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Reactivity of secondary hydroxyl groups in methyl β-D-xylopyranoside toward a β-O-4-type quinone methide

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Abstract Methyl β-D-xylopyranoside was allowed to react with β-O-4-type quinone methide without a catalyst to elucidate the reactivities of secondary hydroxyl groups at the C2, C3, and C4 positions. Benzyl ether-type lignin–carbohydrate complex (LCC) compounds linked at the C2 and C4 positions were predominant, at a ratio of 2:3. However, the reactivity of the hydroxyl group at the C3 position was quite low. These results strongly suggest that the reactivity of the C2 hydroxyl group in xylan toward quinone methide intermediate is higher than that of the C3 hydroxyl group during biosynthesis of LCCs.

Key words Lignin carbohydrate complex · Xylose · Xylan · Model compound · Ozonation

Introduction

The presence of lignin–carbohydrate complexes (LCCs) in wood has been accepted for many years, and there are many reports dealing with their structures and roles. In pulping and bleaching chemistry, nonphenolic benzyl ether-type lignin–carbohydrate linkages have been suggested to prevent the final delignification during kraft pulping because of their stability under alkaline pulping conditions.[1]

Lignin–carbohydrate linkages have not been isolated from woods or pulps, however, and determination of these linkages has been based on methylation or acetylation of free hydroxyl groups prior to the cleavage reaction of the linkages.[2,3] To confirm the existence of LCCs in wood, we have been looking for new techniques to isolate lignin–carbohydrate linkages without their cleavage. Ozonation of glucose-type LCC model compounds was performed in regard to this technique in our previous work.[4]

Benzyl ether-type LCC model compounds have been prepared by several groups.[1,5,6] Tanaka and coworkers reported the formation of benzyl ether-type lignin–carbohydrate linkages between glucose and a quinone methide synthesized from guaiacylglycerol-β-guaiacyl ether.[5] They found that glucose was connected through the primary hydroxyl group at the C6 position. Tanahashi and Higuchi treated the quinone methide with xylose and found that the C5 primary hydroxyl group participated in benzyl ether formation.[7] In some cases secondary hydroxyl groups in sugars also can react with the β-O-4-type quinone methide.[8,9] It is also suggested that some lignins are connected through secondary hydroxyl groups of the xylose unit in hemicellulose.[3] Therefore, an understanding of the reactivity of each secondary hydroxyl group in xylan to quinone methide intermediate would be beneficial in connection with studies on LCC biosynthesis.

Recently, Toikka and Brunow reported the reactivity of methyl β-D-xylopyranoside toward a β-O-4-type quinone methide in the presence of an acid catalyst,[10] but they did not quantify each isomer of the formed LCC compounds. In this study the β-O-4-type quinone methide was allowed to react with methyl β-D-xylopyranoside without a catalyst to investigate the reactivities of secondary hydroxyl groups at the C2, C3, and C4 positions. Methyl β-D-xylopyranoside was used as a model compound for xylan, although xylan has only C2 and C3 hydroxyl groups. The formed xylose-type LCC compounds **1–8** were isolated and analyzed by nuclear magnetic resonance (NMR) spectroscopy. These LCC compounds will be subjected to further ozonation

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studies in regard to the isolation of lignin–carbohydrate linkages in woods and pulps.

Results and discussion

As shown in Fig. 1, synthesized guaiacylglycerol-β-guaiacyl ether **9** was converted to quinone methide **10** in chloroform and then reacted with commercially available methyl β-D-xylopyranoside **11** in dimethylsulfoxide (DMSO) without an acid catalyst until the yellow color of quinone methide **10** disappeared. Figure 2 shows the high-performance liquid chromatography (HPLC) profile of the reaction mixtures. Recovered guaiacylglycerol-β-guaiacyl ether **9** and other impurities were removed by silica gel column chromatography. The overall yield of LCC compounds (a mixture of isomers) from compound **9** was about 20%. The yield of compound **9** formed by the reaction of quinone methide **10** with water in the reaction mixture was low. Water in the reaction system was removed sufficiently and did not affect the yield of LCC compounds.

The yield of LCC compounds was lower than that reported by Toikka and Brunow (48%–56%),[10] but it must be noted that their reaction conditions were different from those in this study. They used dimethylformamide (DMF) instead of $CHCl_3$/DMSO as a solvent, and their reaction temperature seemed to be higher than ours. They also used a strong acid, *p*-toluenesulfonic acid, to accelerate the addition reaction between quinone methide **10** and methyl β-D-xylopyranoside **11**. Under these conditions, quinone methide **10** is activated by the protonation of quinone oxygen at the *p*-position; and the actual reactive species may become a highly reactive benzyl cation.[11] The reaction conditions in this study seemed to be milder than those used by them.

