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A Probable Lignin Structure by Conformational Analysis

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ABSTRACT

This paper reports a simple method of conformational analysis of the most stable known dimers from lignin. The lignin structure is probably situated at the edge of the cellulose unit and is not likely to be inserted between the cellulose layers due to their strong H-bonding. However, this lignin pattern is more likely intermingled with hemicelluloses which also could possess cellulose-like structures, i.e. ribbon in one direction, and sheets in another direction.

INTRODUCTION

Lignins are the most complex polymers of the natural high molecular weight materials, and work has been underway for more than 80 years for the elucidation of their structure. Lignins cannot be isolated without changes in their structures and this has prevented the elucidation of their structures for a long time.

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Although considerable research effort was given to the nature of lignin biodegradation, relatively little was known about the chemistry of lignin biodegradation until recently. However, considerable progress has been made in very recent years on the chemistry of lignin degradation in woods by fungi (Kirk & Chang, 1974, 1975; Chen *et al.*, 1982*a*, *b*, 1983*a*, *b*; Chua *et al.*, 1982; Tai *et al.*, 1982, 1983; Terazawa *et al.*, 1983). *Trichoderma* is not necessarily the most successful coloniser of lignocellulosic materials, possibly because it would appear to lack the ability to degrade lignin (Blanch & Wilke, 1983). On the contrary, *Aspergillus fumigatus* is known to be a proven lignin degrader (Bisaria & Glosh, 1981; Miltein *et al.*, 1983) and therefore has the potential to separate lignin from cellulose and hemicellulose.

Recently, Gold *et al.* (1983) successfully formed viable protoplasts of *P. chrysosporium* which were able to carry out intraspecies protoplast fusion, regenerating stable recombinant organism. Protoplast fusion is a recombinant DNA technique for breeding new hybrid lignin-degrading strains.

New enzyme studies have continued and current research with ligninolytic streptomyces is emphasizing biochemical and genetic studies. Pettey and Crawford (1984) have utilized interspecies and intraspecies protoplast fusion in order to generate stable enhanced lignin-degrading recombinants from *S. viridosporus.*

The structure of the phenylpropanoid lignin polymer and its importance to the biospheric carbon/oxygen cycles have been widely discussed (Crawford, 1981; Higuchi, 1982; Janshekar & Fietcher, 1983). Adler (1977*a*) and Nimz (1974) have published detailed schematic representations of softwood (spruce) and hardwood (birch) lignins. Lignocellulosic materials comprise as much as 95% of the earth's land-produced biomass (Janshekar & Fietcher, 1983). While lignins from most hardwoods and softwoods contain a predictable complement of phenylpropane units derived from corniferyl, sinapyl and coumaryl alcohol monomers, grass lignins also have these, but are also complexed with significant quantities of esterified phenylpropanoid acids (Crawford, 1981; Broda & Paterson, 1983).

Because lignin constitutes approximately 30% of a lignocellulosic feedstock, it represents an important hydrolysis by-product. Potentially, a large variety of products can be produced from lignin (Janshekar & Fietcher, 1983) such as chemical intermediates (benzene, phenols, etc.), hydrolysates (phenols, catechols, etc.), pyrolysates (acetic acid, methane, etc.), polymeric lignins (adhesive, stabilizer, anti-oxidant, etc.).

We, in this paper, describe a simple method of conformational analysis of the most stable known dimers from lignin.

EXPERIMENTAL

Conformational analysis method

We firstly arranged a stable molecule by the symmetry assumption, *cis* for plane of symmetry, *trans* for centre of symmetry, and helix for rotation reflexion through an angle $2\pi/n$ about some axis. Then, we found the Cartesian coordinates of each atom of the molecule and connected each molecule together to form a polymer. All matrix calculations were resolved through a numeric solution using a computer programme made for a HP41CV calculator.

