thioacidolysis and Subsequent Raney Nickel Desulfuration. Thioacidolysis and subsequent Raney nickel desulfuration were performed according to the procedure of Lapiere et al. (11–13). A thioacidolysis reagent was prepared by introducing 5.0 mL of EtSH and 1.25 mL of BF₃ etherate into a 50 mL flask containing a small amount of distilled dioxane, and the final volume was adjusted to 50 mL with dioxane. About 2–5 mg of each lignin sample and 0.5 mL of internal standard solution (0.1 mg/mL docosane in dioxane) were added to 5 mL of the thioacidolysis reagent in a glass tube closed with a Teflon-lined screw-cap. The reaction was performed at 100 °C for 4 h with occasional shaking. The tube was cooled in an ice–water bath. To the ice-cooled reaction mixture was added 2.5 mL of 0.4 M NaHCO₃ to quench the reaction. The pH of the reaction mixture was adjusted to 3–4 by adding diluted aqueous HCl solution, and the reaction mixture was extracted three times with 5 mL of CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄, the volume of the solution was reduced to about 1 mL by evaporation under vacuum, and the final extracts solution was redried over Na₂SO₄. About 10 μL of the final extracts was trimethylsilylated with 30 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 3 μL of pyridine in a vial for monomer analysis.

For dimer analysis, about 1 mL of the final extracts solution was placed with the Raney nickel aqueous slurry (about 2 mL) and dioxane (5 mL) in a glass tube closed with a Teflon-lined screw-cap. Desulfuration was performed at 50 °C for 4 h without shaking. The tube was cooled in an ice–water bath and opened carefully. Distilled water (5 mL) was added to the ice-cooled reaction mixture, and its pH was adjusted to 3–4 by adding diluted aqueous HCl solution. The reaction mixture was extracted three

Table 1. Lignin Content in Extractive-free Wood

wood	S/G ratio	Klason lignin (%)	acid-soluble lignin (%)	total (%)
fir	0	29.0	0.4	29.5
magnolia	1.37	27.2	3.5	30.7
birch	2.49	19.6	7.2	26.7
beech	3.35	18.4	6.6	25.0

Table 2. Yields and Molecular Weights of Dehydrogenation Polymer (DHP) from Mixtures of Various Ratios of Coniferyl Alcohol (CA) and Sinapyl Alcohol (SA)

expt		SA/ (CA + SA)	DHP (%)	ethyl acetate extracts (%)	total (%)	M _n	M _w	M _w / M _n
								M _n
1	G-DHP	0	86.8	13.4	100.3	3180	10440	3.3
2	GS- DHP	0.25	76.0	17.2	93.2	2280	4770	2.1
3		0.5	58.8	7.8	66.7	3810	11450	3.0
4		0.75	38.2	63.1	101.3	1710	2980	1.7
5	S-DHP	1	8.9	59.4	68.3	1620	2230	1.4

times with 5 mL of CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄, the volume of the solution was reduced to about 0.3 mL by evaporation under vacuum, and the final extracts solution was redried over Na₂SO₄. About 10 μL of the final extracts was trimethylsilylated with 30 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 3 μL of pyridine in a vial for dimer analysis.

GC and GC-MS Analysis of Monomeric and Dimeric Products. GC analysis was performed with a GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector. Trimethylsilylated reaction mixtures were injected into a TC-1 fused silica gel capillary column (GL Sciences Inc., 0.25 mm × 30 m). The temperature program was set at 180 °C for 1 min, raised to 280 °C at 2 °C/min, and maintained at this temperature for 30 min. The temperatures of the injection and detector zones were 250 and 300 °C, respectively. The carrier gas was helium with a flow rate of 47.0 cm/s. A response factor of 1.50 (docosane), defined as the ratio of relative concentration (underivatized compounds) to relative area, was used for quantitative analysis of all monomers and dimers (11, 12). Mass spectra were recorded at 70 eV with GCMS-QP2010 (Shimadzu, Japan) under the same analytical conditions.

RESULTS AND DISCUSSION

Lignin Content in Wood Species. The Klason lignin contents of the natural wood species used in this investigation ranged from 29.0% in Japanese fir to 18.4% in Japanese beech (Table 1). The acid-soluble lignin content of the three hardwoods was much higher than that of the softwood. Total lignin content ranged from 25.0 to 30.7%. The S/G ratio of the woods was calculated from monomer yields obtained by thioacidolysis. Klason lignin contents decreased with an increase in the S/G ratio for all species used, which is consistent with the results of Bose et al. (10).

