Carbohydrate Research 388 (2014) 138–146

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis of hydroxycinnamoyl β-D-xylopyranosides and evaluation of their antioxidant properties

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ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 13 December 2013
Accepted 15 December 2013
Available online 21 December 2013

Keywords:
Hydroxycinnamic acids
Hydroxycinnamoyl β-D-xylopyranosides
Lipase
Regioselective transesterification
Antioxidant properties

ABSTRACT

Various hydroxycinnamoyl β-D-xylopyranosides were efficiently prepared from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (TAXB) with amine by amine-promoted glycosylation. The resulted acetylated hydroxycinnamoyl β-D-xylopyranosides with acetoxy groups at C-2, C-3, and C-4 were regioselectively deacetylated at C-4 position with Novozym 435. Antioxidant activities of free hydroxycinnamic acids and the respective β-D-xylopyranosides were evaluated by DPPH· radical scavenging activity as well as their inhibitory effect on autoxidation of bulk methyl linoleate. The radical scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) decreased in the order ferulic acid > caffeic acid ≈ caffeoyl β-D-xylopyranosides ≈ sinapinic acid > sinapoyl β-D-xylopyranosides ≈ feruloyl β-D-xylopyranosides > *p*-coumaric acid > *p*-coumaroyl β-D-xylopyranosides. In bulk methyl linoleate, the antioxidant activity order against autoxidation was almost consistent with the scavenging activity order. The results showed that caffeoyl β-D-xylopyranosides and sinapoyl β-D-xylopyranosides were as effective as free caffeic acid, sinapinic acid, and ferulic acid.

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1. Introduction

Hydroxycinnamic acids such as *p*-coumaric, caffeic, ferulic, and sinapinic acid are very common in fruits and vegetables in the form of esters and glycosides. These compounds have various properties which may be of importance for the remediation of many diseases. Their activities, for example, anti-bacterial, anti-viral, anti-inflammatory, anti-carcinogenic, and anti-microbial agents, are related to their capability to act as antioxidants, metal ion chelating, radical scavenging, and inhibition of prooxidant enzymes.[1–6] Various fruits, including these hydroxycinnamic acids have been used as traditional medicine. For instance, *Rubus* species have been used as antimicrobial, anticonvulsant, muscle relaxant, and radical scavenging agents.[7–10] Hussein et al. found that the extracts provided from *Rubus sanctus* included various caffeoyl sugar esters and an ellagitannin such as 1-*O*-caffeoyl xylose, 3,6-di-*O*-caffeoyl glucose, 2,3-*O*-hexahydroxydiphenoyl-4,6-*O*-sanguisorboy glucose.[11] In addition, 1-*O*-*E*-cinnamoyl-β-D-xylopyranoside, 1-*O*-*E*-cinnamoyl-β-D-rhamnopyranoside and other cinnamoyl glycosides were isolated from ripe fruits of the Chilean strawberry *Fragaria chiloensis* ssp. *Chiloensis*.[12] Cheel et al. investigated antioxidant capacity of these compounds. We previously reported the synthesis of hydroxycinnamoyl β-D-glucopyranosides by amine-promoted glycosylation without heavy metal catalyst and Novozym 435-catalyzed regioselective transesterification and the evaluation of antioxidant properties of these compounds.[13] In this paper, we investigated the synthesis of hydroxycinnamoyl β-D-xylopyranosides, and described the antioxidant activities of hydroxycinnamic acids such as ferulic acid, caffeic acid, and sinapinic acid and its β-D-xylopyranosides. The corresponding hydroxycinnamoyl β-D-xylopyranosides were synthesized with high yield even with xylosyl bromide instead of glucosyl bromide. The availability of amine-promoted glycosylation without heavy metal catalyst was able to be shown. Their antioxidant activity was evaluated on the basis of their inhibitory effects on the autoxidation of methyl linoleate in bulk system and the radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH·). In all tested compounds, caffeoyl β-D-xylopyranosides showed especially high antioxidant activity and long durability of antioxidative potency compared with free caffeic acid. We report the synthesis of hydroxycinnamoyl β-D-xylopyranosides and its antioxidant properties because the importance of xylosides was confirmed.

2. Experimental

2.1. General

Thin layer chromatography (TLC) was performed on precoated F-254 plates (Merck Ltd, Darmstadt, Germany). Flash column

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http://dx.doi.org/10.1016/j.carres.2013.12.014
0008-6215/Published by Elsevier Ltd.

chromatography was performed on silica gel FL60D (Fuji Silysia Chemical Ltd, Aichi, Japan). Optical rotation was determined with a JASCO P-1010 polarimeter (JASCO corp., Tokyo Japan). IR spectroscopy was performed on an FT-IR 460plus spectrometer (JASCO Corp., Tokyo Japan). ^{1}H and ^{13}C NMR spectra were taken on a JNM-ECA-500 spectrometer with tetramethylsilane (Me_4Si) as the internal standard, and chemical shifts were recorded as δ values. Mass spectra were recorded on an AccuTof GCv 4G (JEOL, Tokyo, Japan) mass spectrometer.

2.2. General procedure for glycosylation of hydroxycinnamic acids

A solution of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (TAXB) (678 mg, 2.0 mmol), corresponding hydroxycinnamic acids (6.0 mmol) and *i*-Pr_2NEt (258 mg, 2.0 mmol) in CH_3CN (10 mL) was stirred for 24 h at room temperature under Ar atmosphere, and 1 g 4 Å molecular sieves were used for water binding. Progress of the reaction was monitored by thin layer chromatography. On the completion of the reaction, the reaction mixture was filtered to remove molecular sieves, and the solvent was evaporated under vacuum, and AcOEt was added to the residue. The resulting solution was then neutralized with aqueous $NaHCO_3$, and extracted with AcOEt, followed by washing with brine. The combined extracts were dried over anhydrous $MgSO_4$, and the solvent was removed by evaporating under vacuum. The crude product was purified by flash chromatography (hexane–AcOEt, 6:1 for **1a**, 1:1 for **1b** and **1c**, 3:1 for **1d**, 2:1 for **1e**) to give **1a**–**e**.