The coordinates X can be rotated to another system X′ as follows:

$$\mathrm{X} = B - A \quad \text{coordinates of point B after translation}$$

$$\mathrm{X}' = |A|\mathrm{X} \qquad |A| = \alpha\beta\gamma$$

$$\alpha = \begin{vmatrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{vmatrix} \beta = \begin{vmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \\ 0 & -\sin\theta & \cos\theta \end{vmatrix} \gamma = \begin{vmatrix} \cos\psi & 0 & \sin\psi \\ 0 & 1 & 0 \\ -\sin\psi & 0 & \cos\psi \end{vmatrix}$$

where

α = the matrix rotation factor, rotating ϕ in the xy plane along the Z axis;
β = the matrix rotation factor, rotating θ in the yz plane along the X axis;

and

γ = the matrix rotation factor, rotating ψ in the zx plane along the Y axis.

The original cartesian coordinates of atoms A and B with other atoms can be transformed back as follows:

$$\mathrm{X} = |A|^{-1}\mathrm{X}' \qquad |A|^{-1} = \text{inverse matrix of } |A|$$

$$B = \mathrm{X} + A$$

The two programs of $|A|$ and $|A|^{-1}$, cited above, namely '*XYZ*' and '*XYZI*', were made in order to rotate angle ϕ in the XY plane and angle θ in the YZ plane ($\psi = 0$) as follows:

$$|A|^{-1} = \begin{vmatrix} \cos\phi & -\cos\theta\sin\phi & \sin\phi\sin\theta \\ \sin\phi & \cos\theta\cos\phi & -\cos\phi\sin\theta \\ 0 & \sin\theta & \cos\theta \end{vmatrix} \text{ as '}XYZI\text{'}$$

$$|A| = \left| \begin{matrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi\cos\theta & \cos\phi\ \cos\theta & \sin\theta \\ \sin\phi\sin\theta & -\cos\phi\sin\theta & \cos\theta \end{matrix} \right| \text{ as `}XYZ\text{'}$$

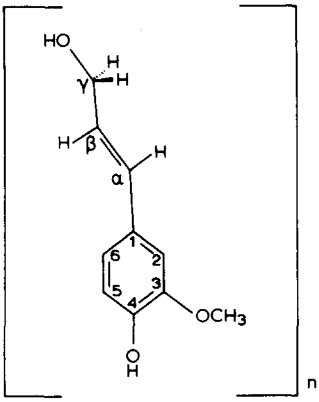
A program $|A'|$ and $|A'|^{-1}$ to rotate θ in the YZ plane and ψ in the XZ plane ($\phi = 0$), is shown as follows: $|A'| = \beta\gamma$

$$|A'| = \left| \begin{matrix} \cos\psi & -\sin\theta\sin\psi & \cos\theta\sin\psi \\ 0 & \cos\theta & \sin\theta \\ -\sin\psi & -\sin\theta\cos\psi & \cos\theta\cos\psi \end{matrix} \right| \text{ as `}YZX\text{'}$$

$$|A'|^{-1} = \left| \begin{matrix} \cos\psi & 0 & -\sin\psi \\ -\sin\psi\sin\theta & \cos\theta & -\sin\theta\ \cos\psi \\ \cos\theta\ \ \sin\psi & \sin\theta & \cos\theta\ \ \cos\psi \end{matrix} \right| \text{ as `}YZXI\text{'}$$

RESULTS AND DISCUSSION

Gymnosperm lignin is believed to be composed mainly of mostly corniferyl alcohol units. This alcohol is linked to the benzene ring through an ethylenic bond in a distance of 1·39 Å (I). The olefinic bond in corniferyl alcohol is represented by XCH═CHX′ where X = CH_2OH and X′ = guaiacyl ring are mostly in *trans* form. As a result, the centre of the gravity of this molecule could be near the ethylenic bond, which



(I)

n = ~68

would not be true if X and X′ were in the *cis* configuration. The HX and HX′ groups projecting out from the double bond are coplanar. The angle between the olefinic bond and C–H, C–X, and C–X′ bonds are all 122°, whilst the angles in the H–C–X and H–C–X′ bonds are 116°.

According to previous proposed lignification mechanism (Higuchi, 1985*a*, *b*; Hwang, 1985) a radical initiates addition polymerization by the attack on the double bond *beta* carbon of corniferyl alcohol monomer, resulting in hydrogen abstraction and peroxide reduction in the presence of peroxidase. The polymerised product, dimer or X-mer, which still has the phenolic –H, oxidizes and polymerizes further in the same manner to form lignin.

