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## Effective combinations of functional groups in chemically modified kraft lignins for reduction of aluminum toxicity

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**Abstract** Plant growth tests were performed with radish (*Raphanus sativa* var. *radicula* Pers.) in culture solutions containing low molecular weight compounds in the presence of aluminum to determine the types of functional groups in kraft lignin (KL) modified with ozone and alkali that contributed to reducing aluminum toxicity. The low molecular weight compounds used in this study contained carboxyl, formyl, methoxyl, alcohol hydroxyl, and phenolic hydroxyl groups. The compounds that had adjacent two carboxyl groups (oxalic acid), carboxyl/alcohol hydroxyl groups (glycolic acid), or carboxyl/formyl groups (glyoxylic acid) were effective in reducing aluminum toxicity. Malonic acid, having two carboxyl groups, also reduced aluminum toxicity. The ability of ozone-treated KLs to reduce aluminum toxicity was considered to be partly due to these chemical structures. Protocatechuic acid, having two adjacent phenolic hydroxyl groups, was also effective in reducing aluminum toxicity. This indicated that the effectiveness of the alkaline-treated KL was partly due to its catechol structure.

**Key words** Aluminum toxicity · Acid soil · Combination of functional groups · Lignin · Soil-conditioning agent

### Introduction

Aluminum is the third most abundant element after silicon and oxygen in the earth's crust. Most aluminum exists in

forms that are harmless to plants at neutral pH, but photo-toxic aluminum ion is eluted into soil by acidification. Aluminum toxicity is known as the major limiting factor of plant growth in acid soil,<sup>1–7</sup> which comprises large areas of the world's land.

Bartlett and Riego<sup>8</sup> reported that maize root did not exhibit the symptom of aluminum toxicity in plant growth tests with Al-citrate and Al-ethylenediaminetetraacetic acid (EDTA) solutions, although it was severely injured in an aqueous solution of Al(OH)<sub>2</sub>Cl. The same results were also obtained by plant growth tests of tomato, buckwheat, soybean, rice, and barley with Al-citrate solution.<sup>8</sup> The complexes of Al-citrate (1:1) and Al-oxalate (1:3) were isolated from cell saps of hydrangea and buckwheat, respectively. Plant growth tests showed that these Al complexes did not inhibit root elongation of corn.<sup>9–12</sup> The complex of Al-malate (1:6–8) was also reported to alleviate the inhibitory effect of aluminum on root elongation.<sup>3</sup>

The findings described above show that complexation with aluminum plays an important role in reducing aluminum toxicity, but combinations of two functional groups that form complexes with aluminum have not been fully elucidated. It is known that complexation with aluminum occurs predominantly with functional groups containing oxygen.<sup>3,13</sup> Hue et al.<sup>14</sup> reported that some acids could reduce aluminum toxicity if they have two carboxyl groups, carboxyl/alcohol hydroxyl groups, or carboxyl/phenolic hydroxyl groups positioned in a manner suitable for a five-membered or six-membered chelate structure with aluminum. However, Schnitzer and Skinner<sup>15</sup> reported that alcohol hydroxyl groups did not take part in complex formation with aluminum after finding that selective blocking of alcohol hydroxyl groups did not affect metal retention capacity of soil organic matter. They also reported that selective blocking of phenolic hydroxyl groups significantly reduced metal retention capacity of soil organic matter.<sup>15</sup> Tam and McColl<sup>16</sup> reported that compounds having adjacent carboxyl/phenolic hydroxyl groups and two phenolic hydroxyl groups had high aluminum binding affinity at pH 4.5. However, Ofei-Manu et al.<sup>17</sup> reported that the

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aluminum binding capacity of compounds having a catechol structure were much lower at pH 4.5 than at pH 7.0. It was reported that salicylic acid has a high stability constant for complexation with aluminum,<sup>13</sup> but it was moderately effective in reducing aluminum toxicity at pH 4.8.<sup>14</sup>

The authors believe that lignin has advantages as an agent for remediation of extensive tracts of acid soil, because lignin is the second most abundant biomass component after cellulose, is reproducible, and forms a part of forest soil as humic substance. The use of lignin derivatives as an acid soil conditioning agent, especially focused on reduction of aluminum toxicity, has been studied in detail.<sup>18–25</sup> Katsumata et al.<sup>18–21</sup> and Wang et al.<sup>22–23</sup> reported that lignins modified with alkaline oxygen or radical sulfonation reduced aluminum toxicity. We reported in previous articles that kraft lignins (KLs) modified by alkaline treatment and ozone oxidation reduced aluminum toxicity.<sup>24,25</sup> It is assumed that reduction of aluminum toxicity with ozone-treated KL is, at least partly, due to complexation with aluminum.