Preparation of DHP with Various Monolignol Ratios. DHP was produced according to the Zutropf (gradual monolignol addition) method from a mixture of CA and SA. Yields and molecular weights of DHPs are summarized in Table 2. The G-DHP yield produced only from CA was 86.8%. The S-DHP yield produced only from SA was 8.9%. The DHP yield decreased with an increase in molar proportion of SA. In contrast, the ethyl acetate extract yields were significantly high when the initial SA ratio was high (experiments 4 and 5). The ethyl acetate extracts were the low molecular weight dehydrogenation products that did not precipitate during DHP formation. These results indicate that SA does not form high molecular weight dehydrogenation polymers when CA is absent. This can be attributed partly to the fact that HRP oxidizes SA less efficiently than CA (17). CA may act as a radical mediator to promote oxidation of SA (18).

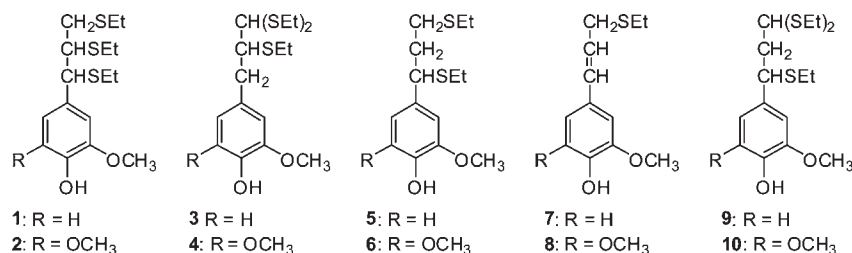


Figure 1. Structures of the monomer products obtained by thioacidolysis.

Table 3. Mass Spectral Characterizations of the Monomer Products Obtained by Thioacidolysis (TMS Derivatives)

compd no. (retention time, min)	molecular ion and prominent fragments <i>m/z</i> (relative intensity)
1 (22.4, 22.6)	418 (1), 295 (4), 270 (20), 269 (100), 239 (3), 235 (9), 204 (4), 131 (5), 75 (14), 73 (16)
2 (25.4, 25.6)	448 (3), 325 (4), 300 (21), 299 (100), 269 (5), 265 (7), 234 (4), 161 (3), 75 (11), 73 (12)
3 (22.8)	418 (2), 356 (6), 284 (10), 283 (47), 262 (4), 223 (15), 222 (53), 192 (11), 137 (8), 135 (100)
4 (25.9)	448 (7), 314 (18), 313 (80), 253 (22), 252 (80), 222 (23), 137 (8), 135 (100), 75 (22), 73 (15)
5 (13.1)	358 (16), 298 (16), 297 (45), 257 (14), 237 (14), 235 (42), 131 (10), 103 (14), 75 (100), 73 (26)
6 (17.1)	388 (23), 327 (43), 328 (13), 267 (14), 265 (36), 222 (7), 207 (9), 161 (8), 75 (100), 73 (26)
7 (9.1)	296 (25), 236 (23), 235 (100), 205 (25), 204 (29), 132 (7), 131 (60), 103 (14), 75 (8), 73 (34)
8 (12.8)	326 (40), 266 (31), 265 (100), 235 (30), 234 (58), 204 (19), 161 (59), 133 (35), 75 (13), 73 (43)
9 (22.0)	418 (11), 356 (11), 295 (14), 270 (19), 269 (100), 233 (12), 209 (11), 207 (10), 135 (30), 73 (39)
10 (25.1)	448 (17), 300 (23), 299 (100), 265 (11), 263 (13), 222 (8), 135 (37), 73 (27), 62 (9), 59 (7)

Characterization of Monomers and Dimers Recovered from Thioacidolysis and Subsequent Desulfuration. Thioacidolysis is a chemical degradation method that offers an estimate of lignin monomer units derived only from β -O-4 structures. Monomer products obtained from thioacidolysis were identified as trimethylsilylated derivatives from thioacidolysis of lignocellulosic materials as shown in **Figure 1**. Among them, compounds **1–4** have been used to estimate internal uncondensed β -O-4 structures in G and S propane units (11). Compounds **5–8** are from coniferyl and sinapyl alcohol end groups. Compounds **9** and **10** are derived from coniferaldehyde and sinapaldehyde end groups. These compounds were identified by comparison of mass spectral data with those reported previously (**Table 3**) (11).