2.2.1. Cinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (1a)

Yield: 70%; white solid; mp = 161–162 °C; R_f = 0.65 (hexane–AcOEt, 1:1); $[\alpha]_D^{25}$ −51.9 (*c* 1.0, THF); IR (KBr): 2950 (C–H), 1752 (C═C—*C*═*O*), 1638 (Ar, C═C), 1380 (*C*—*O*—*C*═O), 1230 (*C*—*O*—*C*═O), 1087 (*C*—*O*—*C*═O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.06 (m, 9H, $CH_3 \times 3$ in OAc at C-2, C-3, and C-4), 3.59 (dd, 1H, *J* = 12.0, 8.7 Hz, H-5a), 4.20 (dd, 1H, *J* = 12.0, 5.0 Hz, H-5b), 5.03 (ddd, 1H, *J* = 8.7, 8.2, 5.0 Hz, H-4), 5.16 (dd, 1H, *J* = 8.7, 6.9 Hz, H-2), 5.27 (dd, 1H, *J* = 8.7, 8.2 Hz, H-3), 5.86 (d, 1H, *J* = 6.9 Hz, H-1), 6.42 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 7.41 (m, 3H, Ph), 7.54 (m, 2H, Ph), 7.76 (d, 1H, *J* = 16.0 Hz, —C(═O)CH═C*H*—); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.7 (–CH_3 in OAc at C-2, C-3, and C-4), 62.8 (C-5), 68.4 (C-4), 69.5 (C-2), 71.0 (C-3), 92.2 (C-1), 116.5 (—C(═O)*C*H═CH—), 128.3, 129.0, 130.9, 133.9 (Ph), 147.0 (—C(═O)CH═*C*H—), 164.7 (—*C*(═O)CH═CH—), 169.3 (—C═O in OAc at C-2), 169.8 (—C═O × 2 in OAc at C-3 and C-4); HRMS: *m*/*z* [M]$^+$ calcd for $C_{20}H_{22}O_9$: 406.1264; found: 406.1281.

2.2.2. *p*-Coumaroyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (1b)

Yield: 67%; white solid; mp = 164–165 °C; R_f = 0.34 (hexane–AcOEt, 1:1); $[\alpha]_D^{25}$ −36.7 (*c* 1.0, THF); IR (KBr): 3405 (O–H), 3006 (Ar, C–H), 1752 (C═C—*C*═*O*), 1607 (Ar, C═C), 1517 (Ar, C═C), 1381 (*C*—*O*—*C*═O), 1239 (*C*—*O*—*C*═O), 1156 (*C*—*O*—*C*═O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.06 (m, 9H, $CH_3 \times 3$ in OAc at C-2, C-3, and C-4), 3.58 (dd, 1H, *J* = 12.6, 8.6 Hz, H-5a), 4.19 (dd, 1H, *J* = 12.6, 5.2 Hz, H-5b), 5.04 (ddd, 1H, *J* = 9.2, 8.6, 5.2 Hz, H-4), 5.15 (dd, 1H, *J* = 8.0, 6.9 Hz, H-2), 5.25 (dd, 1H, *J* = 9.2, 8.0 Hz, H-3), 5.84 (d, 1H, *J* = 6.9 Hz, H-1), 6.25 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 6.86 (m, 2H, Ph), 7.42 (m, 2H, Ph), 7.69 (d, 1H, *J* = 16.0 Hz, —C(═O)CH═C*H*—); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.7 (—CH_3 in OAc at C-2, C-3, and C-4), 62.8 (C-5), 68.4 (C-4), 69.7 (C-2), 71.1 (C-3), 92.1 (C-1), 113.3 (—C(═O)*C*H═CH—), 116.0, 126.4, 130.4 (Ph), 147.1 (—C(═O)CH═*C*H—), 158.7 (Ph), 165.3 (—*C*(═O)CH═CH—), 169.7 (—C═O in OAc at C-2), 170.0 (—C═O × 2 in OAc at C-3 and C-4); HRMS: *m*/*z* [M]$^+$ calcd for $C_{20}H_{22}O_{10}$: 422.1213; found: 422.1204.

2.2.3. Caffeoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (1c)

Yield: 45%; white solid; mp = 172–173 °C; R_f = 0.25 (hexane–AcOEt, 1:1); $[\alpha]_D^{25}$ −30.7 (*c* 1.0, THF); IR (KBr): 3427 (O–H), 2960 (C–H), 1750 (C═C—*C*═*O*), 1602 (Ar, C═C), 1516 (Ar, C═C), 1370 (*C*—*O*—*C*═O), 1226 (*C*—*O*—*C*═O), 1041 (*C*—*O*—*C*═O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.06 (m, 9H, $CH_3 \times 3$ in OAc at C-2, C-3, and C-4), 3.58 (dd, 1H, *J* = 12.0, 8.0 Hz, H-5a), 4.19 (dd, 1H, *J* = 12.0, 5.2 Hz, H-5b), 5.03 (ddd, 1H, *J* = 8.6, 8.0, 5.2 Hz, H-4), 5.15 (dd, 1H, *J* = 9.2, 6.9 Hz, H-2), 5.27 (dd, 1H, *J* = 9.2, 8.6 Hz, H-3), 5.84 (d, 1H, *J* = 6.9 Hz, H-1), 6.20 (d, 1H, *J* = 16.0 Hz, —C(—O)C*H*—CH—), 6.89 (d, 1H, *J* = 8.0 Hz, Ph), 6.99 (dd, 1H, *J* = 8.0, 2.3 Hz, Ph), 7.08 (d, 1H, *J* = 2.3 Hz, Ph), 7.62 (d, 1H, *J* = 16.0 Hz, —C(—O)CH—C*H*—); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.6 (—CH_3 in OAc at C-2, C-3, and C-4), 62.8 (C-5), 68.4 (C-4), 69.7 (C-2), 71.0 (C-3), 92.2 (C-1), 113.6 (—C(—O)*C*H—CH—), 114.4, 115.5, 123.0, 127.0, 144.0, 147.0 (Ph), 147.4 (—C(—O)CH—*C*H—), 165.3 (—*C*(—O)CH—CH—), 169.8 (—C—O in OAc at C-2), 170.0 (—C═O × 2 in OAc at C-3 and C-4); HRMS: *m*/*z* [M]$^+$ calcd for $C_{20}H_{22}O_{11}$: 438.1162; found: 438.1175.

2.2.4. Feruloyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (1d)

Yield: 73%; white solid; mp=141–142 °C; R_f = 0.28 (hexane–AcOEt, 1:1); $[\alpha]_D^{25}$ −26.4 (*c* 1.0, THF); IR (KBr): 3510 (O–H), 2975 (C–H), 1746 (C═C—*C*═*O*), 1595 (Ar, C═C), 1519 (Ar, C═C), 1372 (*C*—*O*—*C*═O), 1227 (*C*—*O*—*C*═O), 1082 (*C*—*O*—*C*═O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.06 (m, 9H, $CH_3 \times 3$ in OAc at C-2, C-3, and C-4), 3.57 (dd, 1H, *J* = 12.4, 9.2 Hz, H-5a), 3.94 (s, 3H, CH_3 in OMe at Ph), 4.19 (dd, 1H, *J* = 12.4, 5.0 Hz, H-5b), 5.03 (ddd, 1H, *J* = 9.2, 8.7, 5.0 Hz, H-4), 5.16 (dd, 1H, *J* = 9.2, 6.9 Hz, H-2), 5.27 (dd, 1H, *J* = 9.2, 8.7 Hz, H-3), 5.84 (d, 1H, *J* = 6.9 Hz, H-1), 6.09 (br s, 1H, OH in Ph), 6.26 (d, 1H, *J* = 15.5 Hz, —C(═O)C*H*═CH—), 6.92 (d, 1H, *J* = 8.0 Hz, Ph), 7.04 (d, 1H, *J* = 2.3 Hz, Ph), 7.08 (dd, 1H, *J* = 8.3, 2.0 Hz, Ph), 7.68 (d, 1H, *J* = 15.5 Hz, —C(═O)CH═C*H*—); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.6 (—CH_3 in OAc at C-2, C-3, and C-4), 56.0 (CH_3 in OMe at Ph), 62.9 (C-5), 68.5 (C-4), 69.7 (C-2), 71.3 (C-3), 92.2 (C-1), 109.4 (Ph), 113.5 (—C(═O)*C*H═CH—), 114.8, 123.7, 126.4, 146.8 (Ph), 147.3 (—C(═O)CH═*C*H—), 148.6 (Ph), 165.1 (—*C*(═O)CH═CH—), 169.5 (—C═O in OAc at C-2), 169.8 (—C═O × 2 in OAc at C-3 and C-4); HRMS: *m*/*z* [M]$^+$ calcd for $C_{21}H_{24}O_{11}$: 452.1319; found: 452.1347.

