

RAPID COMMUNICATION

Olov Karlsson · Tsutomu Ikeda · Takao Kishimoto
Kengo Magara · Yuji Matsumoto · Shuji Hosoya

Ozonation of a lignin–carbohydrate complex model compound of the benzyl ether type

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Abstract Evidence for the presence of lignin-carbohydrate bonds of the benzylic ether type in wood and pulps may be obtained by use of ozonation treatment to selectively degrade the lignin. It was found that the benzyl ether bond in 3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-phenoxy)-3-(methyl- β -D-glucopyranosid-6-*O*-yl)-1-propanol was rather stable during ozonation in acetic acid-water-methanol 16:3:1 at 0°C. The corresponding acid, 3,4-dihydroxy-2-(methyl- β -D-glucopyranosid-6-*O*-yl)-butanoic acid, was found to be the major reaction product. The use of ozonation followed by acid hydrolysis in connection with studies of lignin-carbohydrate linkages is briefly discussed.

Key words Ozone · LCC · Model compounds · Lignin · Benzylic ether · Isolation · Threonic acid · Erythronic acid

The α -ether bond in structural elements of benzylic ether type **1** has been proposed as a link between lignin and

polysaccharides in various lignocellulosic materials (Fig. 1). The presence of such bonds may obstruct selective delignification of the wood during kraft pulping because the α -ether bond in nonphenolic units is stable under pulping conditions.¹ To find evidence for benzylic ether bonds (phenolic and nonphenolics) between lignin and polysaccharide we need techniques that can degrade the wood polymers (lignin and polysaccharide) selectively without cleaving the lignin-carbohydrate bond. Lignin can be selectively degraded by ozonation in a solvent mixture consisting of acetic acid/water/methanol 16:3:1 at 0°C.² Arylglycerol- β -aryl ethers constitute the dominant structural element in lignin. Ozonation of such structures and subsequent saponification lead to the formation of tetric acid (2,3,4-trihydroxybutanoic acid) via degradation of their aromatic nuclei.² If the α -ether bond in benzylic ether **1** is stable during the ozone treatment, corresponding α -etherified tetric acid **2** may be formed (Fig. 1). In this paper we present results from studies on the stability of the α -ether bond in a model compound of benzylic ether type **1** during ozonation.

Synthesis of 3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3-(methyl- β -D-glucopyranosid-6-*O*-yl)-1-propanol (**3**) was performed by applying the procedure for synthesis of the corresponding lignin-carbohydrate, complex (LCC) model compound carrying α -D-glucopyranoside (Fig. 2).³ Formation of structural isomers other than **3** was low. The four diastereomers of **3** were isolated with high performance liquid chromatography (HPLC) using a preparative octadecyl dimethyl silyl (ODS) column (eluent: acetonitrile/water 84:16). Similar amounts of the diastereomers of **3** were formed. The steric structure of each diastereomer was not elucidated. The following nuclear magnetic resonance (NMR) data (recorded in [²H₆]acetone) were obtained from the first eluted diastereomer of **3**.

¹H NMR: δ 3.13 (1H, t, J = 7.8 Hz, C₂-H), 3.22–3.37 (2H, m, C₃-H, and C₄-H), 3.41 (3H, s, OCH₃), 3.44–3.53 (3H, m, C γ -H, C₅-H, and C₆-H), 3.61 (1H, m, C γ -H), 3.76–3.83 (1H, m, C₆-H), 3.84 (6H, s, OCH₃), 4.13 (1H, d, J = 7.8 Hz, C₁-H), 4.32 (1H, m, C β -H), 4.72 (1H, d, J = 5.8 Hz, C α -H), 6.80–

O. Karlsson (✉) · T. Ikeda¹ · T. Kishimoto² · K. Magara · S. Hosoya
Wood Chemistry Laboratory, Wood Chemistry Division, Forestry and Forest Products Research Institute, PO Box 16, Tsukuba Norin Kenkyu Danchi-Nai, Ibaraki 305-8687, Japan
Tel. +298-73-3211 (ext. 524); Fax +298-73-3797
e-mail: olovkarl@ffpri.affrc.go.jp

S. Hosoya
Institute of Agricultural and Forest Engineering, The University of Tsukuba, 1-1-1 Tennoudai, Ibaraki 305-0006, Japan

Y. Matsumoto
Laboratory of Wood Chemistry, Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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Present address:

¹North Carolina State University, Department of Wood and Paper Science, Campus Box 8005, Raleigh, NC 27695-8005, USA

²Laboratory of Wood Chemistry, Research Group of Bioorganic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8598, Japan

Fig. 1. Proposed reaction route for lignin-carbohydrate bonds of the benzylic ether type on treatment with ozone and subsequent acid hydrolysis

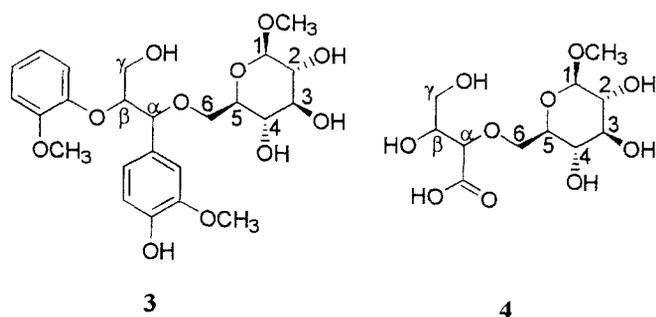
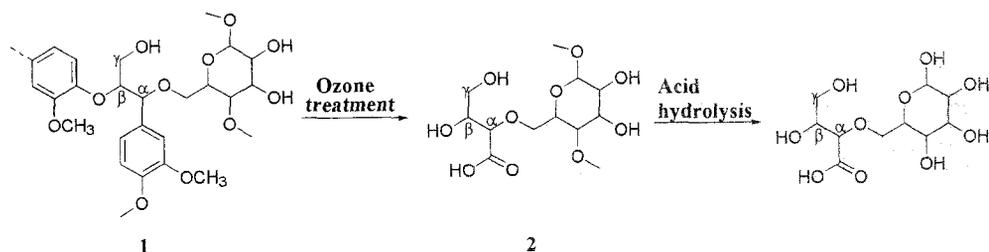


Fig. 2. Benzylic ether **3** and α -ether **4**

7.20 (7H, aromatic protons). ^{13}C NMR: δ 56.2 (1C, Ar-OCH₃), 56.3 (1C, Ar-OCH₃), 56.7 (1C, OCH₃), 62.2 (1C, C_γ), 69.8 (1C, C₆), 71.7 (1C, C₄), 74.7 (1C, C₂), 76.7 (1C, C₅), 78.0 (1C, C₃), 82.9 (1C, C_α), 86.3 (1C, C_β), 105.0 (1C, C₁). Aromatic carbon signals were found at 112.0, 113.4, 115.3, 119.2, 121.4, 121.9, 122.8, 131.1, 147.2, 148.3, 150.1, 151.6 ppm.

Ozone treatment was performed as follows: About 50 mg of benzylic ether was dissolved in 20 ml acetic acid/water/methanol (16:3:1) at 0°C. Ozone (3%) was purged into the solution at the rate of 0.1 l/min for 1 h. After ozonation solvents were removed by evaporation with a rotary evaporator, the sample was saponified in 0.1 M NaOH under nitrogen gas flow for 1 h. Sodium ions were exchanged with ammonium ions by eluting the alkaline solution through a Dowex 50W-X8 cation-exchange column that had been treated with NH₃ (aqueous). Solvents were evaporated, and the product was dried over P₂O₅ in a vacuum overnight. Evidence for formation of 3,4-dihydroxy-2-(methyl- β -D-glucopyranosid-6-O-yl)-butanoic acid (**4**) by ozonation of **3** was obtained (Fig. 2). The diastereomer of **3**, which elutes first during the HPLC analysis, was ozonated according to the procedure described above. A major reaction product was separated from the product mixture by anion-exchange chromatography using a Ashipak NH2P-90 21F column and acetic acid (2M) as eluent. A refractive index (RI) detector was used to monitor the eluted compounds. Following NMR (solvent D₂O, sample concentration ca. 15 mg/ml) data were obtained for the major compound.