Figure 2 shows the structures of the dimer products obtained by thioacidolysis followed by Raney nickel desulfuration. A typical GC chromatogram of the trimethylsilylated dimer products obtained by thioacidolysis followed by Raney nickel desulfuration is shown in **Figure 3**. The dimers were identified by comparing the mass fragmentation patterns (**Table 4**) with those reported previously (12, 19, 20) and those from model compounds: pinosresinol (β - β), syringaresinol (β - β), and dehydroconiferyl alcohol (β -5). Several GS and SG dimers such as compounds **16**, **17**, **23**, **24**, **27**, **28**, **29**, **36**, and **37** were identified in this study. Among them, compounds **17**, **28**, and **37** were newly identified in this study by comparing the mass fragmentation patterns with those of corresponding GG and/or SS dimers. Compounds **28** (GS) and **29** (SG) are isomers with the same molecular mass (*m/z* 550), but they were distinguishable from each other. Compound **28** yielded typical fragment ions at *m/z* 311 and 239, whereas compound **29** produced fragment ions at *m/z* 341 and 209. Structures of compounds **36** and **37** were assigned to SG dimers. Mass fragmentation patterns were similar to those of compounds **38** and **39**, respectively. Fragment ions at *m/z* 306 and 292 were derived from S-type tetrahydronaphthalene moieties in compounds **36** and **37**, respectively.

Evaluation of Thioacidolysis Monomers That Originate from the β -O-4 Bond. **Table 5** shows the monomer yields recovered from thioacidolysis of several natural woods and isolated MWL, which originated from the arylglycerol- β -aryl ether structure in lignin. The total monomer yields from hardwood were higher than those

from softwood, as reported previously (11). These results imply that when both S and G units exist in lignin, the frequency of the β -O-4 structure is higher than that when only G units are present. The same tendency was observed with synthetic DHPs (**Table 6**), although the β -O-4 structure content in the DHPs was significantly lower than that in native lignins. The total monomer yields from GS-DHPs were higher than those from G-DHP and S-DHP.

Monomers that originate from cinnamyl alcohol and aldehyde end groups can be detected by thioacidolysis. The monomer yield was low for natural hardwood samples (**Table 5**), probably because at the initial stage of lignification in the wood cell wall, SA preferentially produces syringaresinol and does not form a structure that yields cinnamyl alcohol and aldehyde end groups. This tendency was more clearly shown with the synthetic DHPs, in which the total yield of the end groups decreased with an increase in the S unit. In contrast, the yield of the end groups from birch MWL was much higher than that from birch wood. Thus, the possibility of forming cinnamyl alcohol and aldehyde end groups during the MWL preparation may not be excluded.

Distribution of Condensed Structures in Natural and Synthetic Lignins with Various S/G Ratios. There was no clear relationship between the S/G ratio and the total yield of dimers in woods and MWL (**Table 7**). However, the proportion of GG dimers in the whole dimer structure decreased, whereas that of SS dimers increased with an increase in the S/G ratio. This same tendency was observed with the synthetic DHPs with various initial SA/(CA + SA) values (**Table 8**).

The β -5 dimer is one of the major dimers obtained from both natural lignins and DHPs by thioacidolysis followed by desulfuration. The relative frequency of the β -5 structure decreased with an increase in the S/G ratio of woods and isolated MWL (**Table 7**). This tendency was basically the same as the synthetic DHPs. The frequency of the β -5 dimer correlated well with the initial proportion of SA (**Table 8**). This may simply be because 3,5-dimethoxy groups in the S unit prevented the formation of a C–C bond at the 5-C position. However, there was a difference between birch wood and birch MWL. In natural wood samples and DHPs, the β -5 dimer with a GS unit was more frequent than that with a GG unit when S and G units coexisted, but the