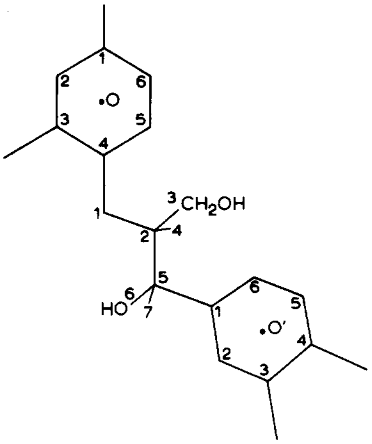
Four dimers are produced, namely: DL-pinoresinol, 1-(4-guaiacyl)-2-(6-guaiacyl)propane-1,3-diol, 1,2-diguaiacylpropane-1,3-diol and guaiacyl-glycerol-β-corniferyl ether. The last one is formed in the largest amount. After the phenoxy radical attack on the double bond of a monomer XHC=CHX′ the produced radical is terminated by a hydroxyl radical. A stable staggered conformation is attained rather than a skew or eclipsed one. The XYH atom or group has the bond angle 109·5° projecting out from the end of the C–C bond, as does the HX′Y′. Each atom of HXY or HX′Y′ is located 120° apart and can be rotated freely along the C–C bond. The guaiacyl ring and phenoxy ring as well as CH_2OH and OH are in the *anti* form. The phenoxy ring and OH as well as the phenyl ring and CH_2OH are in the *gauche* and *trans* form, so they have a centre of symmetry.

Probable dimers forming lignin

The β-O-4 linkage can be either D-(II) or L-(III) configurations. The bond angle of C–O–C is assumed to be 109·5° as that found for the C–C–C bond angle. The attacking radical could be on the plane in *cis* or *trans* position with respect to the original guaiacyl ring. The *cis* form is preferable, because another plane of symmetry could be drawn, bisected with respect to both guaiacyl rings.

The second dimer, β-5 linkage can be either in the D- or L-(IV) configuration and it is found in lower amounts than β-1, β-β', and β-O-5 linkages. The most stable form is with the phenyl ring on the same plane and in the *anti* form.

The third dimer, β-1 linkage is either in D-(V) or L- configuration. This dimer has two guaiacyl rings in *anti* form; CH_2OH and OH are also in *anti* form. The stable form has two benzene rings on a plane with respect to the C–C main backbone. Then, a centre of symmetry of two phenyl rings can be fitted between the cellulose layers.



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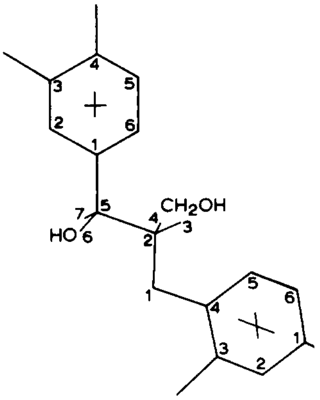

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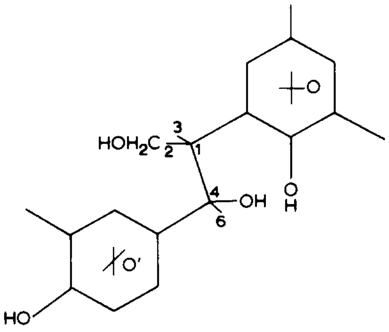

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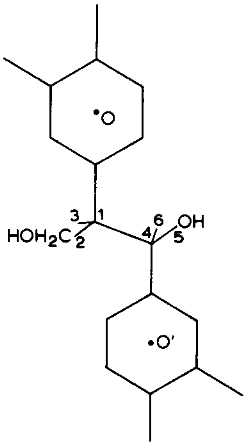

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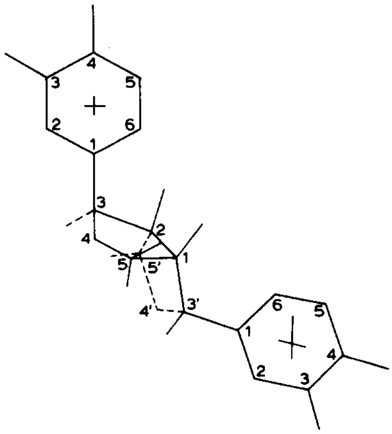