Eriksson and Gierer<sup>26</sup> reported that the reaction of aromatic and olefinic moieties of lignin model compounds with ozone can be interpreted in terms of the Criegee mechanism. According to this mechanism, carbonyl, carboxyl, and ester groups were formed from aromatic or aliphatic double bond moieties of lignin model compounds by ozone oxidation. In the present study, plant growth tests were carried out in culture solutions containing low molecular weight compounds having carboxyl, formyl, alcohol hydroxyl, phenolic hydroxyl, and methoxyl groups to determine why KLs modified by alkaline treatment and ozone oxidation reduce aluminum toxicity.<sup>24,25</sup> The effective combinations of functional groups in reduction of aluminum toxicity with modified KLs are discussed.

## Materials and methods

### Materials

Glycolic acid, glycerol, glyoxylic acid monohydrate, DL-glyceraldehyde, acetic acid, oxalic acid, malonic acid, phthalic acid, and protocatechuic acid were purchased from Wako (Osaka, Japan). Vanillic acid and *p*-hydroxybenzoic acid were purchased from Sigma-Aldrich (Tokyo, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively. These compounds were used directly for plant growth tests without further purification. The compounds were dissolved in nutrient solution prepared for plant growth tests.

### Plant growth test

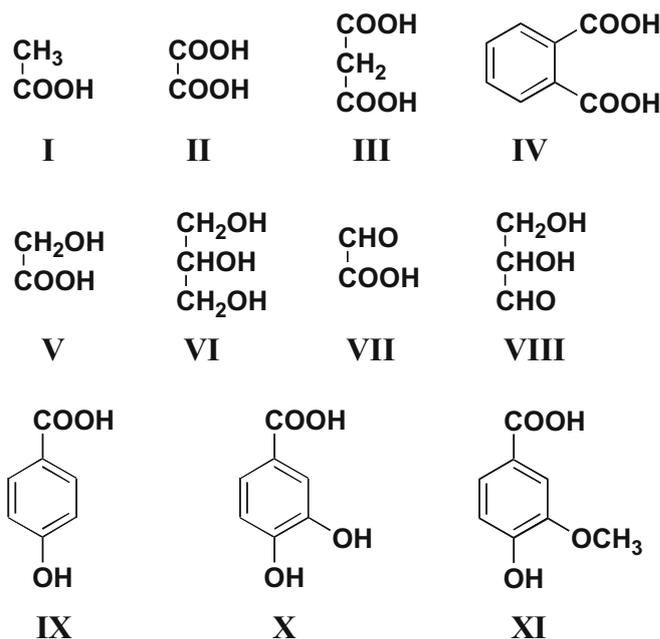
Plant growth tests were conducted as reported in the literature<sup>18–23</sup> with some modifications, and detailed a method was reported in a previous article.<sup>24</sup> Seeds of radish (*Raphanus sativa* var. *radicula* Pers.) were sterilized in 70% ethanol aqueous solution for 30 s and then in 1% sodium hypochlorite solution for 10 min. Ten young seedlings were transplanted on a nylon mesh holder that was floating on 700 ml

of the hydroponic solution. A solution with 1.2 mM CaCl<sub>2</sub> at pH 4.8 was used as nutrient solution. The plant growth test was carried out in the presence and absence of AlCl<sub>3</sub> (37.5 μM) and low molecular weight compounds (18.8, 37.5, and 112.5 μM) at 23.5°C with continuous air bubbling. The pH of each hydroponic solution was adjusted to pH 4.8 with dilute HCl and/or NaOH solution two times a day during the plant growth test. The primary root lengths were measured after a 3-day growth period. Abnormal values were removed using Dixon's Q method (*n* = 10).<sup>27</sup> The results were evaluated statistically by the *t*-test method for assessment of significance.<sup>27</sup>

## Results and discussion

Radish (*Raphanus sativa* var. *radicula* Pers.) was used in our plant growth test because it has been used to evaluate the reduction of aluminum toxicity with modified kraft lignins.<sup>18–25</sup> The solution containing CaCl<sub>2</sub> was used as basal solution in the plant growth tests to avoid interaction between Al and other nutrients such as phosphorus.<sup>11</sup> The chemical structures of the compounds used in this study are shown in Fig. 1. The amounts of compounds used in the plant growth tests were 0.5, 1.0, and 3.0 molar equivalents based on the molar content of AlCl<sub>3</sub>. These compounds were soluble in hydroponic solution and they did not exhibit any toxic effect according to plant growth tests in the absence of aluminum.