2.2.5. Sinapoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (1e)

Yield: 70%; white solid; mp = 138–139 °C; R_f = 0.20 (hexane–AcOEt, 1:1); $[\alpha]_D^{25}$ −26.7 (*c* 1.0, THF); IR (KBr): 3461 (O–H), 2947 (C–H), 1758 (C═C—*C*═*O*), 1603 (Ar, C═C), 1517 (Ar, C═C), 1370 (*C*—*O*—*C*═O), 1221 (*C*—*O*—*C*═O), 1045 (*C*—*O*—*C*═O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.07 (m, 9H, $CH_3 \times 3$ in OAc at C-2, C-3, and C-4), 3.56 (dd, 1H, *J* = 12.4, 9.2 Hz, H-5a), 3.93 (s, 6H, $CH_3 \times 2$ in OMe at Ph), 4.19 (dd, 1H, *J* = 12.4, 5.0 Hz, H-5b), 5.04 (ddd, 1H, *J* = 9.2, 8.7, 5.0 Hz, H-4), 5.17 (dd, 1H, *J* = 9.2, 7.3 Hz, H-2), 5.28 (dd, 1H, *J* = 9.2, 8.7 Hz, H-3), 5.84 (d, 1H, *J* = 7.3 Hz, H-1), 5.92 (br s, 1H, OH in Ph), 6.28 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 6.79 (s, 2H, Ph), 7.66 (d, 1H, *J* = 15.5 Hz, —C(═O)CH═C*H*—); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.6 (—CH_3 in OAc at C-2, C-3, and C-4), 56.3 ($CH_3 \times 2$ in OMe at Ph), 63.0 (C-5), 68.5 (C-4), 69.8 (C-2), 71.4 (C-3), 92.3 (C-1), 105.4 (Ph), 113.9 (—C(═O)*C*H═CH—), 125.3, 137.7, 147.2 (Ph), 147.5 (—C(═O)CH═*C*H—), 164.9 (—*C*(═O)CH═CH—), 169.5 (—C═O in OAc at C-2), 169.8 (—C═O × 2 in OAc at C-3 and C-4); HRMS: *m*/*z* [M]$^+$ calcd for $C_{22}H_{26}O_{12}$: 482.1424; found: 482.1397.

2.3. General procedure for regioselective transesterification with Novozym 435

A solution of the corresponding hydroxycinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides (**1a**–**e**) (1.0 mmol), MeOH (384 mg,

12.0 mmol), and Novozym 435 (0.8 g) in *tert*-butyl methyl ether (MTBE) (40 mL) was stirred for 24 h at 50 °C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, Novozym 435 was filtered off the reaction mixture, and the solvent was then removed under vacuum. The residue was purified with flash chromatography ($CHCl_3$–MeOH, 100:1 for **2a**, 20:1 for **2d** and **2e**) or by recrystallized from $CHCl_3$ for **2b**, **2c** to give **2a–e**.

2.3.1. Cinnamoyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (2a)

Yield: 85%; white solid; mp = 183–184 °C; R_f = 0.25 ($CHCl_3$–MeOH, 20:1); $[\alpha]_D^{25}$ −66.6 (*c* 1.0, THF); IR (KBr): 3416 (O–H), 2937 (C–H), 1747 (C═C—*C*═*O*), 1637 (Ar, C═C), 1381 (*C*—*O*—*C*═O), 1240 (*C*—*O*—C═O), 1164 (O–H), 1078 (*C*—*O*—*C*═O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 1.97 (s, 3H, CH_3 in OAc at C-2), 2.02 (s, 3H, CH_3 in OAc at C-3), 3.51 (dd, 1H, *J* = 11.5, 10.3 Hz, H-5a), 3.74 (m, 1H, H-4), 3.90 (dd, 1H, *J* = 11.5, 5.7 Hz, H-5b), 4.90 (dd, 1H, *J* = 9.2, 8.0 Hz, H-2), 5.08 (dd, 1H, *J* = 10.3, 9.2 Hz, H-3), 5.55 (d, 1H, *J* = 3.4 Hz, OH at C-4), 5.83 (d, 1H, *J* = 8.0 Hz, H-1), 6.63 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 7.45 (m, 3H, Ph), 7.70 (d, 1H, *J* = 16.0 Hz, —C(═O)CH═C*H*—), 7.74 (m, 2H, Ph); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.3, 20.7 (—CH_3 × 2 in OAc at C-2 and C-3), 65.9 (C-5), 66.9 (C-4), 70.4 (C-2), 74.5 (C-3), 92.3 (C-1), 116.6 (—C(═O)*C*H═CH—), 128.7, 129.0, 131.0, 133.6 (Ph), 146.6 (—C(═O)CH═*C*H—), 164.4 (—*C*(═O)CH═CH—), 169.2 (—C═O in OAc at C-2), 169.6 (—C═O in OAc at C-3); HRMS: *m*/*z* $[M]^+$ calcd for $C_{18}H_{20}O_8$: 364.1158; found: 364.1170.

2.3.2. *p*-Coumaroyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (2b)

