Phytochemistry, Vol. 28, No. 5, pp. 1401–1403, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

ON THE MOLECULAR ORIGIN OF THE ALKALI SOLUBILITY OF GRAMINEAE LIGNINS

CATHERINE LAPIERRE, DOMINIQUE JOUIN and BERNARD MONTIES

Laboratoire de Biochimie, INRA, Institut National Agronomique Paris-Grignon, 78850 Thiverval-Grignon, France

(*Received* 26 *September* 1988)

Key Word Index—*Triticum aestivum*; *Secalotriticum*; Gramineae; straw lignin; lignin free phenolic groups; alkali solubility of lignin.

Abstract—The molecular origin of the alkali solubility of Gramineae lignins was investigated, in the case of wheat and triticale straws. The role of the phenolic hydroxyl groups was particularly examined. Straw samples were therefore thoroughly methylated with diazomethane to etherify these groups. It was found that this mild methylation step drastically decreased the dissolution of straw lignins by alkali. The molecular mechanism of such an effect is discussed.

INTRODUCTION

It has been known for a long time that Gramineae lignins have a characteristic alkali solubility, as compared to wood lignins [1]. Such a property is currently reported to explain the beneficial effect of alkali treatment on the nutritional value of cereal straws [2, 3]. Detailed structural data on Gramineae lignins are scarce. In depth investigations on these macromolecules are however the key step to understanding the structural basis of their alkali solubility. Scalbert and co-workers have presented evidence that ferulic acid was ether-linked to wheat alkali-soluble lignin, and might be involved in the solubilization mechanism [4–6].

The functionality of lignin preparations exerts a pronounced effect on their solubility properties [7]. For example, the free phenolic hydroxyl groups in spruce methanol lignin were shown to be responsible for its solubility in sodium hydroxide [8].

Using thioacidolysis of diazomethane–methylated samples, we have recently shown that wheat straw lignins have twice as many free phenolic groups in their guaiacyl units as pine lignins [9]. Similar results have been obtained with various straw lignins and with reference to pine or poplar samples (unpublished results).

In the present work, we have examined whether the content of free phenolic groups in cereal straw lignins is the structural parameter responsible for their alkali solubility. Consequently, straw samples were treated with diazomethane to etherify the phenolic hydroxyl groups present in lignin. Alkali solubilization of liginins from original and diazomethane-methylated straws was then compared, in the case of wheat and triticale.

RESULTS AND DISCUSSION

Lignin content

Before alkali extraction, original and methylated wheat straws have similar Klason lignin [10] contents (Table 1). For wheat straw, it is substantially decreased by the alkali treatment; in contrast, and in the case of the methylated wheat sample, it is slightly enhanced (Table 1).

Table 1. Klason lignin contents in wheat samples (wt %)

	Wheat straw	Permethylated wheat straw
Before and	16.35	16.45
After alkali extraction	14.45	17.00

A more detailed compositional determination of the alkali-treated samples has confirmed the data of Table 1. It was performed by the sulphuric acid fractionation procedure of cell walls, which includes a prehydrolysis step [11]. The amount of material soluble in dilute sulphuric acid, which mainly corresponds to hemicellulose, is similarly reduced in the original and methylated alkali-treated wheat straws; on the other hand, the lignin content measured by this procedure is decreased only for the first sample (Table 2).

These gravimetric data suggest that the alkali treatment causes a substantial leaching of lignins and hemicellulose from original wheat straw, whereas it mainly results in the solubilization of hemicellulose from the methylated sample.

Thioacidolysis of the alkali-soluble material

Thioacidolysis (i.e. solvolysis in dioxane–ethanethiol, with boron trifluoride etherate) of original or methylated lignin samples causes the cleavage of aryl alkyl ether bonds, but does not attack the aryl methyl ether linkages [9, 12]. From the characteristic lignin structure outlined in Fig. 1, it allows the recovery of monomeric compounds from the guaiacyl (G) or syringyl (S) β-aryl ether linked

Table 2. Sulphuric acid fractionation [11] of wheat straw samples (wt %)

	Fraction soluble in 5% H_2SO_4*	Cellulosic residue	Lignin residue	Ash
Original wheat straw	34.3	50.4	13.6	1.7
Wheat straw after alkali extraction	17.8	69.8	11.5	0.6
Wheat straw after CH_2N_2 permethylation then alkali extraction	19.6	65.2	14.6	0.6

*Mainly constituted of hemicellulose.