Methyl β-D-xylopyranoside **11** has secondary hydroxyl groups at the C2, C3, and C4 positions. The formed LCC compounds might be a mixture of 12 isomers because each of the expected LCC compounds linked at the C2, C3, and C4 positions may have four diastereomers due to asymmetric carbons at the Cα and Cβ positions in the propane side chain. Indeed, Toikka and Brunow reported that 12 isomers were formed under acid-catalyzed conditions.[10]

Only eight isomers were found to be predominant in this study, as shown in Fig. 2. The formed LCC compounds **1–8** were isolated successfully by HPLC with a preparative octadecyl dimethylsilyl (ODS) column. Some fractions were separated repeatedly by HPLC to obtain pure compounds. After isolation of individual isomers, LCC compounds **1–8** were analyzed by ^{1}H-NMR and ^{13}C-NMR spectroscopy. All NMR data are reported in the Experimental section. The ^{13}C-NMR data for compounds **1–8** are in good agreement with the data reported by Toikka and Brunow, but some ^{1}H-NMR data are not. This is probably because the solvents used for the NMR measurements were different from each other. We used acetone-d_6 for compounds **1–8** and $CDCl_3$ for acetates of compounds **1–8**, whereas they used acetone-d_6 with D_2O for all compounds.

The hydroxyl groups participated in the benzyl ether formation were confirmed by the chemical shifts of C2-C4 protons after acetylation of LCC compounds **1–8**, as shown in Table 1. For example, compound **1** was assigned to be

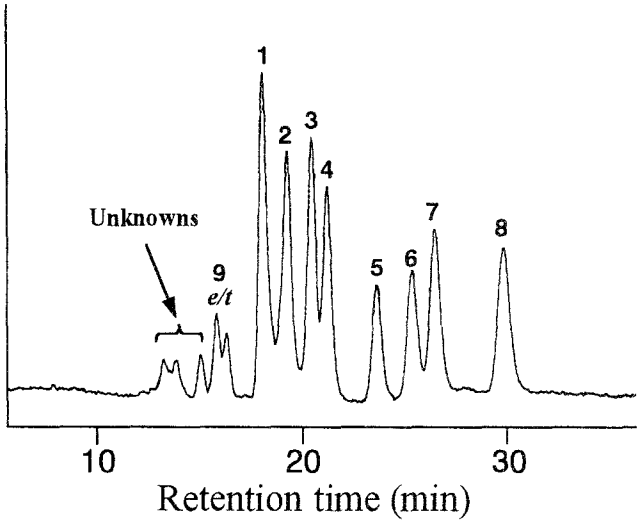

Fig. 2. High-performance liquid chromatography (HPLC) of the formed lignin–carbohydrate complex (LCC) compounds mixture from the reaction of β-*O*-4-type quinone methide **10** with methyl β-D-xylopyranoside **11**. *e/t*, erythro/threo

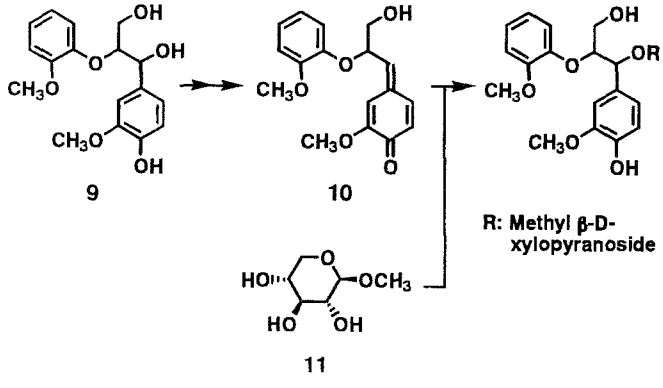

Fig. 1. Reaction of β-*O*-4-type quinone methide **10** with methyl β-D-xylopyranoside **11** in anhydrous $CHCl_3$/dimethylsulfoxide (DMSO) under air at 4°C for 2 days

Table 1. ^{1}H-NMR spectral data (chemical shifts) of acetates of LCC compounds **1–8**

Acetates of compounds **1–8** (relative yields)	Binding sites	C2-H	C3-H	C4-H
1 (18%)	C4 (*t*)	4.74	5.08	3.70
2 (16%)	C4 (*e*)	4.74	5.12	3.74–3.80
3 (15%)	C2 (*t*)	3.37	5.16	4.79
4 (12%)	C2 (*e*)	3.36	5.04	4.76
5 (7%)	C2 (*t*)	3.64	5.20	4.89
6 (8%)	C4 (*t*)	4.78	5.18	3.70–3.77
7 (11%)	C2 (*e*)	3.56	5.25	4.91
8 (11%)	C4 (*e*)	4.83	5.19	3.68–3.77

NMR, nuclear magnetic resonance; LCC, lignin–carbohydrate complex; *t*, threo form; *e*, erythro form

connected through oxygen at the C4 position because C2 and C3 protons shifted downfield owing to electron-withdrawing acetyl groups. The assignment can be done by the chemical shifts of C2-C4 carbons before acetylation.[1] The carbons participating in ether formation were found mostly down-field, as shown in Table 2. It was found that LCC compounds **1**, **2**, **6**, and **8** were linked at the C4 positions; and LCC compounds **3**, **4**, **5**, and **7** were linked at the C2 position, as shown in Fig. 3. Assignments of *erythro* (*e*) and *threo* (*t*) forms were made by analogy with the reported ^{1}H-NMR data.[6,8,12] γ-Protons in *threo* forms of β-*O*-4 structures are more shielded than those in *erythro* forms.