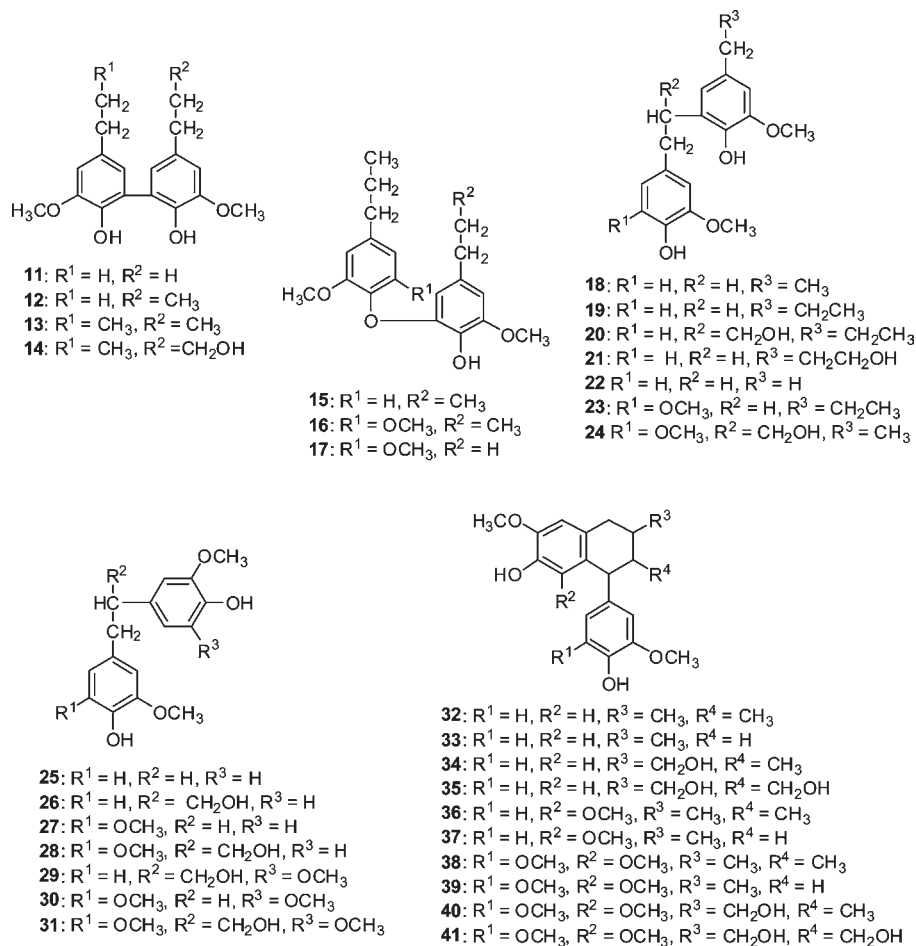


Figure 2. Structures of the dimer products obtained by thioacidolysis followed by Raney nickel desulfuration.

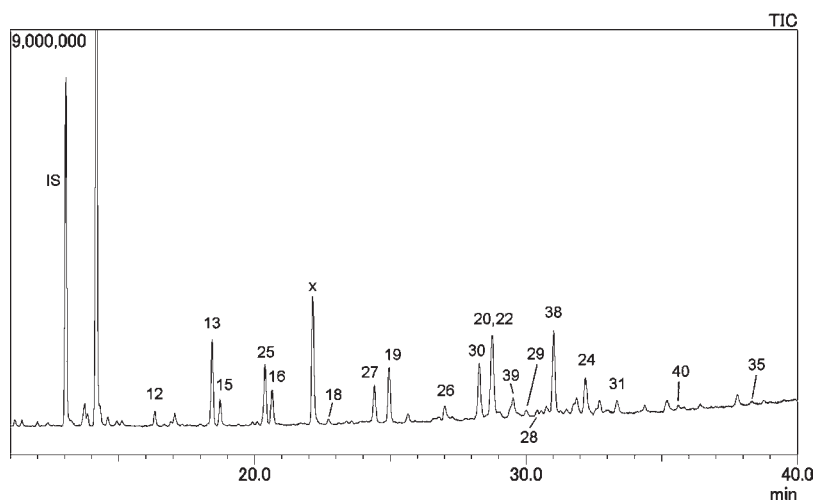


Figure 3. Gas chromatogram of trimethylsilylated dimer products obtained by thioacidolysis followed by Raney nickel desulfuration of magnolia. The numbered peaks are assigned to TMS derivatives of the structures represented in **Figure 2**. IS, internal standard.

opposite results were obtained from isolated birch MWL. This difference may have been caused by the MWL isolation process, which accounts for only 27% of whole lignin in birch wood.