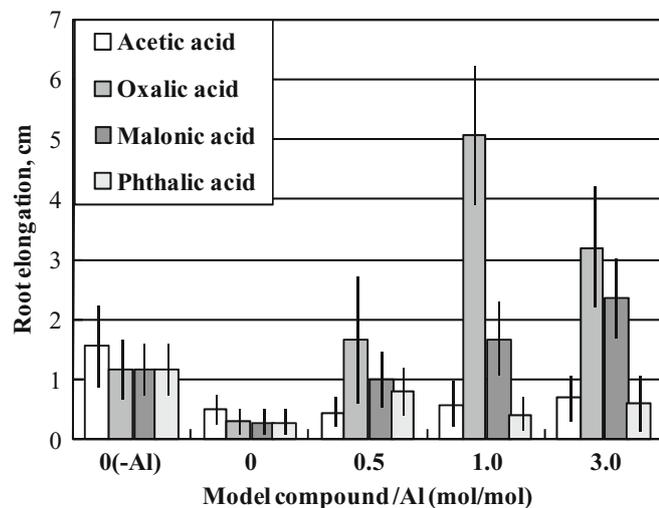
At first, plant growth tests were carried out in the culture solutions containing acetic, oxalic, malonic, and phthalic acids (I–IV in Fig. 1) to confirm the importance of the rela-



**Fig. 1.** Chemical structures of the compounds used in this study. **I**, Acetic acid; **II**, oxalic acid; **III**, malonic acid; **IV**, phthalic acid; **V**, glycolic acid; **VI**, glycerol; **VII**, glyoxylic acid; **VIII**, glyceraldehyde; **IX**, *p*-hydroxybenzoic acid; **X**, protocatechuic acid; **XI**, vanillic acid

tive position of carboxyl groups for reducing aluminum toxicity. The results are shown in Fig. 2. Recoveries of root elongation up to the level of the condition without Al [0(-Al)] were observed with oxalic and malonic acids, showing that they were effective in reducing aluminum toxicity. Hue et al.<sup>14</sup> reported that some acids could be Al detoxifiers if they could form five-membered or six-membered chelate rings with aluminum. Oxalic and malonic acids have two carboxyl groups suitably positioned to make five-membered or six-membered rings with aluminum. Acetic and phthalic acids had no effect in reducing aluminum toxicity. Acetic acid has only one carboxyl group and is not able to complex with aluminum. Seven-membered ring structures, such as that found in the aluminum complex by two carboxyl groups in phthalic or succinic acid, have low stability because of steric hindrance.<sup>13</sup> It should be noted that root elongation was accelerated with oxalic and malonic acids. These phenomena have also been observed in the case of KLs oxidized with alkaline oxygen or ozone.<sup>20,24,25</sup> It is not clear at present why root elongation was accelerated with these compounds. It was reported in previous articles that the content of oxalic acid increased in KL as the ozone treatment progressed.<sup>24,25</sup> Malonic acid was detected as one of the minor reaction products in the ozone-treated KLs by gas liquid chromatography (GLC) after trimethylsilylation.<sup>24,25</sup>

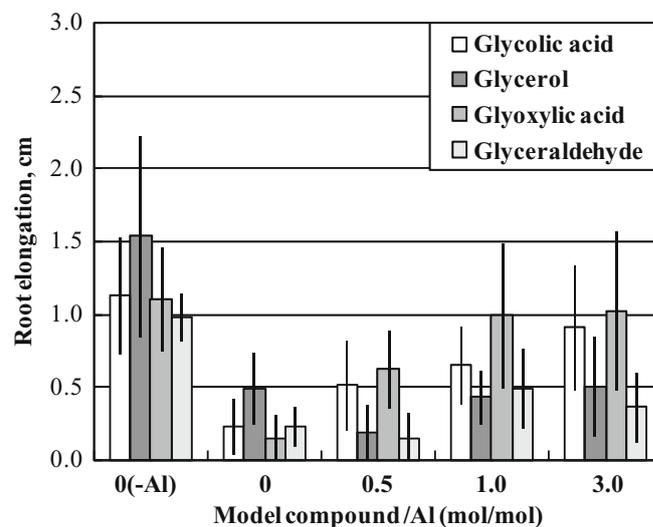
Next, plant growth tests were carried out in the culture solutions containing glycolic acid, glycerol, glyoxylic acid, and glyceraldehyde (V–VIII in Fig. 1) to confirm the contributions of carbonyl groups and alcohol hydroxyl groups in ozone-treated KL and alkaline-treated KL in reducing aluminum toxicity. The results are shown in Fig. 3. It was observed that root elongation was recovered up to the level of the condition [0 (-Al)] with glycolic acid at the dosage of 112.5  $\mu\text{M}$  and glyoxylic acid at dosages of 37.5 and 112.5  $\mu\text{M}$ .