The relative yields of the formed LCC compounds **1–8** are shown in Table 1, as determined by HPLC analysis. It is assumed that the molar absorptivities of the LCC compounds are the same. The unidentified products shown in the HPLC profile were not purified. The possibility that the unknown compounds might be LCC compounds linked at the C3 position cannot be ruled out. However, the amount of the unknown products was less than 5% on the total amount of LCC compounds **1–8**.

Toikka and Brunow reported the formation of 12 isomers of LCC compounds, but they did not quantify each isomer. On the other hand, we found that LCC compounds linked at the C2 and C4 positions were predominant under the conditions used. The ratio of the LCC compounds linked at the C2 and C4 positions was 2:3. The difference in the yield of each regioisomer should be attributed to the reactivity of each secondary hydroxyl group toward quinone methide **10**. The order of steric hindrance of these secondary hydroxyl groups is in the following order: C2 > C3 > C4 because methyl β-D-xylopyranoside **11** has a relatively bulky methoxyl group at the C1 position. Therefore, the least hindered C4 hydroxyl group had the highest reactivity. However, the most hindered C2 hydroxyl group had a higher reactivity than the less hindered C3 hydroxyl group. These results are well explained by pKa values of secondary hydroxyl groups in methyl β-D-xylopyranoside. The pKa values of C2, C3, and C4 hydroxyl groups were calculated to be 12.7, 13.2, and 13.6, respectively, using the Taft equation.[13] The pKa value at the C2 hydroxyl group is the smallest owing to the electron-withdrawing effect of ring oxygen. Therefore, the most hindered C2 hydroxyl group has higher reactivity than the C3 hydroxyl group. Both the steric factor and the dissociation ability of hydroxyl groups are important in the regioselective reaction of methyl β-D-xylopyranoside with β-*O*-4-type quinone methides.

These results obtained by model experiments cannot be directly applied to the biosynthesis of LCC because the steric factor and other conditions during biosynthesis of LCC are different from those used in this study. However, the results obtained in this study strongly suggest that the reactivity of the C2 hydroxyl group in xylan toward the quinone methide intermediate is higher than that of the C3 hydroxyl group during biosynthesis of LCCs. The formed xylose-type LCC compounds **1–8** are ozonized in connection with the isolation of lignin–carbohydrate linkages in woods and pulps. Ozonation of compounds **1–8** would result in the selective degradation of aromatic moieties. The subsequent acid hydrolysis would lead to the formation of erythronic or threonic acid derivatives linked with xylose.[4] These products are used as authentic samples for the

Table 2. ^{13}C-NMR spectral data (chemical shifts) of LCC compounds **1–8**

Compounds	Binding sites	C-2	C-3	C-4
1	C4 (*t*)	74.0	75.3	76.3
2	C4 (*e*)	73.9	75.1	76.0
3	C2 (*t*)	78.6	76.2	70.9
4	C2 (*e*)	78.2	76.2	70.8
5	C2 (*t*)	81.5	77.7	70.6
6	C4 (*t*)	74.1	77.4	78.7
7	C2 (*e*)	81.7	77.9	70.9
8	C4 (*e*)	74.3	77.3	78.7

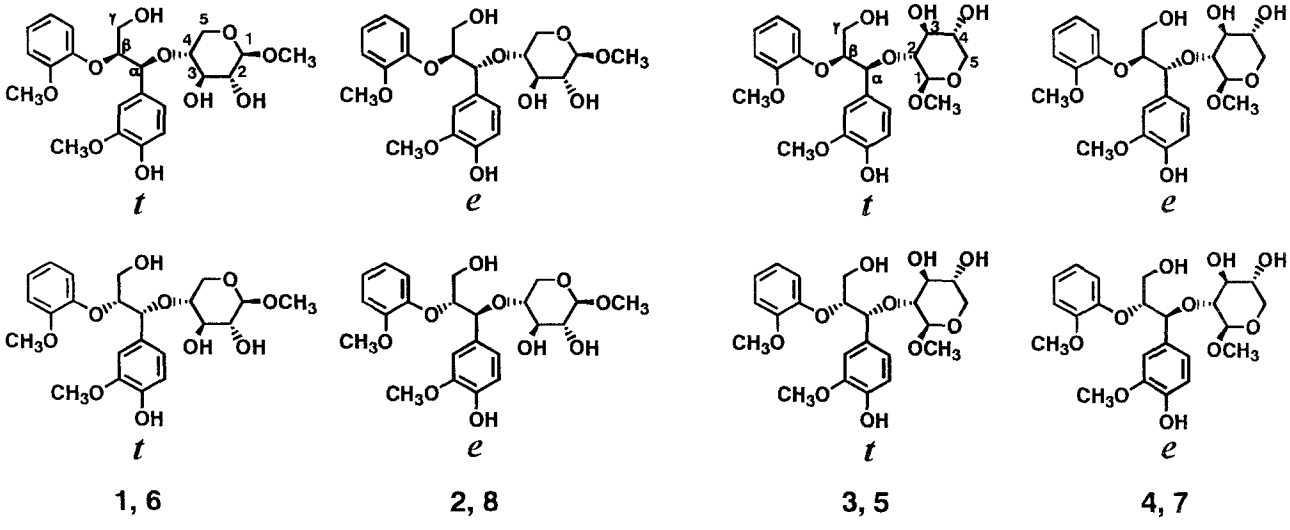

Fig. 3. Formed LCC compounds linked at C4 positions **1**, **2**, **6**, **8** and at C2 positions **3**, **4**, **5**, **7**