The relative proportion of β - β dimer that originated only from the resinol structure is shown in **Tables 7** and **8**. Only limited amounts of the β - β dimer with a GG unit from the pinoresinol structure were detected in natural woods of fir and magnolia, whereas it was not detected in natural birch and beech woods.

However, a significant amount of the β - β dimer with a GG unit was observed in the synthetic G-DHP. These results were in good agreement with previous reports (13). Although there were some differences in the frequency of the β - β structure between natural lignins and DHPs, a similar tendency was observed in connection with the S/G ratio. The β - β dimer yield increased with an increase in the S/G ratio in natural woods and isolated lignins. It was more evident in the case of DHPs, which reflects the fact that SA

Table 4. Mass Spectral Characterizations of the Dimer Products Obtained by Thioacidolysis Followed by Raney Nickel Desulfuration (TMS Derivatives)

compd no. (retention time, min)	molecular ion and prominent fragments <i>m/z</i> (relative intensity)
11 (14.6)	446 (80), 447 (35), 432 (20), 431 (40), 417 (23), 416 (33), 207 (18), 147 (9), 75 (30), 73 (100)
12 (16.3)	460 (100), 461 (40), 446 (17), 445 (46), 431 (26), 430 (40), 371 (14), 207 (9), 75 (12), 73 (99)
13 (18.4)	474 (100), 475 (38), 460 (16), 459 (41), 445 (32), 444 (28), 415 (8), 385 (8), 357 (8), 73 (81)
14 (27.8)	562 (100), 563 (46), 548 (9), 547 (14), 457 (8), 446 (8), 445 (9), 383 (7), 357 (12), 73 (68)
15 (18.7)	402 (100), 403 (35), 388 (18), 387 (51), 374 (12), 373 (39), 343 (15), 209 (9), 157 (11), 73 (34)
16 (20.7)	432 (100), 433 (32), 418 (14), 417 (42), 403 (27), 402 (21), 373 (8), 209 (13), 207 (8), 73 (31)
17 (18.3)	418 (100), 419 (31), 404 (14), 403 (43), 390 (9), 389 (29), 359 (9), 207 (16), 193 (8), 73 (27)
18 (22.7)	446 (30), 447 (11), 237 (19), 222 (6), 210 (17), 209 (100), 207 (34), 179 (7), 75 (7), 73 (39)
19 (25.0)	460 (3), 461 (12), 251 (14), 210 (17), 209 (100), 207 (18), 193 (6), 179 (7), 73 (18)
20 (28.8)	562 (48), 563 (22), 472 (24), 352 (17), 263 (25), 209 (70), 207 (16), 191 (76), 149 (13), 73 (100)
21 (34.6)	548 (39), 549 (22), 339 (15), 223 (14), 210 (17), 209 (100), 207 (11), 193 (8), 179 (9), 73 (47)
22 (20.9)	432 (26), 433 (9), 223 (12), 210 (12), 209 (100), 193 (11), 179 (10), 177 (10), 75 (8), 73 (64)
23 (28.7)	490 (33), 491 (12), 251 (5), 240 (18), 239 (100), 223 (5), 209 (14), 207 (13), 191 (9), 73 (36)
24 (32.2)	592 (54), 593 (25), 352 (9), 263 (13), 253 (42), 240 (39), 239 (100), 223 (10), 191 (42), 73 (95)
25 (20.4)	418 (17), 419 (6), 210 (17), 209 (100), 193 (3), 179 (9), 74 (3), 73 (37)
26 (27.0)	520 (22), 521 (8), 505 (6), 312 (27), 311 (100), 223 (11), 209 (28), 179 (10), 149 (21), 73 (79)
27 (24.4)	448 (25), 449 (8), 433 (4), 240 (18), 239 (100), 210 (7), 209 (40), 179 (7), 73 (43)
28 (30.6)	550 (41), 551 (14), 535 (9), 312 (26), 311 (100), 240 (12), 239 (45), 209 (19), 75 (10), 73 (64)
29 (30.0)	550 (23), 551 (9), 535 (6), 516 (9), 342 (26), 341 (100), 253 (16), 238 (8), 209 (23), 73 (58)
30 (28.3)	478 (20), 479 (7), 463 (3), 240 (17), 239 (100), 223 (3), 209 (9), 73 (30)
31 (33.4)	580 (29), 581 (13), 342 (27), 341 (100), 310 (9), 253 (14), 240 (8), 239 (37), 209 (14), 73 (68)
32 (27.6)	472 (100), 473 (38), 457 (9), 415 (9), 386 (18), 385 (59), 276 (11), 261 (7), 178 (10), 73 (40)
33 (25.5)	458 (100), 459 (43), 443 (9), 428 (10), 386 (18), 385 (44), 263 (9), 262 (24), 247 (14), 73 (50)
34 (33.2)	560 (23), 470 (26), 455 (22), 440 (48), 439 (100), 428 (19), 381 (15), 261 (17), 209 (13), 73 (59)
35 (38.3)	648 (14), 558 (45), 528 (21), 527 (50), 468 (28), 456 (20), 455 (59), 437 (46), 428 (19), 73 (100)
36 (28.3)	502 (100), 503 (35), 487 (13), 472 (11), 415 (18), 307 (13), 306 (47), 193 (10), 187 (11), 73 (33)
37 (26.7)	488 (100), 489 (18), 473 (12), 293 (19), 292 (74), 263 (11), 262 (10), 173 (19), 97 (12), 73 (50)
38 (31.0)	532 (100), 533 (40), 517 (12), 445 (9), 307 (13), 306 (40), 275 (11), 239 (11), 187 (12), 73 (31)
39 (29.5)	518 (100), 519 (38), 503 (12), 488 (11), 487 (12), 293 (16), 292 (52), 262 (12), 173 (11), 73 (32)
40 (35.6)	620 (82), 621 (40), 606 (23), 500 (29), 499 (67), 355 (25), 354 (86), 266 (78), 239 (21), 73 (100)
41 (40.3)	708 (43), 618 (26), 587 (24), 497 (21), 354 (44), 327 (42), 289 (21), 239 (28), 103 (23), 73 (100)