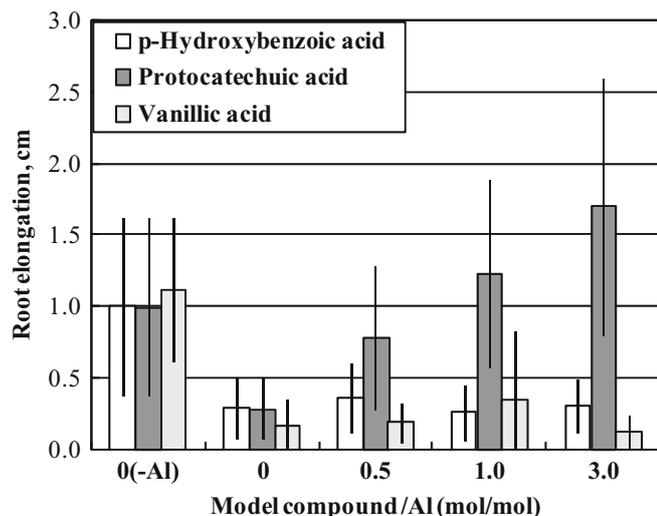
**Fig. 2.** Effect of acetic acid, oxalic acid, malonic acid, and phthalic acid on root elongation of radish (*Raphanus sativa* var. *radicola* Pers.). Hydroponic solution contained 1.2 mM  $\text{CaCl}_2$  and 37.5  $\mu\text{M}$   $\text{AlCl}_3$  at pH 4.8. Dosages of compounds were 18.8, 37.5, and 112.5  $\mu\text{M}$  and that of  $\text{AlCl}_3$  was 37.5  $\mu\text{M}$ . Growth period was 3 days ( $n = 10$ ). 0(-Al), hydroponic solution without aluminum; error bars, standard deviation

These results show that the adjacent carboxyl/alcohol hydroxyl groups and carboxyl/formyl groups contribute to reduction of aluminum toxicity. Effective combinations of functional groups in reducing aluminum toxicity have not been fully elucidated. Combination of carboxyl/alcohol hydroxyl groups was reported to be effective in reducing aluminum toxicity.<sup>14</sup> However, the contribution of carbonyl groups to reducing aluminum toxicity has not been clarified to date. This is the first report of the effectiveness of adjacent carboxyl/formyl groups in reducing aluminum toxicity. Glycerol, having adjacent two alcohol hydroxyl groups, did not exhibit any effect, confirming the findings of Schnitzer and Skinner<sup>15</sup> that alcohol hydroxyl groups did not participate in complex formation with aluminum. Glyceraldehyde, which has adjacent formyl/alcohol hydroxyl groups, also showed no effect in reducing toxicity. It was reported that compounds having carbonyl groups were formed by ozone treatment of lignin model compounds and KL.<sup>26,28,29</sup> Glyoxylic acid was detected as one of the minor reaction products in ozone-treated KLs after analysis by GLC after trimethylsilylation.<sup>24,25</sup> Glycolic, glyceric, erythronic, threonic, tartronic, and malic acids have adjacent carboxyl/alcohol hydroxyl groups and they were found to be present in the reaction products of lignin with ozone. Erythronic and threonic acids are known as the major low molecular weight products formed by ozone treatment of lignin model compounds and lignins including KL.<sup>30–34</sup> Glyceric and glycolic acids were also found as major low molecular weight products of ozone-treated KL, milled wood lignin (MWL), klason lignin, and soda lignin.<sup>30,32</sup> Tartronic and malic acids were found to be present in the reaction products of lignin model compound, klason lignin, and KL treated with ozone.<sup>24,25,30</sup>

Finally, plant growth tests were performed in the culture solutions containing *p*-hydroxybenzoic, protocatechuic, and