ozonation products of woods. It is direct evidence of the existence of LCCs if the erythronic or threonic acid derivatives are detected in the ozonation products of woods.

Experimental

The ^{1}H-NMR and ^{13}C NMR spectra were recorded with a Jeol JNM-LA400 FT-NMR spectrometer in acetone-d_6 or chloroform-d with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) and coupling constants (J) are given in δ values (ppm) and Hz, respectively. Some chemical shifts assignments were made by correlation spectroscopy (^{1}H-COSY) and heteronuclear multiple quantum coherence (HMQC) spectra; others were made by analogy with similar model compounds. Thin-layer chromatography (TLC) was performed with Whatman PK6F silica gel 60A. Column chromatography was performed with Wakogel C-200 (Wako). HPLC analysis and separation were performed on a Shimadzu liquid chromatograph, LC-10A with UV-VIS detector, SPD-10A$_{VP}$ (280nm). A Supelco Discovery C18 column (15cm × 4.6mm) (eluent: acetonitrile/water 20:80; flow rate 0.6ml/min) was used for analysis. The Supelco Discovery C18 column (25cm × 21.2mm) (eluent: acetonitrile/water 18.5:81.5; flow rate 7.0ml/min) was used to separate the iomers of LCC compounds.

Preparation of quinone methide **10**

Guaiacylglycerol-β-guaiacyl ether **9** was synthesized according to the method of Hosoya et al.[14] Quinone methide **10** was prepared by the method of Ralph and Young.[15] In a typical experiment, bromotrimethylsilane (1.67ml, 3Eq) was added to a stirred solution of compound **9** (1.35g) in anhydrous chloroform (30ml) at 0°C. The reaction mixture was kept at 0°C for 3.5h and then was shaken with saturated aqueous sodium bicarbonate (30ml) at room temperature. The $CHCl_3$ layer containing quinone methide **10** was immediately dried over $MgSO_4$ at room temperature.

Reaction of quinone methide **10** with methyl β-D-xylopyranoside **11**

Quinone methide **10** in $CHCl_3$ (30ml) was added to the solution of methyl β-D-xylopyranoside **11** (10.38g, 15Eq) in anhydrous DMSO (20ml). The reaction mixture was kept at 4°C for 2 days until the yellow color of quinone methide **10** disappeared. An aliquot was withdrawn for HPLC analysis, and water (200ml) was then added to the reaction mixture to stop further reaction. The reaction mixture was extracted with $CHCl_3$ (80ml × 2) and ethyl acetate (80ml × 2). Each organic layer was analyzed by TLC. Almost all LCC compounds were extracted with $CHCl_3$. The extraction with ethyl acetate was performed to ensure complete extraction of LCC compounds. The organic layer was combined, dried over Na_2SO_4, and concentrated to dryness in vacuo using a rotary evaporator equipped with a vacuum pump to afford a syrup (about 1.6g). The aqueous layer was also concentrated to dryness in vacuo to afford a crude crystalline solid, methyl β-D-xylopyranoside. The obtained syrup was purified on a silica gel column with 5% methanol/$CHCl_3$ to afford a mixture of LCC compounds **1–8**. The overall yield of LCC compounds **1–8** from compound **9** was about 20%. The LCC compounds **1–8** were analyzed and separated by HPLC with an ODS column eluted by acetonitrile/water. Some fractions were separated repeatedly by HPLC to obtain pure compounds.