Table 3. Yields of the main G and S monomeric products recovered from the thioacidolysis of the soda-solubilized material from cereal straw samples

	Wheat straw		Triticale straw	
	Original	CH_2N_2-methylated	Original	CH_2N_2-methylated
G	21.4	3.2	22.1	3.4
S	27.6	3.7	25.1	3.6
Total	49.0	6.9	47.2	7.0

Yields are expressed as μmol of compounds recovered from the material alkali washed from 1 g of straw.*

*For original straw, the quantified G or S products are those with $R^3 = H$ in Fig. 1; For methylated samples, indicated yields correspond to the sum of G or S products with $R^3 = H$ and $R^3 = Me$ in Fig. 1.

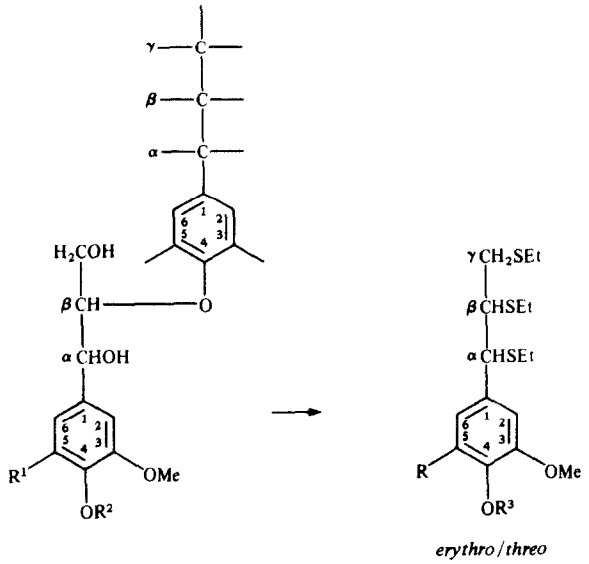

Fig. 1. Thioacidolysis of β-aryl ether linked guaiacyl ($R^1 = H$) or syringyl ($R^1 = OMe$) structures in original or methylated lignins. In original lignins, $R^2 = H$ or Alk and $R^3 = H$. In methylated lignins, $R^2 = R^3 = Me$ when the phenolic group at C-4 is initially free then CH_2N_2-methylated; $R^2 = Alk$ and $R^3 = H$ when this group is initially etherified to another lignin sidechain.

units with free or alkylated phenolic hydroxyl group at C-4, according to the usual lignin nomenclature. These compounds are recovered with a similar high reaction yield from the lignin structures depicted in Fig. 1, whatever the substituents R^1 and R^2.

Thioacidolysis was directly performed on the alkali-soluble material extracted from original and methylated straw samples, in order to compare the amounts of β-aryl ether linked lignin structures present in these extracts that are specifically degraded into the monomeric compounds depicted in Fig. 1. For wheat and triticale samples, the thioacidolysis yields of the alkali extracts are considerably reduced by the methylation step (Table 3). This shows that there is *ca* seven times less β-aryl ether-linked lignin structures dissolved by alkali from the methylated straws than from the original samples.

Both results from lignin determination and thioacidolysis are in good agreement in showing that the solubilization of cereal straw lignins in sodium hydroxide is drastically reduced after a mild methylation step of the straw with diazomethane. As a straightforward hypothesis, it can be suggested that the high content of free phenolic groups previously reported in the guaiacyl units of Gramineae lignins [9] plays a key role in the alkali solubilization of these polymers. When ionized, the free phenolic groups, present in the guaiacyl terminal units of the highly branched Gramineae lignin macromolecules, should efficiently contribute to this solubilization. On the other hand, methylation of these groups with diazomethane should largely prevent Gramineae lignins from dissolving in alkali. Although it seems strongly supported by the experimental data reported here, it is likely that this mechanism is not the only one which might be involved in the alkali solubility of Gramineae lignins.