**Fig. 3.** Effect of glycolic acid, glycerol, glyoxylic acid, and glyceraldehyde on root elongation of radish (*Raphanus sativa* var. *radicola* Pers.). Hydroponic solution contained 1.2 mM  $\text{CaCl}_2$  and 37.5  $\mu\text{M}$   $\text{AlCl}_3$  at pH 4.8. Dosages of compounds were 18.8, 37.5, and 112.5  $\mu\text{M}$  and that of  $\text{AlCl}_3$  was 37.5  $\mu\text{M}$ . Growth period was 3 days ( $n = 10$ )



**Fig. 4.** Effect of *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic acid on root elongation of radish (*Raphanus sativa* var. *radicula* Pers.). Hydroponic solution contained 1.2 mM  $\text{CaCl}_2$  and 37.5  $\mu\text{M}$   $\text{AlCl}_3$  at pH 4.8. Dosages of compounds were 18.8, 37.5, and 112.5  $\mu\text{M}$  and that of  $\text{AlCl}_3$  was 37.5  $\mu\text{M}$ . Growth period was 3 days ( $n = 10$ )

vanillic acids (IX–XI in Fig. 1). These tests examined the contributions of phenolic hydroxyl groups and its methyl ether (methoxyl group) in alkaline-treated KL in reducing aluminum toxicity. The results are shown in Fig. 4. Only protocatechuic acid reduced the aluminum toxicity, showing that the catechol structure contributed to reducing the aluminum toxicity. It was reported in a previous article that original KL was insoluble in water and had no effect on reducing aluminum toxicity.<sup>24</sup> However, KL become effective in reducing aluminum toxicity after enhancement of its water solubility by alkaline treatment.<sup>25</sup> This indicates that water solubility is an important factor in reducing aluminum toxicity with the catechol structure in KL. The catechol structure was reported to be formed by cleavage of a methyl aryl ether linkage at the methoxyl position of lignin during cooking treatment.<sup>35</sup> One phenolic hydroxyl group or guaiacyl nuclei did not reduce aluminum toxicity. It was suggested by Vance et al.<sup>13</sup> and Barceló and Poschenrieder<sup>36</sup> that the contribution of phenolic hydroxyl groups to reducing aluminum toxicity was, in acidic pH, less apparent than that of carboxyl groups. On the other hand, Schnitzer<sup>15</sup> and Tam and McColl<sup>16</sup> reported the importance of phenolic hydroxyl groups in complex formation with aluminum. It was reported that the combination of carboxyl/phenolic hydroxyl groups was moderately effective in reducing aluminum toxicity.<sup>14</sup>