Table 5. Monomer Yields Recovered from Thioacidolysis of Natural Lignins^a

sample	S/G ratio	main monomer ($\mu\text{mol/g}$)			end group ($\mu\text{mol/g}$)					total
		G	S	total	S/ (G + S)	Galc	Gald	Salc	Sald	
wood fir	0	1183	0	1183	0	63	27	0	0	90
magnolia	1.37	813	1112	1925	0.578	0	6	0	0	6
birch	2.49	621	1546	2167	0.713	0	0	0	0	0
beech	3.35	462	1546	2008	0.777	0	0	0	0	0
MWL fir	0	1026	0	1026	0	51	43	0	0	94
birch	1.76	577	1015	1592	0.638	0	12	29	11	52

^a Galc, monomer from coniferyl alcohol end group; Gald, monomer from coniferyl aldehyde end group; Salc, monomer from sinapyl alcohol end group; Sald, monomer from sinapyl aldehyde end group.

Table 6. Monomer Yields Recovered from Thioacidolysis of Synthetic Lignins (DHPs)^a

expt	SA/ (CA + SA)	main monomer ($\mu\text{mol/g}$)			end group ($\mu\text{mol/g}$)					total
		G	S	total	S/ (G + S)	Galc	Gald	Salc	Sald	
1	0	488	0	488	0	220	0	0	0	220
2	0.25	716	98	814	0.120	79	0	0	0	79
3	0.50	504	236	740	0.319	13	0	0	0	13
4	0.75	585	445	1031	0.432	0	0	0	0	0
5	1.00	0	665	665	1.00	0	0	8	0	8

^a Galc, monomer from coniferyl alcohol end group; Gald, monomer from coniferyl aldehyde end group; Salc, monomer from sinapyl alcohol end group; Sald, monomer from sinapyl aldehyde end group.

preferentially produces syringaresinol by oxidation with the HRP/H₂O₂ system. It should also be noted that the β - β dimer

with a GS unit was not detected in the birch and other woods, but it was detected in trace amounts in the birch MWL and DHPs. The β - β dimer derived from secoisolaricresinol structure (12) was also detected in the fir MWL, fir wood, and magnolia wood, although the data were not included in the tables.

Another difference between natural lignin and DHP is that the relative abundance of the β -1 dimer from thioacidolysis followed by desulfuration was much higher in native woods and isolated lignins than in synthetic DHPs as reported previously (13, 21). There was no clear relationship between the relative proportion of the β -1 dimer and the S/G ratio in natural woods and isolated lignins. However, the β -1 dimer with a GG unit decreased and that with a SS unit increased with an increase in the S/G ratio in natural lignins and DHPs.