Compound **1** (C4-*t*). ^{1}H-NMR (acetone-d_6): δ 3.11 (t, 1H, $J_{2,3}$ = 7.6, C2-H), 3.29 (dd, 1H, $J_{5a,5b}$ = 11.7, $J_{4,5a}$ = 9.2, C5a-H), 3.36–3.41 (m, 2H, C4-H, Cγa-H), 3.38 (s, 3H, OCH_3), 3.48 (t, 1H, $J_{3,4}$ = 8.1, C3-H), 3.53–3.60 (1H, Cγb-H), 3.85, 3.85 (s, 6H, Ar-OCH_3), 4.07 (dd, 1H, $J_{4,5b}$ = 4.4, $J_{5a,5b}$ = 11.7, C5b-H), 4.14 (d, 1H, $J_{1,2}$ = 6.8, C1-H), 4.30 (m, 1H, Cβ-H), 4.85 (d, 1H, $J\alpha,\beta$ = 6.0, Cα-H), 6.79–7.13 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): δ 56.3, 56.3, 56.4 (OCH_3), 61.9 (Cγ), 63.0 (C5), 74.0 (C2), 75.3 (C3), 76.3 (C4), 80.4 (Cα), 86.6 (Cβ), 105.4 (C1), 112.3, 113.5, 115.3, 119.1, 121.7, 121.9, 122.9, 130.9, 147.3, 148.2, 150.0, 151.7 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.62, 1.93, 1.98, 2.29 (s, 12H, $COCH_3$), 3.30 (t, 1H, $J_{4,5a}$ = 11.0, C5a-H), 3.44 (s, 3H, OCH_3), 3.70 (m, 1H, C4-H), 3.83, 3.84 (s, 6H, Ar-OCH_3), 3.87 (dd, 1H, $J\beta,\gamma_a$ = 5.4, $J\gamma_a,\gamma_b$ = 12.2, Cγa-H), 4.09 (dd, 1H, $J\gamma_a,\gamma_b$ = 12.0, $J\beta,\gamma_b$ = 4.2, Cγb-H), 4.25 (dd, 1H, $J_{4,5b}$ = 5.4, $J_{5a,5b}$ = 12.2, C5b-H), 4.29 (d, 1H, $J_{1,2}$ = 7.8, C1-H), 4.42 (m, 1H, Cβ-H), 4.65 (d, 1H, $J\alpha,\beta$ = 7.3, Cα-H), 4.74 (dd, 1H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.3, C2-H), 5.08 (t, 1H, $J_{3,4}$ = 9.1, C3-H), 6.86–7.03 (m, 7H, Ar-H).

Compound **2** (C4-*e*): ^{1}H-NMR (acetone-d_6): δ 3.13 (dd, 1H, $J_{1,2}$ = 6.8, $J_{2,3}$ = 7.8, C2-H), 3.32 (dd, 1H, $J_{4,5a}$ = 9.3, $J_{5a,5b}$ = 10.8, C5a-H), 3.33–3.39 (m, 1H, C4-H), 3.39 (s, 3H, OCH_3), 3.52 (t, 1H, $J_{3,4}$ = 8.1, C3-H), 3.74 (dd, 1H, $J\beta,\gamma_a$ = 4.0, $J\gamma_a,\gamma_b$ = 12.0, Cγa-H), 3.78–3.84 (m, 1H, Cγb-H), 3.85, 3.85 (s, 6H, Ar-OCH_3), 4.09 (dd, 1H, $J_{5a,5b}$ = 10.0, $J_{4,5b}$ = 3.2, C5b-H), 4.17 (d, 1H, $J_{1,2}$ = 6.9, C1-H), 4.31 (m, 1H, Cβ-H), 4.84 (d, 1H, $J\alpha,\beta$ = 5.8, Cα-H), 6.77–6.95 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.3, 56.4 (OCH_3), 61.4 (Cγ), 63.0 (C5), 73.9 (C2), 75.2 (C3), 76.1 (C4), 79.7 (Cα), 85,9 (Cβ), 105.4 (C1), 112.4, 113.6, 115.0, 119.2, 121.8, 121.9, 123.1, 131.2, 147.0, 148.1, 149.2, 151.8 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.56, 1.96, 2.00, 2.29 (s, 12H, $COCH_3$), 3.43 (dd, 1H, $J_{4,5a}$ = 10.4, $J_{5a,5b}$ = 12.0, C5a-H), 3.48 (s, 3H, OCH_3), 3.74–3.80 (m, 1H, C4-H), 3.80, 3.82 (s, 6H, Ar-OCH_3), 4.28 (dd, 1H, $J\beta,\gamma_a$ = 3.2, $J\gamma_a,\gamma_b$ = 11.5, Cγa-H), 4.37 (d, 1H, $J_{1,2}$ = 7.6, C1-H), 4.39–4.48 (m, 2H, Cβ-H, Cγb-H), 4.46 (dd, 1H, $J_{4,5b}$ = 5.4, $J_{5a,5b}$ = 11.7, C5b-H), 4.74 (dd, 1H, $J_{1,2}$ = 7.4, $J_{2,3}$ = 9.3, C2-H), 4.83 (d, 1H, $J\alpha\beta$ = 4.3, Cα-H), 5.12 (t, 1H, $J_{3,4}$ = 9.3, C3-H), 6.79–6.99 (m, 7H, Ar-H).