(VI)

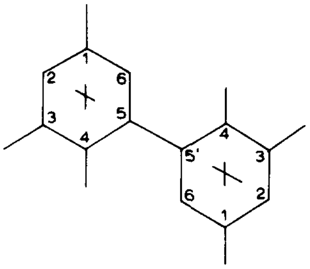
The fourth dimer, β–β' linkage (VI) is in either D- or L-configuration with two guaiacyl rings in equatorial position (Ludwig *et al.*, 1954), which is the stable form. At the same time coplanar biphenyl rings are formed.

Both 5–5′ (VII) and 5–O–4 (VIII) modes are the fifth and sixth dimers which can be either in D- or L- configuration. The stable form is two phenyl rings on the same plane to fit in cellulose layers.

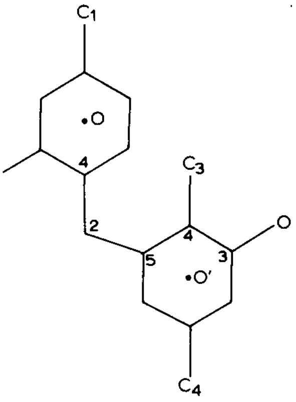
The α–6′ linkage (IX) is formed in a non-common manner, where the hydroxyl radical has not terminated on the *alpha* carbon. Then, it is possible to shift this radical to the C–6 position and to combine the other radical at the *alpha* carbon.

Linkages between dimers and cellulose layers

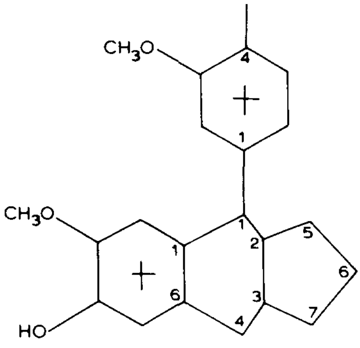
It is known that the distance between the cellulose layers is 2·7–3·0 Å. Since the CH_2OH of cellulose forms an ether bond with the *alpha*



VII

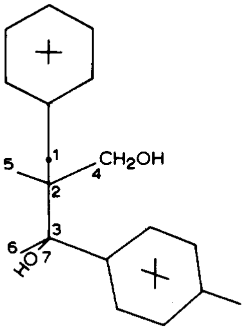


(VIII)

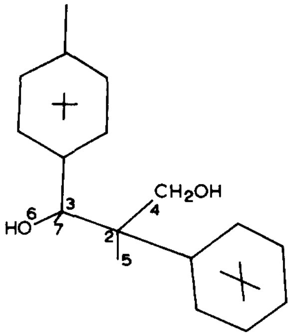


IX

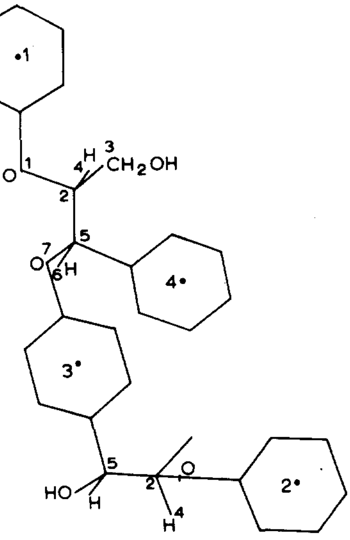
carbon of the α–O–4 model in the lignin chain, it can be imagined that the phenyl rings of lignin are situated at half distance between the cellulose layers. From this study it can be concluded that if two benzene rings of the first set are inserted between cellulose layers, the benzene rings of the second set cannot exist 1·4 Å apart with continuously connecting oligomers. It is most probable therefore that one of the phenyl rings, the β–O–4 model, is not in the same plane as the other phenyl ring. This kind of step polymer (X and XI) has a centre of symmetry that can be developed as the *trans* form.