^1H NMR: δ 3.14 (1H, d.t, $J = 7.3, 2.4$ Hz, C₂-H), 3.33–3.39 (2H, m, C₃-H, and C₄-H), 3.44 (3H, s, OCH₃), 3.40–3.50 (1H, m, C₅-H), 3.54 (1H, d.d, $J = 11.6, 6.7$, C_γ-H), 3.60 (1H, d.d, $J = 11.6, 5.5$, C_γ-H), 3.69–3.76 (2H, m, C₆-H), 3.88–3.92 (1H, m, C_β-H), 3.98 (1H, d, $J = 3.0$ Hz, C_α-H), 4.24 (1H, d, $J = 8.0$ Hz, C₁-H). ^{13}C NMR: δ 58.3 (1C, OCH₃), 63.4 (1C,

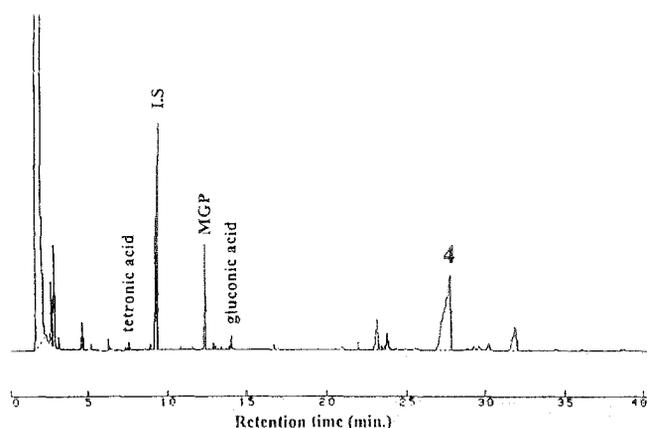


Fig. 3. Gas chromatography of product mixture from ozonation of one diastereomer of benzylic ether **3**. MGP, methyl- β -D-glucopyranoside

C_γ), 70.6 (1C, C₄), 70.7 (1C, C₆), 73.6 (1C, C_β), 74.2 (1C, C₂), 75.9 (1C, C₅), 76.6 (1C, C₃), 82.2 (1C, C_α), 104.4 (1C, C₁), 177.5 (1C, COOH).

C_α and COOH could be easily observed in the ^{13}C spectrum when the broadening factor was increased. The ether linkage between C_α and C₆ in compound **4** was confirmed by 2D-NMR spectroscopy [heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC)]. A sample from the product mixture of the ozonated benzyl ether was trimethyl silylated with 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane in 1 ml dimethylsulfoxide (DMSO) (dried over molecular sieves 3 Å) and analyzed with gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The chromatographed compounds were detected with chemical ionization (CI) [ammonia (g)] and electron impact (EI). A dominating high-molecular-weight peak at 762 m/z ($M + \text{NH}_4$) corresponding to α -ether **4** was found in the mass spectra of the major reaction product. EI gave mass fragments: M-15 and M-132. Similar data were obtained for the ozone-treated diastereomeric mixture **3**. Methyl- β -D-glucopyranoside (MGP) was also found in the product mixture of the ozone-treated benzylic ether (Fig. 3). The formation of gluconic acid was limited under the conditions used for ozonation of benzylic ether, but it was formed to a somewhat larger extent when the ozone charge was increased. Tetronic acid is rather stable during ozonation.⁴ It was found in a separate experiment that less than one-fifth of the threonic acid was degraded during ozone treatment. Only a small amount of tetronic acid was found in the product mixture of ozonated benzylic

ether (Fig. 3). Tetronic acid was not formed⁵ or was formed to only a small extent⁶ during ozonation of benzylic ether, 3-(3,4-dimethoxyphenyl)-3-ethoxy-2-(2-methoxyphenoxy)-1-propanol. The latter compound was mainly converted to the corresponding α -etherified tetronic acid.^{5,6}

Considerable parts of the benzylic ether bond in **3** were not cleaved during the ozone treatment. It is interesting to note that the benzylic ether bond in compounds of the Ph-CH₂-OR type is easily cleaved by ozone.⁷ If benzylic ether **1** constitutes the bonding fragment between lignin and polysaccharide in wood, tetronic acid etherified to sugar residues **2** may be formed after ozonation-saponification (Fig. 1). Because the resulting α -ether bond in **2** is no longer activated by an aromatic substituent, it should be more stable to acid treatment. A preliminary experiment indicates that the anomeric methoxy group in compound **4** can be selectively cleaved by acid hydrolysis. Thus, it may be possible to hydrolyze glycosidic bonds in the polysaccharide chain with acids or carbohydrate-degrading enzymes without cleavage of the α -ether bond (Fig. 1). We are aiming to use such treatments for isolation and characterization of lignin-carbohydrate bonds from lignocellulosic materials.

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