Yield: 86%; white solid; mp = 194–195 °C; R_f = 0.05 ($CHCl_3$–MeOH, 20:1); $[\alpha]_D^{25}$ −74.9 (*c* 1.0, THF); IR (KBr): 3410 (O–H), 3007 (Ar, C–H), 1732 (C═C—*C*═*O*), 1607 (Ar, C═C), 1517 (Ar, C═C), 1384 (*C*—*O*—*C*═O), 1263 (*C*—*O*—*C*═O), 1164 (O–H), 1080 (*C*—*O*—*C*═O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 1.96 (s, 3H, CH_3 in OAc at C-2), 2.02 (s, 3H, CH_3 in OAc at C-3), 3.48 (dd, 1H, *J* = 11.5, 10.3 Hz, H-5a), 3.71 (ddd, 1H, *J* = 10.3, 9.7, 5.7 Hz, H-4), 3.88 (dd, 1H, *J* = 11.5, 5.7 Hz, H-5b), 4.88 (dd, 1H, *J* = 9.2, 8.0 Hz, H-2), 5.06 (dd, 1H, *J* = 9.7, 9.2 Hz, H-3), 5.54 (br s, 1H, OH at C-4), 5.79 (d, 1H, *J* = 8.0 Hz, H-1), 6.36 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 6.80 (d, 2H, *J* = 8.6 Hz, Ph), 7.57 (d, 2H, *J* = 8.6 Hz, Ph), 7.60 (d, 1H, *J* = 17.2 Hz, —C(═O)CH═C*H*—), 10.12 (br s, 1H, OH in Ph); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.3, 20.7 (—CH_3 × 2 in OAc at C-2 and C-3), 65.9 (C-5), 66.9 (C-4), 70.5 (C-2), 74.6 (C-3), 92.1 (C-1), 112.5 (—C(═O)*C*H═CH—), 115.8, 124.7, 130.8 (Ph), 146.9 (—C(═O)CH═*C*H—), 160.4 (Ph), 164.8 (—*C*(═O)CH═CH—), 169.2 (—C═O in OAc at C-2), 169.6 (—C═O in OAc at C-3); HRMS: *m*/*z* $[M]^+$ calcd for $C_{18}H_{20}O_9$: 380.1107; found: 380.1125.

2.3.3. Caffeoyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (2c)

Yield: 83%; white solid; mp = 178–179 °C; R_f = 0.14 ($CHCl_3$–MeOH, 20:1); $[\alpha]_D^{25}$ −77.6 (*c* 1.0, THF); IR (KBr): 3512 (O–H), 2949 (C–H), 1732 (C═C—*C*═*O*), 1609 (Ar, C═C), 1516 (Ar, C═C), 1381 (*C*—*O*—*C*═O), 1257 (*C*—*O*—*C*═O), 1035 (*C*—*O*—*C*═O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 1.95 (s, 3H, CH_3 in OAc at C-2), 2.01 (s, 3H, CH_3 in OAc at C-3), 3.48 (dd, 1H, *J* = 11.5, 9.7 Hz, H-5a), 3.71 (ddd, 1H, *J* = 9.7, 9.2, 5.2 Hz, H-4), 3.90 (dd, 1H, *J* = 11.5, 5.2 Hz, H-5b), 4.87 (dd, 1H, *J* = 9.7, 8.0 Hz, H-2), 5.05 (dd, 1H, *J* = 9.7, 9.2 Hz, H-3), 5.77 (d, 1H, *J* = 8.0 Hz, H-1), 6.21 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 6.78 (d, 1H, *J* = 8.0 Hz, Ph), 7.02 (dd, 1H, *J* = 8.0, 2.3 Hz, Ph), 7.05 (d, 1H, *J* = 2.3 Hz, Ph), 7.51 (d, 1H, *J* = 15.5 Hz, —C(═O)CH═C*H*—), 9.30 (br s, 1H, OH in Ph); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.2, 20.5 (—CH_3 × 2 in OAc at C-2 and C-3), 65.8 (C-5), 66.9 (C-4), 70.4 (C-2), 74.5 (C-3), 92.1 (C-1), 112.3 (—C(═O)*C*H═CH—), 115.0, 115.7, 121.7, 125.1 (Ph), 147.1 (—C(═O)CH═*C*H—), 148.9 (Ph), 164.5 (—*C*(═O)CH═CH—), 169.1 (—C═O in OAc at C-2), 169.5 (—C═O in OAc at C-3); HRMS: *m*/*z* $[M]^+$ calcd for $C_{18}H_{20}O_{10}$: 396.1057; found: 396.1057.

2.3.4. Feruloyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (2d)

Yield: 89%; white solid; mp = 74–75 °C; R_f = 0.22 ($CHCl_3$–MeOH, 20:1); $[\alpha]_D^{25}$ −60.7 (*c* 1.0, THF); IR (KBr): 3442 (O–H), 2941 (C–H), 1738 (C═C—*C*═*O*), 1592 (Ar, C═C), 1515 (Ar, C═C), 1376 (*C*—*O*—*C*═O), 1243 (*C*—*O*—*C*═O), 1080 (*C*—*O*—*C*═O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 1.97 (s, 3H, CH_3 in OAc at C-2), 2.03 (s, 3H, CH_3 in OAc at C-3), 3.50 (dd, 1H, *J* = 12.0, 10.9 Hz, H-5a), 3.73 (m, 1H, H-4), 3.83 (s, 3H, CH_3 in OMe at Ph), 3.91 (dd, 1H, *J* = 12.0, 5.7 Hz, H-5b), 4.91 (dd, 1H, *J* = 8.0, 9.7 Hz, H-2), 5.08 (dd, 1H, *J* = 10.3, 9.7 Hz, H-3), 5.56 (br s, 1H, OH at C-4), 5.83 (d, 1H, *J* = 7.4 Hz, H-1), 6.47 (d, 1H, *J* = 16.0 Hz, —C(═O)C*H*═CH—), 6.82 (d, 1H, *J* = 8.0 Hz, Ph), 7.13 (dd, 1H, *J* = 8.6, 1.7 Hz, Ph), 7.36 (d, 1H, *J* = 1.7 Hz, Ph), 7.61 (d, 1H, *J* = 15.5 Hz, —C(═O)CH═C*H*—), 9.72 (br s, 1H, OH in Ph); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.4, 20.7 (—CH_3 × 2 in OAc at C-2 and C-3), 55.7 (CH_3 in OMe at Ph), 65.9 (C-5), 67.0 (C-4), 70.6 (C-2), 74.7 (C-3), 92.1 (C-1), 111.3 (Ph), 112.8 (—C(═O)*C*H═CH—), 115.5, 124.0, 125.3 (Ph), 147.3 (—C(═O)CH═*C*H—), 148.0, 149.9 (Ph), 164.9 (—*C*(═O)CH═CH—), 169.3 (—C═O in OAc at C-2), 169.7 (—C═O in OAc at C-3); HRMS: *m*/*z* $[M]^+$ calcd for $C_{19}H_{22}O_{10}$: 410.1213; found: 410.1258.