(X)



XI



(XII)

In structure XII a plane of ring 1 and 4 is 1·32 Å apart with respect to the oxygen of the α–O–4 linkage. This means that there is 1·32 Å between the plane of ring 1 and the plane of ring 3 or the plane of the cellulose sheet (see Fig. 1). A plane of ring 1 is 2·75 Å distant from the plane of ring 2.

In the peroxidase reaction, which includes phenolic oxidation and hydrogen peroxide reduction, the radical produced in the β–O–4 linkage in the D- configuration can be dimerized via reaction with the α–O–4 linkage of another aroxyl radical in the L- configuration. On the other hand, the radical produced in the β–O–4 linkage in L- configuration can be dimerized with the α–O–4 linkage in the D- configuration (XII).

The branch chain β–O–4 mode in the α–O–4 combination should have a structure similar to a step ladder. It can be assumed that the C–O bond is 1·51 Å, bond angle 109·5 ± 5°, and conformation angle 60 ± 5°. The lower bond angle and the higher conformation angle bring out the high pitch (Table 1).

Each unit that has been found in a lignin molecule contains 2 phenyl rings and can connect with each other as shown in Table 2 (Goldstein, 1951).

From the Freudenberg model (see Fig. 2) the monomer or X-mers have reactive functional units with an ethylenic bond as a head (H), and the

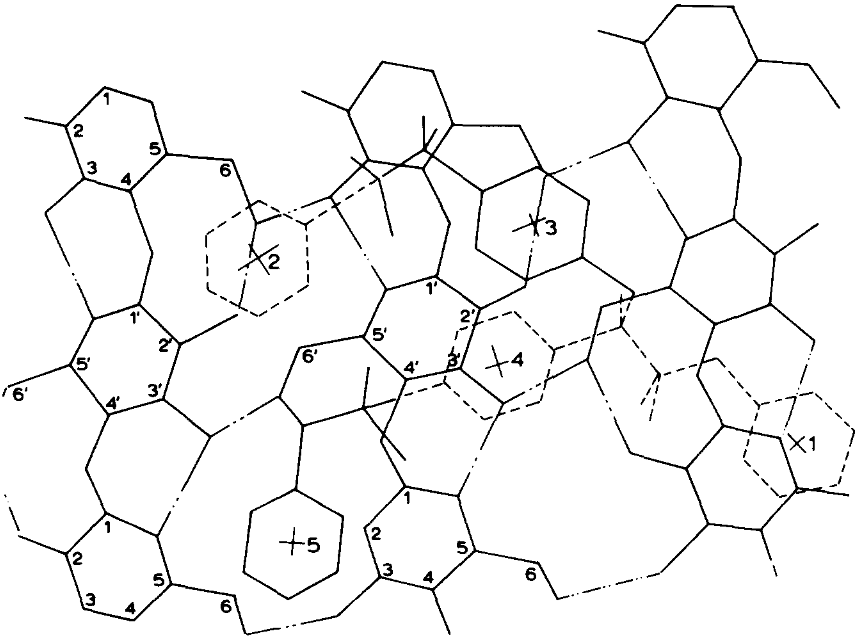


Fig. 1. α–O–4 mode fitted in cellulose layers.

TABLE 1
Bond and Conformation Angles of β-O-4 Dimer

Bond angle	*Conformation angle*	*X*	*Y*	*Z*
109·5	60	1·2327	0·7117	0·5041
104·5	65	1·3249	0·6138	0·3781

TABLE 2
The Connections Between Benzene Rings of the Dimers

No. of rings	*Connection type*	*Configuration*	*Mode*
1–4	T–H	D	β-O-4
4–5	T–H	D	β-O-4 Stepped
5–6	T–H	D	β-O-4 Stepped
6–7	T–T	D	5-O-4
7–8	H–T	D	β-O-4
8–9	H–H	D	β-β′
9–10	T–T	D	5-5′
10–12	H–T	L	β-O-4
12–15	H–T	L	β-O-4 Stepped
15–16	H–T	L	β-O-4 Stepped
16–19	H–T	L	β-O-4
12–13	H–T	L	α-O-4
13–14	H–T	D	β-O-4 Stepped
15–17	H–T	D	α-O-4
17–18	H–T	D	β-5
3–2	H–T	L	β-O-4 Stepped
4–3	H–T	L	β-O-4

phenolic-H and the 5-carbon adjacent phenol unit as a tail (T). The connections are usually head–tail or tail–head as in β-O-4 and β-5′; head–head as in β-β′; and tail–tail as in 5-5′ and 5-O-4 (Table 2). In Fig. 2 it can be observed that some linkages are connected but not shown. However, if the main chain is kept in the D mode, the branch chains must be in the L mode. We would propose that a plant makes D and L configurations in pairs.