Compound **3** (C2-*t*): ^{1}H-NMR (acetone-d_6): δ 3.06 (t, 1H, $J_{2,3}$ = 7,6, C2-H), 3.17 (dd, 1H, $J_{4,5a}$ = 9.1, $J_{5a,5b}$ = 11.5, C5a-H), 3.35–3.49 (m, 3H, C3-H, C4-H, Cγa-H), 3.42 (s, 3H, OCH_3), 3.59–3.65 (1H, Cγb-H), 3.80 (dd, 1H, $J_{4,5b}$ = 4.7, $J_{5a,5b}$ = 11.5, C5b-H), 3.84, 3.85 (s, 6H, Ar-OCH_3), 4.32 (d, 1H, $J_{1,2}$ = 6.8, C1-H), 4.37 (m, 1H, Cβ-H), 5.22 (d, 1H, $J\alpha\beta$ = 5.8, Cα-H), 6.76–7.16 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.2, 56.3 (Ar-OCH_3), 56.5 (OCH_3), 62.3 (Cγ), 66.1 (C5), 70.9 (C4), 76.2 (C3), 78.6 (C2), 80.8 (Cα), 86.3 (Cβ), 106.0

(C1), 112.6, 113.5, 115.0, 118.8, 121.8, 122.1, 122.7, 130.7, 147.1, 148.1, 150.1, 151.5 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.69, 1.94, 1.97, 2.29 (s, 12H, $COCH_3$), 3.30 (t, 1H, $J_{4,5a}$ = 11.0, C5a-H), 3.37 (dd, 1H, $J_{1,2}$ = 7.1, $J_{2,3}$ = 9.1, C2-H), 3.49 (s, 3H, OCH_3), 3.81, 3.82 (s, 6H, Ar-OCH_3), 3.99 (dd, 1H, $J\beta,\gamma_a$ = 6.8, $J\gamma_a,\gamma_b$ = 11.7, Cγa-H), 4.01 (dd, 1H, $J_{4,5a}$ = 5.2, $J_{5a,5b}$ = 12.0, C5b-H), 4.28 (dd, 1H, $J\gamma_a,\gamma_b$ = 12.0, $J\beta,\gamma_b$ = 4.2, Cγb-H), 4.36 (d, 1H, $J_{1,2}$ = 7.3, C1-H), 4.69 (m, 1H, Cβ-H), 4.79 (m, 1H, C4-H), 4.94 (d, 1H, $J\alpha\beta$ = 4.9, Cα-H), 5.16 (t, 1H, $J_{3,4}$ = 9.3, C3-H), 6.89–7.06 (m, 7H, Ar-H).

Compound **4** (C2-*e*): ^{1}H-NMR (acetone-d_6): δ 3.06 (dd, 1H, $J_{1,2}$ = 7.1, $J_{2,3}$ = 8.6, C2-H), 3.17 (dd, 1H, $J_{4,5a}$ = 9.5, $J_{5a,5b}$ = 11.5, C5a-H), 3.34–3.43 (2H, C3-H, C4-H), 3.40 (s, 3H, OCH_3), 3.57–3.66 (1H, Cγa-H), 3.73–3.86 (2H, Cγb-H, C5b-H), 3.78, 3.83 (s, 6H, Ar-OCH_3), 4.31 (d, 1H, $J_{1,2}$ = 7.3, C1-H), 4.34 (m, 1H, Cβ-H), 5.14 (d, 1H, $J\alpha,\beta$ = 5.9, Cα-H), 6.74–6.92 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.2, 56.3 (Ar-OCH_3), 56.5 (OCH_3), 62.3 (Cγ), 66.1 (C5), 70.8 (C4), 76.2 (C3), 78.2 (C2), 80.8 (Cα), 85,9 (Cβ), 105.8 (C1), 113.0, 113.5, 114.9, 118.8, 121.7, 122.8, 122.8, 131.0, 147.1, 148.1, 149.6, 151.6 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.69, 1.97, 2.05, 2.28 (s, 12H, $COCH_3$), 3.27 (dd, 1H, $J_{4,5a}$ = 9.7, $J_{5a,5b}$ = 11.7, C5a-H), 3.36 (dd, 1H, $J_{1,2}$ = 7.3, $J_{2,3}$ = 9.3, C2-H), 3.55 (s, 3H, OCH_3), 3.65, 3.69 (s, 6H, Ar-OCH_3), 4.01 (dd, 1H, $J_{4,5a}$ = 5.3, $J_{5a,5b}$ = 11.7, C5b-H), 4.31 (d, 1H, $J_{1,2}$ = 7.3, C1-H), 4.42 (dd, 1H, $J\beta,\gamma_a$ = 6.4, $J\gamma_a,\gamma_b$ = 11.8, Cγa-H), 4.49 (dd, 1H, $J\beta,\gamma_b$ = 3.4, $J\gamma_a,\gamma_b$ = 11.7, Cγb-H), 4.58 (m, 1H, Cβ-H), 4.76 (m, 1H, C4-H), 4.89 (d, 1H, $J\alpha,\beta$ = 7.3, Cα-H), 5.04 (t, 1H, $J_{3,4}$ = 9.3, C3-H), 6.86–7.07 (m, 7H, Ar-H).