2.3.5. Sinapoyl 2,3-di-*O*-acetyl-β-D-xylopyranoside (2e)

Yield: 83%; pale yellow solid; mp = 85–86 °C; R_f = 0.18 ($CHCl_3$–MeOH, 20:1); $[\alpha]_D^{25}$ −52.1 (*c* 1.0, THF); IR (KBr): 3455 (O–H), 2944 (C–H), 1731 (C═C—*C*═*O*), 1605 (Ar, C═C), 1516 (Ar, C═C), 1376 (*C*—*O*—*C*═O), 1225 (*C*—*O*—*C*═O), 1081 (*C*—*O*—*C*═O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 1.97 (s, 3H, CH_3 in OAc at C-2), 2.02 (s, 3H, CH_3 in OAc at C-3), 3.50 (t, 1H, *J* = 10.6 Hz, H-5a), 3.73 (br s, 1H, H-4), 3.81 (s, 6H, CH_3 × 2 in OMe at Ph), 3.90 (q, 1H, *J* = 5.7 Hz, H-5b), 4.89 (dd, 1H, *J* = 9.7, 8.0 Hz, H-2), 5.07 (t, 1H, *J* = 9.5 Hz, H-3), 5.55 (br s, 1H, OH at C-4), 5.84 (d, 1H, *J* = 8.0 Hz, H-1), 6.53 (d, 1H, *J* = 15.5 Hz, —C(═O)C*H*═CH—), 7.06 (s, 2H, Ph), 7.62 (d, 1H, *J* = 15.5 Hz, —C(═O)CH═C*H*—), 9.07 (br s, 1H, OH in Ph); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.4, 20.7 (—CH_3 × 2 in OAc at C-2 and C-3), 56.1 (CH_3 × 2 in OMe at Ph), 65.9 (C-5), 67.0 (C-4), 70.6 (C-2), 74.8 (C-3), 92.0 (C-1), 106.7 (Ph), 113.2 (—C(═O)*C*H═CH—), 124.0, 138.9 (Ph), 147.7 (—C(═O)CH═*C*H—), 148.0 (Ph), 164.9 (—*C*(═O)CH═CH—), 169.3 (—C═O in OAc at C-2), 169.7 (—C═O in OAc at C-3); HRMS: *m*/*z* $[M]^+$ calcd for $C_{20}H_{24}O_{11}$: 440.1319; found: 440.1344.

2.4. Evaluation of scavenging activity for DPPH· radicals

The antioxidant activity of hydroxycinnamoyl β-D-xylopyranosides was estimated by measuring their free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH·) as free radical according to modification of the method of Silva et al.[14] The reaction mixture (10 mL) comprised of freshly made 0.15 mM DPPH· in ethanol (7000 μL), different concentrations of each hydroxycinnamoyl β-D-xylopyranosides (1, 5, and 10 μmol) in 300 μL DMSO, and Tris–HCl buffer (pH 7.4, 100 mM). The reaction mixture was kept for 30 min in a water bath at 25 °C under dark and optical density was measured at 517 nm. DPPH· has an unpaired electron, which gave purple color, and when this electron is balanced, the color is lost. The compounds which can give an electron to the DPPH· can bleached as the decrease in absorbance of the DPPH· solution without test compounds. All analyses were carried out in triplicate.

2.5. Evaluation of the inhibitory effect on autoxidation of methyl linoleate

To 1 g of methyl linoleate, 25 μL of the acetone solution of the test compound was mixed in a 50 mL vial, and the mixture was

agitated under ultrasonic wave for 30 s. A 25 μL aliquot of acetone without sample was added for control. After purging the acetone with nitrogen, the mixture was place in an oven at 40 °C in dark. Final concentration of each sample was 0.05 μmol/g oil. An aliquot of oil sample was dissolved in ethanol, and the conjugated diene absorbance was measured at 234 nm with an UVmini-2400 UV–vis spectrophotometer every 24 h at 20 °C. All tests were run in triplicate.

3. Results and discussion

3.1. Effect of solvent for glycosylation of ferulic acid

We had previously reported on the synthesis of hydroxycinnamoyl β-D-glucopyranosides by amine-promoted glycosylation and feruloyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside was successfully obtained with 74% yield.[13] Therefore, synthesis of feruloyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**1d**) was attempted under the same conditions (Scheme 1). However, the yield was 53% and it was 20% lower compared with the case of feruloyl glucoside. This result showed that optimum reaction conditions are different depending on the type of sugar. Effect of solvent was confirmed by using various types of solvent (Table 1). In the case of using acetone and THF, the yields were 44% and 38%, respectively. These results was about 10% lower compared with those of DMSO and CH_3CN, which were 50% and 53%, respectively. The donor number of DMSO was highest 29.8. However, there was no great difference of donor numbers between other solvents.[15] The acceptor numbers of THF and acetone were smaller compared with those of DMSO and CH_3CN. Generally, Lewis acid as a catalyst such as Ag_2CO_3, $Hg(CN)_2$ and Cs_2CO_3 was used in glycosylation by Koenigs–Knorr method.[16–18] Because solvents that had large acceptor number acts Lewis acid, reactivity of TAXB was improved by bonding between solvent and Br in TAXB. On the other hand, solvents with large donor number was bonded with hydrogen atom in carboxyl group, and promoted the production of carboxylate anion. It was suggested that the reactivity of carboxylate anion was decreased, because solvents had large acceptor number solvated with carboxylate anion and stabilized. Large donor number did not reduce its reactivity by electrostatic repulsion between carboxylate anion. Therefore, the yields of DMSO and CH_3CN were higher than those of THF and acetone.

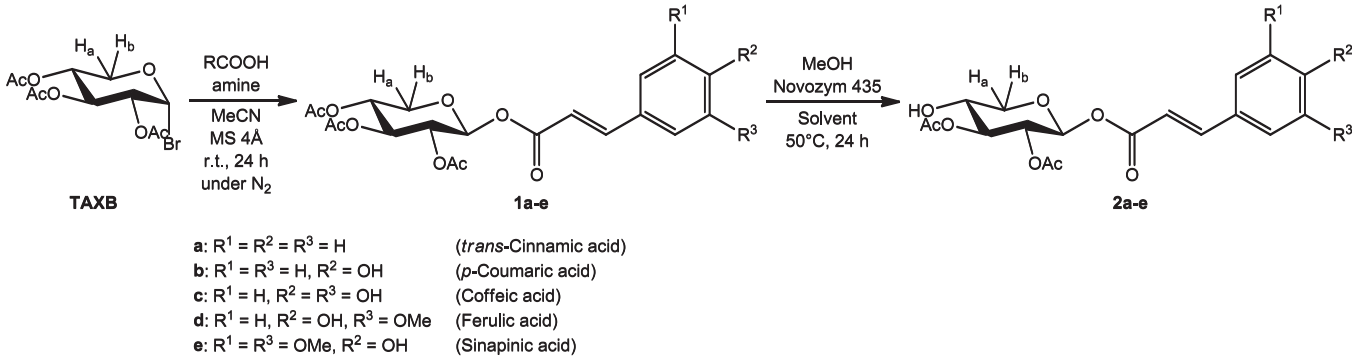
Table 1
Solvent effect on glycosylation of xylosyl bromide with ferulic acid[a]

Entry	Solvent	Donor number[b]	Acceptor number[b]	Yield (%)
1	THF	20.0	8.0	38
2	Acetone	17.0	12.5	44
3	DMSO	29.8	19.3	50
4	MeCN	14.1	18.9	53

[a] Xylosyl bromide: 2.0 mmol, ferulic acid: 4.0 mmol, *i*-Pr_2NEt: 6.0 mmol, MS 4 Å: 1 g, solvent: 10 mL, under N_2, rt, 24 h.
[b] Ref. 15.