The relative frequency of the 5-5 dimer correlated well with the S/G ratio of woods and MWL as well as synthetic DHPs. The 5-5 dimer yield decreased with an increase in the S/G ratio. This is reasonably understood by the fact that two methoxy groups at the 3-C and 5-C positions in the S unit prevent the formation of the 5-5 structure.

When both S and G units were present in lignins, the relative proportion of the 4-O-5 dimer in natural woods and DHPs was higher than that when only a G unit was involved in lignin structure. These results were somewhat different from the β -5 and 5-5 dimers in which the dimer yields decreased clearly with an increase in the S/G ratio. The S unit with 3,5-dimethoxy groups cannot connect with other units at its 5-C position, but it has more chances to couple with other units in its 4-O position. In fact, the relative frequency of the 4-O-5 dimer with an SG unit increased with an increase in the S/G ratio in woods and DHPs.

Conclusions. Interunit linkages of natural and synthetic lignins with various S/G ratios were determined by thioacidolysis and

Table 7. Total Dimer Yields (Micromoles per Gram of Klason Lignin or Isolated Lignin) and the Relative Frequencies with Which They Were Recovered from Thioacidolysis Followed by Desulfuration of Natural Lignins^a

sample	S/G ratio	total dimer yield (GG/GS/SS) ($\mu\text{mol/g}$)	relative frequency of dimers from main interunit linkages (GG/GS/SS)				
			β -5	β - β	β -1	5-5	4-O-5
wood	fir	234 (100/0/0)	38 (100/0/0)	1 (100/0/0)	28 (100/0/0)	28 (100/0/0)	5 (100/0/0)
	magnolia	286 (44/31/25)	29 (44/55/0)	15 (7/0/93)	32 (42/24/34)	13 (100/0/0)	11 (34/66/0)
	birch	232 (33/32/35)	24 (37/63/0)	18 (0/0/100)	40 (31/25/44)	8 (100/0/0)	10 (28/72/0)
	beech	222 (27/29/44)	14 (39/61/0)	28 (0/0/100)	35 (28/27/45)	8 (100/0/0)	15 (26/74/0)
MWL	fir	237 (100/0/0)	35 (100/0/0)	1 (100/0/0)	32 (100/0/0)	28 (100/0/0)	4 (100/0/0)
	birch	220 (46/19/35)	20 (67/33/0)	14 (0/1/99)	52 (39/21/41)	11 (100/0/0)	3 (75/25/0)

^a GG, guaiacyl–guaiacyl dimer; GS, mixed guaiacyl–syringyl dimer; SS, syringyl–syringyl dimer.

Table 8. Total Dimer Yields (Micromoles per Gram of Synthetic Lignin) and the Relative Frequencies with Which They Were Recovered from Thioacidolysis Followed by Desulfuration of Synthetic Lignins (DHPs)^a

expt	DHP SA/(CA + SA)	total dimer yield (GG/GS/SS) ($\mu\text{mol/g}$)	relative frequency of dimers from main interunit linkages (GG/GS/SS)				
			β -5	β - β	β -1	5-5	4-O-5
1	0	210 (100/0/0)	56 (100/0/0)	13 (100/0/0)	7 (100/0/0)	22 (100/0/0)	2 (100/0/0)
2	0.25	355 (41/33/27)	41 (31/69/0)	32 (11/5/84)	3 (70/30/0)	20 (100/0/0)	5 (58/42/0)
3	0.5	311 (27/23/50)	17 (10/90/0)	47 (0/1/99)	5 (17/20/63)	18 (100/0/0)	12 (46/54/0)
4	0.75	395 (5/12/83)	6 (1/99/0)	77 (0/1/99)	8 (1/20/79)	3 (100/0/0)	5 (32/68/0)
5	1	410 (0/0/100)	0	83 (0/0/100)	17 (0/0/100)	0	0

^a GG, guaiacyl–guaiacyl dimer; GS, mixed guaiacyl–syringyl dimer; SS, syringyl–syringyl dimer.

subsequent Raney nickel desulfuration. The frequency of interunit linkages in lignin was highly dependent on the S/G ratio with some exceptions. Basically, similar tendencies were observed in the variation of lignin structures deriving from differences in the S/G ratio, although there were huge differences in the relative frequency of interunit linkages between natural and synthetic lignins. These results suggest that the frequency of interunit linkages in natural lignin can be modified to some extent by biotechnological manipulation of the S/G ratio.

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