Compound **5** (C2-*t*): ^{1}H-NMR (acetone-d_6): δ 3.14–3.19 (1H, C5a-H), 3.17 (s, 3H, OCH_3), 3.19 (t, 1H, $J_{1,2}$ = 6.8, C2-H), 3.39 (dd, 1H, $J\beta,\gamma_a$ = 4.9, $J\gamma_a,\gamma_b$ = 11.7, Cγa-H), 3.49 (m, 1H, C4-H), 3.55 (t, 1H, $J_{3,4}$ = 8.2, C3-H), 3.72 (dd 1H, $J\beta,\gamma_b$ = 4.4, $J\gamma_a,\gamma_b$ = 11.7, Cγb-H), 3.80 (dd, 1H, $J_{5a,5b}$ = 11.2, $J_{4,5b}$ = 4.9, C5b-H), 3.85, 3.87 (s, 6H, Ar-OCH_3), 4.14 (d, 1H, $J_{1,2}$ = 6.8, C1-H), 4.41 (m, 1H, Cβ-H), 5.13 (d, 1H, $J\alpha,\beta$ = 6.8, Cα-H), 6.77–7.08 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.2, 56.2, 56.3 (OCH_3), 61.1 (Cγ), 66.1 (C5), 70.6 (C4), 77.7 (C3), 81.5 (C2), 82.8 (Cα), 85.4 (Cβ), 104.7 (C1), 112.4, 113.2, 114.9, 118.0, 121.5, 121.8, 122.8, 131.8, 146.9, 147.9, 149.2, 151.4 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.79, 1.92, 2.00, 2.30 (s, 12H, $COCH_3$), 3.12 (s, 3H, OCH_3), 3.33 (dd, 1H, $J_{4,5a}$ = 8.6, $J_{5a,5b}$ = 12.0, C5a-H), 3.64 (dd, 1H, $J_{1,2}$ = 6.7, $J_{2,3}$ = 8.6, C2-H), 3.84, 3.84 (s, 6H, Ar-OCH_3), 3.94 (dd, 1H, $J\beta,\gamma_a$ = 5.8, $J\gamma_a,\gamma_b$ = 12.2, Cγa-H), 4.00 (dd, 1H, $J_{5a,5b}$ = 12.0, $J_{4,5a}$ = 5.2, C5b-H), 4.14 (dd, 1H, $J\beta,\gamma_b$ = 3.9, $J\gamma_a,\gamma_b$ = 11.7, Cγb-H), 4.17 (d, 1H, $J_{1,2}$ = 6.9, C1-H), 4.48 (q, 1H, Cβ-H), 4.89 (m, 1H, C4-H), 5.03 (d, 1H, $J\alpha\beta$ = 5.9, Cα-H), 5.20 (t, 1H, $J_{3,4}$ = 8.8, C3-H), 6.86–7.07 (m, 7H, Ar-H).

Compound **6** (C4-*t*): ^{1}H-NMR (acetone-d_6): δ 3.10–3.21 (2H, C2-H, C5a-H), 3.25–3.46 (1H, C4-H), 3.35 (s, 3H, OCH_3), 3.47–3.54 (m, 2H, C5b-H, Cγa-H), 3.54 (t, 1H, $J_{3,4}$ = 8.8, C3-H), 3.76 (dd, 1H, $J\beta,\gamma_b$ = 4.4, $J\gamma_a,\gamma_b$ = 11.8, Cγb-H), 3.85 (s, 6H, Ar-OCH_3), 4.06 (d, 1H, $J_{1,2}$ = 7.3, C1-H), 4.38 (q, 1H, Cβ-H), 5.00 (d, 1H, $J\alpha\beta$ = 5.8, Cα-H), 6.79–7.01 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.1, 56.2, 56.5 (OCH_3), 61.4 (Cγ), 64.9 (C5), 74.1 (C2), 77.4 (C3), 78.7 (C4), 83.4 (Cα), 85.1 (Cβ), 105.4 (C1), 112.1, 113.1, 115.4, 117.3, 121.4, 121.7, 122.4, 131.8, 147.4, 148.2, 149.7, 151.0 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 1.85, 1.93, 2.01, 2.31 (s, 12H, $COCH_3$), 3.20 (m, 1H, C5a-H), 3.42 (s, 3H, OCH_3), 3.70–3.77 (m, 2H, C4-H, C5b-H), 3.82, 3.83 (s, 6H, Ar-OCH_3), 3.94 (dd, 1H, $J\beta,\gamma_a$ = 5.8, $J\gamma_a,\gamma_b$ = 11.7, Cγa-H), 4.15 (dd, 1H, $J\beta,\gamma_b$ = 4.2, $J\gamma_a,\gamma_b$ = 12.0, Cγb-H), 4.30 (d, 1H, $J_{1,2}$ = 7.4, C1-H), 4.44 (q, 1H, Cβ-H), 4.76 (d, 1H, $J\alpha\beta$ = 6.3, Cα-H), 4.78 (dd, 1H, $J_{1,2}$ = 7.3, $J_{2,3}$ = 9.2, C2-H), 5.18 (t, 1H, $J_{3,4}$ = 8.6, C3-H), 6.84–7.04 (m, 7H, Ar-H).