3.2. Optimization of amine and amount added

The equal amount and the type of amines were investigated (Table 2). When *i*-Pr_2NEt was used as an amine, **1d** was obtained at the highest 53% yield compared with other types of amines (entry 4). There was no great difference in the case of using Et_3N (entry 6). In contrast, the yields was decreased, and showed 14% and 34%, respectively, in the case of using DBU and Ph_3N (entries 7 and 8). pK_a in acetonitrile of Et_3N and *i*-Pr_2NEt are 18.8 and 18.1, respectively, there is no great difference in basicity.[19,20] However, because *i*-Pr_2NEt is bulky structure compared with Et_3N, nucleophilicity of Et_3N is higher. Nucleophilic attack of carboxylate anion of ferulic acid potentially competed against that of Et_3N. It was assumed that this caused the somewhat decrease of yield of **1d**. The yield was the lowest 14% in the case of using Ph_3N (entry 7). pK_a of Ph_3N was the lowest in the amine used here.[21] When three equal amounts of Ph_3N for TAXB were used, it did not completely dissolve in acetonitrile. It was suggested that proton drawing of the carboxyl group in ferulic acid is difficult for low basicity of Ph_3N, and the decrease of the yield resulted. DBU that had the highest basicity was used, and the yield was somewhat lower compared with *i*-Pr_2NEt and Et_3N (entry 8). It was known that DBU caused dehydrogenation to give alkene.[22–24] It was assumed that because the production of carboxylate anion by dehydrogenation of ferulic acid and the production of alkene occurred competitively, the decrease of the yield was caused. From the above results, *i*-Pr_2NEt is the best as the amine. The optimization of the amine additive amount was investigated. The yield of **1d** was 53% when *i*-Pr_2NEt was added three equal amounts for TAXB (entry 4). However, the yield was decreased and 36% in the case of a 0.5 equal amount (entry 1). *i*-Pr_2NEt improve the reactivity of ferulic acid by drawing a proton of the carboxylic acid to the carboxylate anion. Additionally, it captures HBr produced from TAXB into *i*-$Pr_2NEt{\cdot}HBr$ salt. However, the added amount of *i*-Pr_2NEt is less; it did not completely capture HBr, and the reaction solution becomes acidic. Dissociation constant of ferulic acid is higher than that of HBr. Therefore, the yield is reduced because the formation of carboxylate anion was inhibited, and the reactivity of ferulic acid is reduced to lead the low yield. Two and five equal amounts of *i*-Pr_2NEt caused 52% and 47% yield, respectively (entries 3 and 5). These yields were almost the same with the use of three equal amounts regardless of added amounts. However, the maximum yield of 65% was shown in the case of one equal amount (entry 2). These results showed that the optimum amounts of *i*-Pr_2NEt was one equal amount for TAXB.



Scheme 1. Glycosylation of hydroxycinnamic acids and regioselective transesterification using Novozym 435.

Table 2
Effect of amine and its amount on glycosylation of ferulic acid[a]

Entry	Amine/amount[b] (equiv)	pK_a (MeCN)	Yield (%)
1	*i*-Pr_2NEt/0.5	18.1[c]	36
2	*i*-Pr_2NEt/1.0		65
3	*i*-Pr_2NEt/2.0		52
4	*i*-Pr_2NEt/3.0		53
5	*i*-Pr_2NEt/5.0		47
6	Et_3N/3.0	18.8[d]	49
7	Ph_3N/3.0	1.3[e]	14
8	DBU/3.0	24.3[d]	34

[a] Xylosyl bromide: 2.0 mmol, ferulic acid: 4.0 mmol, MS 4 Å: 1 g, MeCN: 10 mL, under N_2, rt, 24 h.
[b] Amine amount was based on xylosyl bromide.
[c] Ref. 19.
[d] Ref. 20.
[e] Ref. 21.

3.3. Optimization of ferulic acid amount added and glycosylation using other hydroxycinnamic acids

It could be performed to improve the yield of **1d** by optimizing equal amounts of *i*-Pr_2NEt. Optimization of equal amounts of ferulic acid added was investigated to increase the yield of **1d** (Table 3). **1d** was obtained at 65% yield when ferulic acid of two equal amounts to TAXB added (entry 6). The yields were 73% and 69%, respectively, in the case of the addition of three and four equal amounts. There was no great difference in the yield of **1d** compared with the addition of two equal amounts (entries 7 and 8). However, ferulic acid of three equal amounts gave the maximum yield, and these conditions were most suitable. Therefore, glycosylation was subjected using cinnamic acid, *p*-coumaric acid, caffeic acid, and sinapinic acid in addition to ferulic acid. All hydroxycinnamic acid derivatives except *p*-coumaric acid and caffeic acid gave **1** with about 70% yields (entries 1, 7, and 9). Galland et al. reported that glycosylation of hydroxycinnamic acid derivatives that were methylated at carboxyl group with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide was investigated.[25] Methyl esters of 4-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-glucopyranosyl coumarate, ferulate, and caffeate were obtained at 75%, 65%, and 30%, respectively. These results indicated that the competition of carboxylate anion and phenoxy anion caused the yield to decrease when *p*-coumaric acid and caffeic acid reacted with TAXB. It was suggested that the yield of **1** in caffeic acid was decreased compared with *p*-coumaric acid because hydroxy group at ortho position stabilized phenoxy anion and the reactivity of phenoxy anion was increased. Ferulic acid and sinapinic acid had methoxy groups unlike *p*-coumaric acid and caffeic acid. It was seemed that steric hindrance of these methoxy groups inhibited the reaction of TAXB and phenoxy anion, and the decrease of the yield was not occurred. In the case of *p*-coumaric acid, the yield was increased compared with *i*-Pr_2NEt when Et_3N was used (entries 2 and 3). On the other hand, when caffeic acid was used, there was no difference in the yield even using Et_3N compared with *i*-Pr_2NEt (entries 4 and 5). Basicity of Et_3N is slightly lower than that of *i*-Pr_2NEt. *i*-Pr_2NEt was easy to salt formation with phenolic hydroxyl that had high acidity, and salt formation of Et_3N was difficult. The yield was increased by these reasons (entries 2 and 3). Acidity of phenolic hydroxy group in caffeic acid was higher than that of *p*-coumaric acid because caffeic acid had hydroxyl group at ortho position, the difference in the yield did not occur to form salt regardless of the type of amines. All **1** were obtained in about 70% yields except caffeic acid.

Table 3
Effect of amount of ferulic acid on glycosylation and applied the optimal conditions for synthesis of other hydroxycinnamoyl β-D-xylopyranosides[a]

Entry	Carboxylic acid/amount (equiv)	Amine	Product	Yield (%)
1	Cinnamic acid/3.0	*i*-Pr_2NEt	**1a**	70
2	*p*-Coumaric acid/3.0	*i*-Pr_2NEt	**1b**	53
3		Et_3N		67
4	Caffeic acid/3.0	*i*-Pr_2NEt	**1c**	45
5		Et_3N		46
6	Ferulic acid/2.0	*i*-Pr_2NEt	**1d**	65
7	Ferulic acid/3.0			73
8	Ferulic acid/4.0			69
9	Sinapinic acid/3.0	*i*-Pr_2NEt	**1e**	70

[a] Xylosyl bromide: 2.0 mmol, amine: 6.0 mmol, MS 4 Å: 1 g, MeCN: 10 mL, under N_2, rt, 24 h.