A comparison between the proposed model and others

It is possible that cellulose is endowed with a helical form (Viswansham & Shenda, 1971). However, the chain folding becomes a problem in the

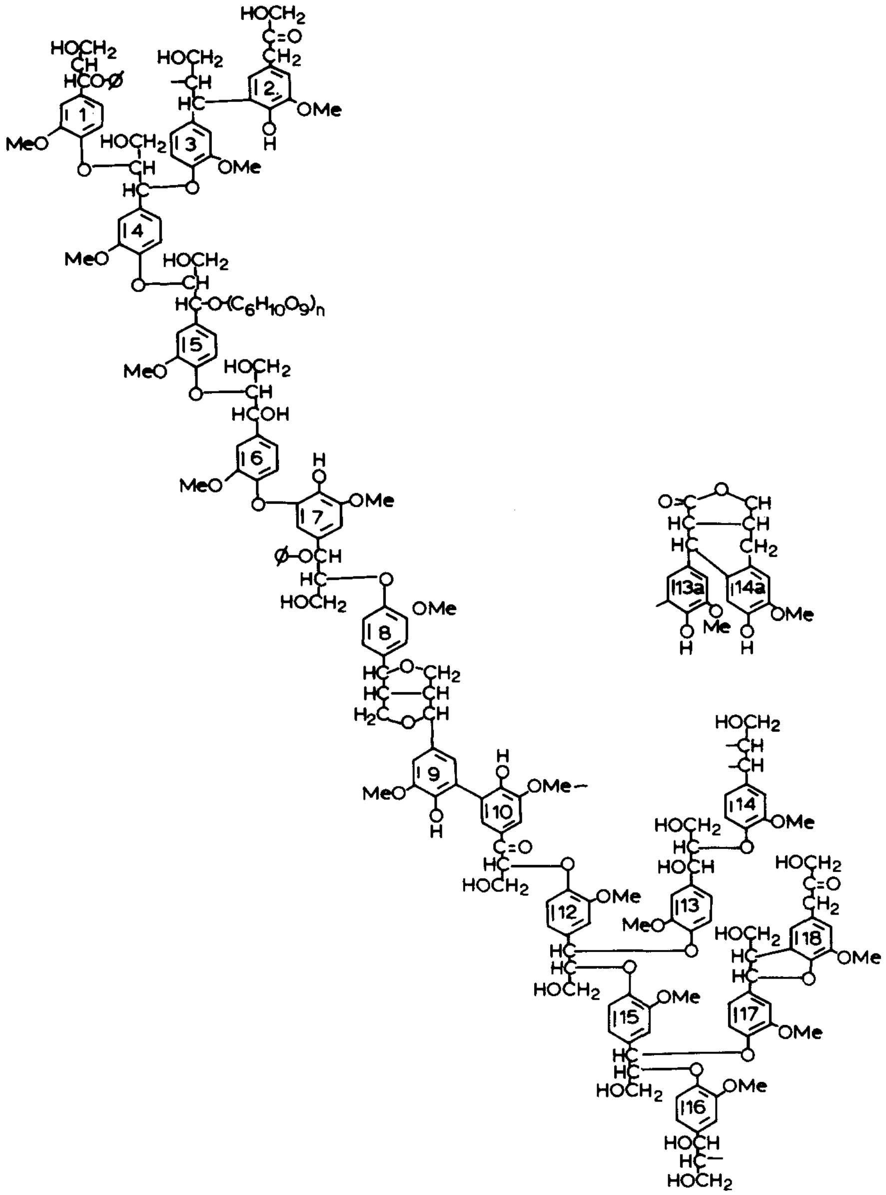


Fig. 2. Revised Freudenberg's lignin model.

sheet form. We can assume that the benzene rings of lignin are on the same plane (see Fig. 3).