EXPERIMENTAL

Plant material. Wheat (*Triticum aestivum* L., cv Capitole) and Triticale (*Secalotriticum*, cv Montcalm 118) were harvested at full maturity. Air-dried and ground straws (stem and leaves)

were exhaustively extracted in a Soxhlet apparatus with toluene–EtOH (2:1), followed by 96% EtOH and then H_2O.

CH_2N_2-*methylation.* The CH_2N_2-methylation of extractive-free straws was performed on *ca* 3 g of sample swollen in 30 ml dioxane for one week. Diazomethane soln in 100 ml Et_2O–MeOH (9:1), prepared by the reaction of 2 g of *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (Aldrich) with 10 ml KOH 60% (w/v), was added to the suspension of straw. The yellow suspension was kept for 5 days with magnetic stirring, new ethereal CH_2N_2 being added each day. After the last methylation, the methylated straw was washed with Et_2O, then MeOH and finally freeze-dried.

NaOH *extraction.* For subsequent lignin determinations, extractive-free straw (2.5 g) was stirred in 1.0 M aq. NaOH (75 ml) under N_2 in a sealed vial for 2 hr at 40°. The residue recovered by filtration was washed with 1.0 M NaOH (10 ml), 0.1 M MeCOOH (100 ml) and then water (200 ml) and freeze-dried. For subsequent thioacidolysis of NaOH extract, a lower amount of straw (30 mg) was stirred in 1 ml 1.0 M aq. NaOH under N_2 in a Teflon-lined screw cap glass tube for 2 hr at 40°. An aliquot (0.5 ml) of the NaOH extract was recovered in a 10 ml Teflon-lined screw cap glass tube, by filtration under red. pres., neutralized with 1.0 M aq. HCl and evapd to dryness under red. pres. at 40°, in the same tube.

Gravimetric analyses of the cell wall residues. They were only performed for the wheat samples. The Klason lignin determination was performed from 300 mg of original or alkali-treated wheat straw, according to ref. [11]. The determination of acid-insoluble lignin, cellulosic residue and soluble fraction in 5% H_2SO_4 (% wt) was carried out on the same amount and according to ref. [11], but without MeOH washing of the lignin residue. Standard deviations between triplicate experiments were lower than 10%.

Thioacidolyses. They were directly performed in the glass tube containing the dried material, alkali dissolved from original or methylated wheat and triticale straws. This dried material was resuspended in 5 ml dioxane–ethanethiol (9:1), 0.2 M BF_3 etherate. The thioacidolysis was then allowed to proceed in N_2, with magnetic stirring, at 100° for 4 hr. The general analysis procedure was identical to the one previously described [9]. Thioacidolysis monomeric products were identified by GC-MS and quantified by GC, as their TMSi derivatives according to [12]. The amount of GC internal standard (hexacosane), used for quantification, was 0.1 mg per assay. Standard deviation between 4 independent experiments was *ca* 10% for each straw sample.

REFERENCES

1. Beckmann, E., Liesche, O. and Lehmann, F. (1923) *Biochem. Z* **139**, 491.
2. Jackson, M. G. (1977) *Anim. Feed Sci. Technol.* **2**, 105.
3. Chesson, A. (1981) *J. Sci. Food Agric.* **32**, 745.
4. Scalbert, A., Monties, B., Lallemand, J. Y., Guittet, E. and Rolando, C. (1985) *Phytochemistry* **24**, 1359.
5. Scalbert, A., Monties, B., Rolando, C. and Sierra-Escudero, A. (1986) *Holzforschung* **40**, 191.
6. Scalbert, A. and Monties, B. (1986) *Holzforschung* **40**, 249.
7. Whiting, P. and Goring, D. A. I. (1982) *Pap. Puu* **64**, 592.
8. Brauns, F. E. and Brauns, D. A. (1960) *The Chemistry of Lignin*, p. 247. Academic Press, New York.
9. Lapierre, C., Monties, B. and Rolando, C. (1988) *Holzforschung* **42**, 409.
10. Effland, M. E. (1977) *Tappi* **60**, 143.
11. Monties, B. (1984) *Agronomie*, **4**, 387.
12. Lapierre, C. and Rolando, C. (1988) *Holzforschung* **42**, 1.