3.4. Novozym 435-catalyzed regioselective transesterification of 1

It was known that high polar antioxidants exhibit high antioxidative activity for autoxidation of bulk oil such as methyl linoleate.[26,27] It was seemed that deprotection of acetyl group at C-2, C-3, and C-4 positions without transesterification of aglycon at C-1 position caused the increase of molecular polarity, and antioxidant activities in aqueous and lipophilic medium might been developed. The reaction using base such as NaOH and NaOMe hydrolyzed aglycon at C-1 position because this position had high reactivity. It was reported that acetyl xylan esterase catalyzed the deacetylation in sugar moieties.[28–30] Although acetyl xylan esterase was useful enzyme, the purchase of it was difficult. In constant, lipase is a same category as hydrolase with acetyl xylan esterase, and it can be purchased comparatively cheaply. Novozym 435 had wide substrate specificity. Therefore, Novozym 435-catalyzed transesterification was investigated for the deprotection of acetyl group regioselectively. Additionally, the reactivity and selectivity of Novozym 435 for hydroxycinnamoyl β-D-xyropyranosides (**1**) was compared with that of hydroxycinnamoyl β-D-glucopyranosides. We previously reported lipase-catalyzed regioselective hydrolysis of feruloyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside.[13] Regioselective deacetylation at C-4 and C-6 position was successfully achieved by Novozym 435 without transesterificating ferulic acid ester at C-1 position. Therefore, deacetylation of hydroxycinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides was also performed using Novozym 435 (Table 4). **1d**, **1b**, and **1c** did not dissolve completely in MTBE (entries 2, 3, and 4). Mixed solvent (MTBE; 30 mL and benzene; 10 mL) was used. Reaction progressed with a yield of approximately 80% in all substrates at 24 h. These results indicated that there was no affect for the reaction by mixing of benzene. High resolution mass analysis and 2D NMR of the product obtained was analyzed, and these results showed that these products were hydroxycinnamoyl 2,3-di-*O*-acetyl-β-D-xylopyranosides (**2**) deacetylated at only C-4 position. Compound **2** transesterified only C-4 positioned acetyl group were obtained with about 80% yields in all substrate. These results showed that structural difference in hydroxycinnamic acid derivatives bound to C-1 position did not affect the substrate specificity of Novozym 435. Hydroxycinnamoyl 2,3-di-*O*-acetyl-β-D-glucopyranosides were obtained in a yield of 80–90% at 24 h, and the

Table 4
Novozym 435-catalyzed regioselective transesterification of **1**[a]

Entry	Substrate	Solvent	Product	Yield (%)
1	**1a**	MTBE (40 mL)	**2a**	85
2	**1b**	MTBE/PhH (30 mL/10 mL)	**2b**	86
3	**1c**	MTBE/PhH (30 mL/10 mL)	**2c**	71
4	**1d**	MTBE/PhH (30 mL/10 mL)	**2d**	89
5	**1e**	MTBE (40 mL)	**2e**	83

[a] **1**: 1.0 mmol, MeOH: 12.0 mmol, Novozym 435: 0.8 g, 50 °C, 24 h.

transesterification did not further progress even extending the reaction time. These results indicated that Novozym 435 recognized the whole molecular of substrates, and transesterified only acetyl groups at C-4 and C-6 position. In other words, hydroxycinnamoyl 2,3-di-*O*-acetyl-β-D-glucopyranosides was not fully complying with the substrate specificity of Novozym 435. Reaction mechanism of lipase-catalyzed transesterification was suggested.[31–33] The crystal structure of *Candida antarctica* lipase B (Novozym 435) has been resolved by Uppenberg et al.[34] The active site of *Candida antarctica* lipase B is composed by Ser105, His187, and Asp224. Transesterification was started by attack oxygen atom of serine to carbonyl carbon at acetyl group in substrate. Naoshima et al. reported that it is easy to react as oxygen of serine that is active center of lipase and carbonyl carbon of ester group in substrates is close.[35] It was previously considered that only acetyl groups of hydroxycinnamoyl glucosides at C-4 and C-6 position were selectively transesterified because steric hindrance around these acetyl groups was small. Hydroxycinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides had no *O*-acetyl carbinol group at C-5 position unlike glucopyranosides. The vicinity of C-5 position was vacant spatially. Carbonyl carbon in acetyl group at C-4 position was lower steric hindrance compared with other acetyl groups, and only C-4 acetyl group was transesterified. Acetyl group at C-3 position was close to acetyl group at C-2 position, and acetyl group at C-2 position was close to hydroxycinnamic acids moiety. Distance between carbonyl carbon and oxygen of serine was long because phenyl propane structure of ferulic acid was high steric hindrance, which ferulic acid ester at C-1 position was not reacted. Ferulic acid ester and acetyl groups at C-2 and C-3 position did not transesterify even extend for more than 24 h. Lemke et al. suggested that lipase recognized not size but shape of substrates, and this recognition related to reactivity and selectivity.[36] C-2, C-3, and C-4 position were the same acetyl groups in hydroxycinnamoyl β-D-xylopyranosides (**1**) although C-1 position was ferulic acid ester. In spite of that, Novozym 435 was transesterified only acetyl group at C-4 position. From these results, it was assumed that Novozym 435 recognized the shape of substrate molecule. Hydroxycinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides (**1**) were consistent with substrate specificity of Novozym 435, and hydroxycinnamoyl 2,3-di-*O*-acetyl-β-D-xylopyranosides (**2**) do not consistent was indicated.