From the Freudenberg model (see Fig. 4), we rearrange the benzene rings (see Fig. 5), and then make the calculation of the Cartesian coordinates.

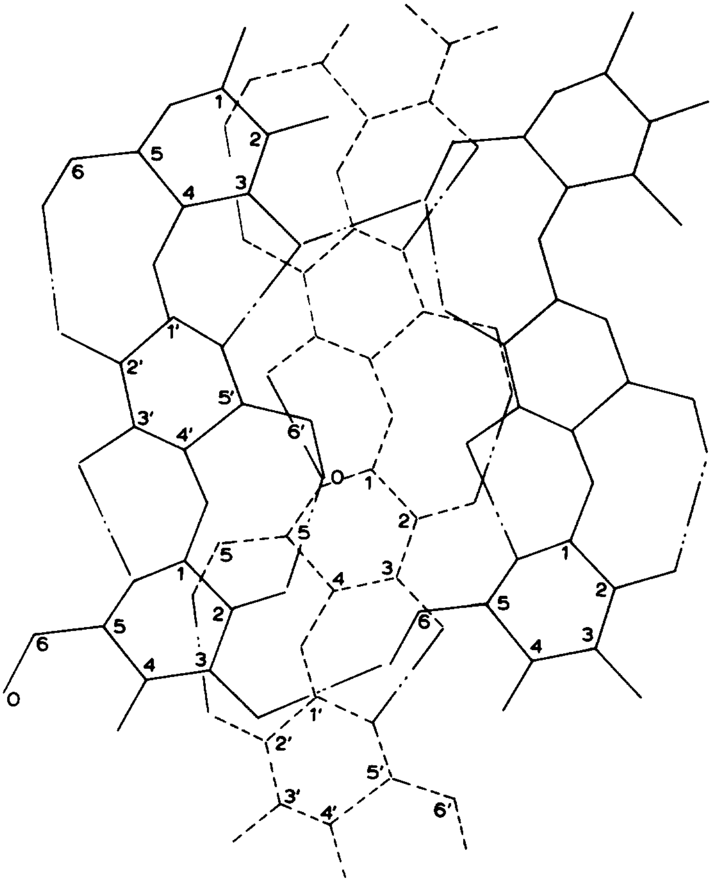


Fig. 3. Cellulose.

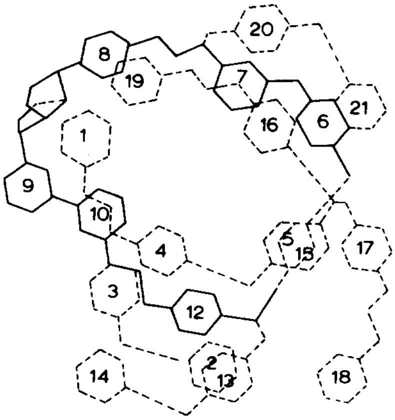


Fig. 4. Freudenberg's lignin model interwoven among cellulose layers.

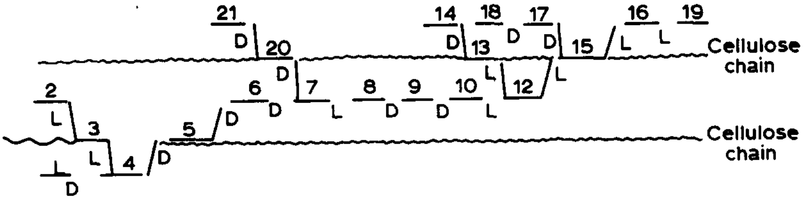


Fig. 5. Proposed lignin model between cellulose layers.