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## References

- Matsumoto H (2000) Cell biology of aluminum toxicity and tolerance in higher plants. *Int Rev Cytol* 200:1–46
- Aimi R, Murakami T (1964) Cell-physiological studies on the effect of aluminum on the growth of crop plants (in Japanese). *Bull Nat Inst Agr Sci* D11:331–396
- Ma JF (2000) Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol* 41:383–390
- Ma JF, Ryan PR, Delhaize E (2001) Aluminum tolerance in plants and the complexing role of organic acids. *Trends Plant Sci* 6:273–278
- Ma JF, Furukawa J (2003) Recent progress in the research of external Al detoxification in higher plant: a minireview. *J Inorg Biochem* 97:46–51
- Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu Rev Plant Physiol Plant Mol Biol* 46: 237–260
- Kochian LV, Hoekenga OA, Piñeros MA (2004) How do crop plants tolerate acid soil? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol* 55:459–493
- Bartlett RJ, Riego DC (1972) Effect of chelation on the toxicity of aluminum. *Plant Soil* 37:419–423
- Ma JF, Hiradate S, Nomoto K, Iwashita T, Matsumoto H (1997) Internal detoxification mechanism of Al in hydrangea. *Plant Physiol* 113:1033–1039
- Ma JF, Zheng SJ, Matsumoto H (1997) Detoxifying aluminium with buckwheat. *Nature* 390:569–570
- Zheng SJ, Ma JF, Matsumoto H (1998) High aluminum resistance in buckwheat. I. Al-induced specific secretion of oxalic acid from root tip. *Plant Physiol* 117:745–751
- Shen R, Ma JF, Kyo M, Iwashita T (2002) Compartmentation of aluminum in leaves of an Al-accumulator, *Fagopyrum esculentum* Moench. *Planta* 215:394–398
- Vance GF, Stevenson FJ, Sikora FJ (1996) Environmental chemistry of aluminum-organic complexes. In: Sposito G (ed) *The environmental chemistry of aluminum*, 2nd. edn. Lewis, Boca Raton, pp 169–220
- Hue NV, Graddock GR, Adams F (1986) Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci Soc Am J* 50:28–34
- Schnitzer M, Skinner SIM (1965) Organo-metallic interactions in soils: 4. Carboxyl and hydroxyl groups in organic matter and metal retention. *Soil Sci* 99:278–284
- Tam SC, McColl JG (1990) Aluminum- and calcium-binding affinities of some organic ligands in acidic conditions. *J Environ Qual* 19:514–520
- Ofei-Manu P, Wagatsuma T, Ishikawa S, Tawaraya K (2001) The plasma membrane strength of the root-tip cells and root phenolic compounds are correlated with Al tolerance in several common woody plants. *Soil Sci Plant Nutr* 47:359–375
- Katsumata K, Meshitsuka G (2002) Modified kraft lignin and its use for soil preservation. In: Hu TQ (ed) *Chemical modification, properties and usage of lignin*. Kluwer, New York, pp 151–165
- Saito K, Nakanishi MT, Matsubayashi M, Meshitsuka G (1997) Development of new lignin derivatives as soil conditioning agents by radical sulfonation and alkaline-oxygen treatment. *Mokuzai Gakkaishi* 43:669–677
- Katsumata SK, Maruyama M, Meshitsuka G (2001) Reduction of aluminum toxicity to radish by alkaline oxygen treated kraft lignin. *J Wood Sci* 47:129–134
- Katsumata SK, Shintani H, Meshitsuka G (2003) Mechanism of detoxification of aluminum ions by kraft lignin treated with alkaline oxygen. *J Wood Sci* 49:93–99
- Wang D, Katsumata SK, Meshitsuka G (2005) Characterization of lignin fragments in alkaline oxygen-stage waste liquor as soil-conditioning agent. *J Wood Sci* 51:357–362
- Wang D, Katsumata SK, Meshitsuka G (2005) Effect of low molecular weight lignin fragments including oxalic acid in alkaline-oxygen stage waste liquor on Al toxicity. *J Wood Sci* 51:634–639
- Aimi H, Ohmura S, Kato T, Nakahara T, Shimizu K (2008) Development of acid soil conditioning agent from lignin by ozone treatment I. *J Wood Sci* 54:214–219
- Aimi H, Ohmura S, Uetake M, Shimizu K (2009) Development of acid soil conditioning agent from lignin by ozone treatment II. *J Wood Sci* 55:121–125
- Eriksson T, Gierer J (1985) Studies on the ozonation of structural elements in residual kraft lignins. *J Wood Chem Technol* 5:53–84
- Miller JN, Miller JC (1988) *Statistics and chemometrics for analytical chemistry*, 2nd edn. Ellis Horwood, Chichester, UK

28. Kaneko H, Hosoya S, Nakano J (1980) Degradation of lignin with ozone. *Mokuzai Gakkaishi* 26:752–758
29. Kratzl K, Claus P, Reichel G (1976) Reactions of lignin and lignin model compounds with ozone. *TAPPI* 59:86–87
30. Tsutsumi Y, Islam A, Anderson CD, Sarkanen KV (1990) Acidic permanganate oxidations of lignin and model compounds: comparison with ozonolysis. *Holzforschung* 44:59–66
31. Sarkanen KV, Islam A, Anderson CD (1992) Ozonation. In: Lin SY, Dence CW (eds) *Methods in lignin chemistry*. Springer, Berlin Heidelberg New York, pp 387–406
32. Matsumoto Y, Ishizu A, Nakano J (1986) Studies on chemical structure of lignin by ozonation. *Holzforschung* 40(Suppl):81–85
33. Matsumoto Y, Minami K, Ishizu A (1993) Structural study on lignin by ozonation. The *erythro* and *threo* ratio of the  $\beta$ -O-4 structure indicates how lignin polymerizes. *Mokuzai Gakkaishi* 39:734–736
34. Akiyama T, Sugimoto T, Matsumoto Y, Meshitsuka G (2002) *Erythro/threo* ratio of  $\beta$ -O-4 structures as an important structural characteristic of lignin I. Improvement of ozonation method for the quantitative analysis of lignin side-chain structure. *J Wood Sci* 48:210–215
35. Gierer J (1970) The reactions of lignin during pulping. A description and comparison of conventional pulping processes. *Sven Papperstidn* 73:571–596
36. Barceló J, Poschenrieder C (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: a review. *Environ Exp Bot* 48:75–92