Compound **7** (C2-*e*): ^{1}H-NMR (acetone-d_6): δ 3.13 (s, 3H, OCH_3), 3.18 (dd, 1H, $J_{5a,5b}$ = 11.7, $J_{4,5a}$ = 9.3, C5a-H), 3.24 (dd, 1H, $J_{1,2}$ = 6.8, $J_{2,3}$ = 8.3, C2-H), 3.51 (m, 1H, C4-H), 3.58 (t, 1H, $J_{3,4}$ = 8.3, C3-H), 3.75–3.85 (2H, C5b-H, Cγa-H), 3.80, 3.85 (s, 6H, Ar-OCH_3), 3.98 (dd, 1H, $J\beta,\gamma_b$ = 2.4, $J\gamma_a,\gamma_b$ = 11.6, Cγb-H), 4.15 (d, 1H, $J_{1,2}$ = 6.8, C1-H), 4.31 (m, 1H, Cβ-H), 5.07 (d, 1H, $J\alpha\beta$ = 6.4, Cα-H), 6.75–6.93 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.2, 56.3, 56.4 (OCH_3), 61.3 (Cγ), 66.0 (C5), 70.9 (C4), 77.9 (C3), 81.7 (C2), 82.6 (Cα), 85.6 (Cβ), 104.9 (C1), 112.2, 113.5, 114.8, 118.5, 121.5, 121.7, 122.9, 132.6, 146.7, 147.8, 148.7, 151.6 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 2.00, 2.06, 2.09, 2.29 (s, 12H, $COCH_3$), 2.97 (s, 3H, OCH_3), 3.34 (dd, 1H, $J_{4,5a}$ = 8.3, $J_{5a,5b}$ = 11.7, C5a-H), 3.56 (dd, 1H, $J_{1,2}$ = 6.1, $J_{2,3}$ = 8.1, C2-H), 3.77, 3.81 (s, 6H, Ar-OCH_3), 4.02 (dd, 1H, $J_{4,5b}$ = 4.8, $J_{5a,5b}$ = 12.2, C5b-H), 4.14 (d, 1H, $J_{1,2}$ = 5.9, C1-H), 4.37 (s, 3H, Cβ-H, Cγa-H, Cγb-H), 4.91 (m, 1H, C4-H), 5.00 (d, 1H, $J\alpha\beta$ = 4.9, Cα-H), 5.25 (t, 1H, $J_{3,4}$ = 8.1, C3-H), 6.75–7.01 (m, 7H, Ar-H).

Compound **8** (C4-*e*): ^{1}H-NMR (acetone-d_6): δ 3.11 (dd, 1H, $J_{4,5a}$ = 9.8, $J_{5a,5b}$ = 11.2, C5a-H), 3.17 (dd, 1H, $J_{1,2}$ = 7.6, $J_{2,3}$ = 9.0, C2-H), 3.35 (s, 3H, OCH_3), 3.33–3.42 (m, 1H, C4-H), 3.48 (dd, 1H, $J_{4,5a}$ = 5.4, $J_{5a,5b}$ = 11.3, C5b-H), 3.55 (t, 1H, $J_{3,4}$ = 8.8, C3-H), 3.73, 3.84 (s, 6H, Ar-OCH_3), 3.83–3.89 (1H, Cγa-H), 3.99 (dd, 1H, $J\beta,\gamma_b$ = 3.2, $J\gamma_a,\gamma_b$ = 12.4, Cγb-H), 4.06 (d, 1H, $J_{1,2}$ = 7.3, C1-H), 4.33 (m, 1H, Cβ-H), 4.88 (d, 1H, $J\alpha\beta$ = 7.9, Cα-H), 6.73–6.95 (m, 7H, Ar-H). ^{13}C-NMR (acetone-d_6): 56.2, 56.3, (Ar-OCH_3), 56.5 (OCH_3), 61.6 (Cγ), 64.9 (C5), 74.3 (C2), 77.3 (C3), 78.7 (C4), 82.9 (Cα), 84.6 (Cβ), 105.4 (C1), 112.3, 113.7, 115.2, 118.7, 121.6, 121.7, 122.9, 132.7, 147.2, 148.0, 148.7, 151.7 (Ar). ^{1}H-NMR ($CDCl_3$) (acetate): δ 2.00, 2.06, 2.09, 2.30 (s, 12H, $COCH_3$), 3.11–3.17 (m, 1H, C5a-H), 3.41 (s, 3H, OCH_3), 3.68–3.77 (m, 2H, C4-H, C5b-H), 3.73, 3.78 (s, 6H, Ar-OCH_3), 4.30 (d, 1H, $J_{1,2}$ = 7.2, C1-H), 4.34 (dd, 1H, $J\beta,\gamma_a$ = 6.4, $J\gamma_a,\gamma_b$ = 12.0, Cγa-H), 4.38–4.43 (m, 2H, Cβ-H, Cγb-H), 4.76 (d, 1H, $J\alpha,\beta$ = 6.0, Cα-H), 4.83 (dd, 1H, $J_{1,2}$ = 7.0, $J_{2,3}$ = 8.6, C2-H), 5.19 (t, 1H, $J_{3,4}$ = 8.2, C3-H), 6.66–6.99 (m, 7H, Ar-H).

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