TABLE 3
List of the Renewed Adler's Lignin Model

No. of rings	*Connection*	*Configuration*	*Mode*
1-2	T-H	D	β-O-4 Stepped
2-3	T-H	D	β-O-4 Stepped
3-4	T-H	D	β-5
4-5	T-H	D	β-O-4
5-6	T-T	D	5-5′
6-7	H-T	L	β-O-4 Stepped
7-8	H-T	L	β-O-4 Stepped
8-9	H-H	L	β-1
8-10	T-T	L	5-O-4
10-11	H-H	D	β-β′
11-12	T-T	D	5-5′
3-13	T-H	L	α-O-4
13-14	T-H	D	β-O-4 Stepped
14-15	H-T	L	β-O-4
15-17	T-H	D	β-O-4

After necessary correction in compliance with the proposed mechanism and conformation study of Adler's lignin model (Adler, 1977*b*), which refer to the numbering of the benzene rings (Table 3 and Fig. 6), we have made a rearrangement among the cellulose layers as shown in Fig. 7.

CONCLUSION

Lignin is a statistical polymer which cannot be studied by the X-ray method due to its amorphous structure. From the revised Freudenberg's model, it can be concluded that the α-5 linkage between benzene rings 2 and 3 is highly impossible, because if one set of benzene rings lies between the cellulose layers, the other set of benzene rings, which

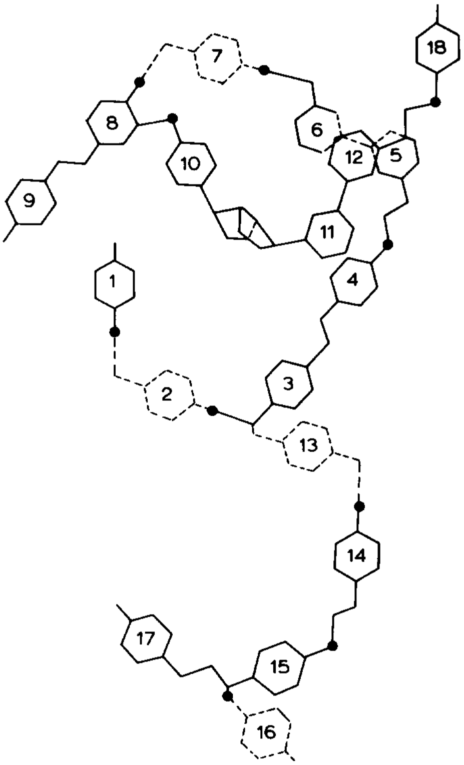


Fig. 6. Adler's lignin model interwoven among cellulose layers.

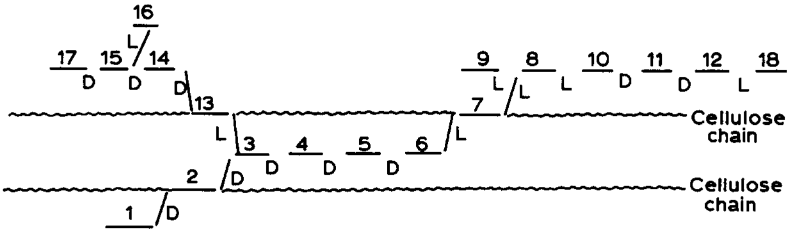


Fig. 7. Rearranged lignin model before cellulose layers.

projects at 109·5°, will not fit between the cellulose layers. The β–1, β–5 linkages should also be included in the lignin structure, because the former has $\Delta\text{L} = 2{\cdot}43$ (–β). The latter has $\Delta\text{L} = 1{\cdot}79$ (–β) which enables further addition–polymerization reactions. The various linkage formations depend on the ΔL value, on the functional groups' surroundings and on the space available to react. The lignin model has guaiacyl rings sandwiched between the cellulose layers. The β–O–4 formation is present in the largest amount. If the glucose units of a cellulose chain are in the same plane, the guaiacyl group can sometimes pass through the cellulose layers. With the β–O–4 model a staircase conformation is formed which allows guaiacyl rings to become inserted between the neighbouring 2 glucose units.

It has been concluded that the lignin structure is probably situated at the edge of the cellulose unit and is likely to be inserted between the cellulose layers due to the strong lignin–cellulose H-bonding. However, this lignin pattern is more likely intermingled with hemicelluloses which also could possess cellulose-like structures, i.e. ribbon in one direction, and sheets in another direction.

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