3.5. Structural elucidation of xylopyranoside 1d and 2d

The structural elucidation of the product **1d** was based on the 2D NMR spectrum. The assignment of sugar part was described. Because two oxygen atoms bond to the C-1 position of glycoside, H-1 shifts to the downfield compared with the corresponding signals in simple carbohydrates. In addition, the doublet at δ_H 5.84 was assigned to H-1 in the ^{1}H NMR spectrum. The doublet–doublet at δ_H 5.16 correlated with H-1 signal and was assigned to H-2 from the H–H COZY spectrum. In the same way, H-2 was correlated with H-3, H-3 was correlated with H-4 and H-4 was correlated with H-5a and H-5b, respectively. Therefore, the signals at δ_H 5.27, 5.03, 3.57, and 4.19 were assigned to H-3, H-4, H-5a, and H-5b, respectively. The assignment of signals in ^{13}C NMR was based on the HMQC spectrum. The signals C-1 (δ_C 92.2), C-2 (δ_C 69.7), C-3 (δ_C 71.3), C-4 (δ_C 68.5), C-5 (δ_C 62.9), and –CH_3 in OAc (δ_C 20.6) were determined by the corresponding cross-peaks of the ^{1}H and ^{13}C NMR spectra. In addition, the assignment of the carbon signals at δ_C 169.5 and 169.8 were based on the HMBC spectrum. The signal at δ_C 169.8 was correlated with H-3 at δ_H 5.27 and H-4 at δ_H 5.03, and was assigned to —C═O in OAc on C-3 and C-4, respectively. The signal at δ_C at 169.5 was correlated with H-2 at δ_H 5.16, and was assigned to —C═O in OAc on C-2. The sugar part of xylopyranoside **2d** was elucidated in the same way. The doublet at δ_H 5.83 was assigned to H-1. The doublet–doublet at δ_H 4.91 correlated with H-1 in the H–H COZY spectrum was assigned to H-2. Similarly, the signal of H-3 was correlated with H-2, the signal of H-4 was correlated with H-3, the signal of H-5a and H-5b was correlated with H-4. From the H–H COZY spectrum of xylopyranoside **2d**, the doublet–doublet at δ_H 5.08, the multiplet at δ_H 3.73, the doublet–doublet at δ_H 3.50 and the doublet–doublet at δ_H 3.91 were assigned to H-3, H-4, H-5a, and H-5b, respectively. The assignment of ^{13}C NMR was based on the HMQC spectrum. The assignment of signals for C-1 (δ_C 92.1), C-2 (δ_C 70.6), C-3 (δ_C 74.7), C-4 (δ_C 67.0), C-5 (δ_C 65.9), and –OCH_3 (δ_C 55.7) was based on the cross-peak of the ^{1}H and ^{13}C NMR spectra. The assignments of acetoxyl groups of the carbon signals at δ_C 169.3 and 169.7 were based on the HMQC and HMBC spectra. The signal at δ_C 169.3 correlated with H-2 (δ_H 4.91) in the HMBC spectrum was assigned to —C═O in OAc at C-2, while the signal at δ_H 1.97 correlated with —C═O at δ_C 169.3 and was assigned to –CH_3 in OAc at C-2. The signal in δ_C 20.4 correlated with –CH_3 in OAc at H-2 (δ_H 1.97) in the HMQC spectrum was assigned to –CH_3 in OAc at C-2. In addition, the signal at δ_C 169.7 was correlated with H-3 (δ_H 5.08) in the HMBC spectrum, and was assigned to —C═O in OAc at C-3. The signal at δ_H 2.03 correlated with —C═O in OAc at C-3 (δ_C 169.7) in the HMBC spectrum was assigned to –CH_3 in OAc at C-3, while the signal at δ_C 20.7 correlated with –CH_3 in OAc at C-3 (δ_H 2.03) in the HMQC spectrum was assigned to –CH_3 in OAc at C-3. Additionally, the δ_H of H-4 were most shifted 5.03 to 3.73 between **1d** and **2d** compared with the δ_H of H-2 and H-3. Thus, it was elucidated that xylopyranoside **1d** was regioselectively deacetylated at C-4 with Novozym 435 to afford to xylopyranoside **2d**. Other xylopyranosides were elucidated in the same way.

3.6. Radical scavenging of hydroxycinnamic acids and respective β-D-xylopyranosides

The antioxidant properties of hydroxycinnamic acids and its β-D-xylopyranosides synthesized were evaluated by the demonstration of DPPH· radical scavenging activity and the inhibitory effect on autoxidation of methyl linoleate in bulk phase. Figure 1 shows the DPPH· radical scavenging activity of hydroxycinnamic acids and the respective β-D-xylopyranosides. Scavenging activity of all tested compounds increased with concentration in the range of 0.1–1.0 μmol/mL to compare the previous data.[13] Ferulic acid had the best radical scavenging activity compared with other tested compounds, with the activity about 90% in all concentration. The activity decreased in the order ferulic acid > caffeic acid ≈ caffeoyl β-D-xylopyranosides (**1c** ≈ **2c**) ≈ sinapinic acid > sinapoyl β-D-xylopyranosides (**2e** > **1e**) ≈ feruloyl β-D-xylopyranosides (**1d** ≈ **2d**) > *p*-coumaric acid > *p*-coumaroyl β-D-xylopyranosides (**1b** ≈ **2b**). The scavenging activities of both caffeoyl β-D-xylopyranosides (**1c** and **2c**) were almost the same as that of quercetin. Feruloyl β-D-xylopyranosides (**1d** and **2d**) and sinapoyl β-D-xylopyranosides (**1e** and **2e**) had high DPPH· radical scavenging activity similar to that of α-tocopherol. Antioxidants that have many phenolic hydroxy groups show high antioxidant activities. DPPH· radical scavenging activities of ferulic and sinapinic acids showed similar activities compared with that of caffeic acid. Ortho methoxy groups for hydroxyl group at benzene ring improve antioxidative activity in order to stabilize phenoxy radical. Ferulic acid and sinapinic acid showed high radical scavenging activity compared with *p*-coumaric acid because these carboxylic acids had ortho methoxy groups. Although the order of DPPH· radical scavenging activity was almost the same as the case of glucopyranosides, radical scavenging activity of xylopyranosides was generally low compared with that of glucopyranosides. Glucopyranosides had hydroxyl group and relatively high polar acetoxy group at C-6 position. Xylopyranosides did not have C-6 position. Therefore, the water solubility of

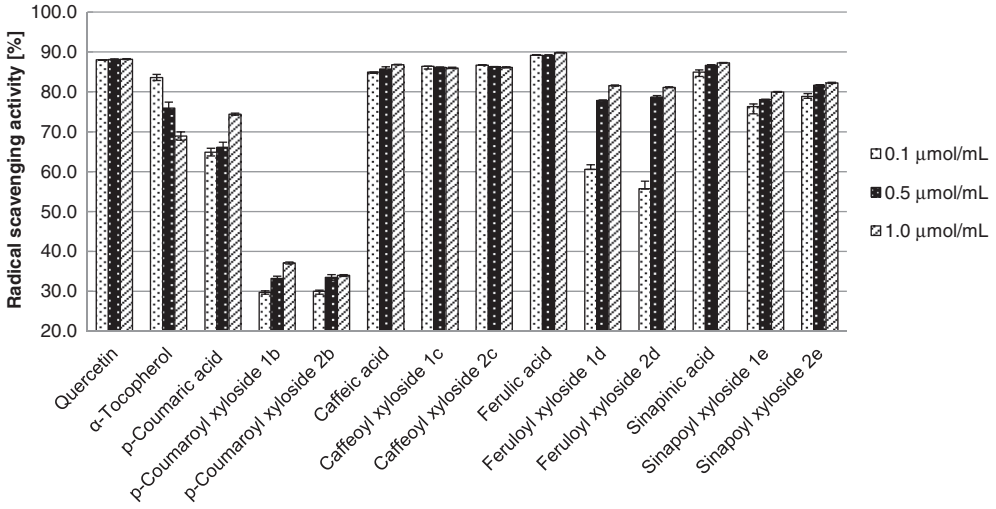


Figure 1. DPPH· radical scavenging activity of free hydroxycinnamic acids and its β-D-xylopyranosides.

glucopyranosides was higher than that of xylopyranosides. Glucopyranosides were easy to scavenge the water-soluble DPPH· radical, and DPPH· radical scavenging activity of xylopyranosides was lower. The radical scavenging activities of all hydroxycinnamoyl β-D-xylopyranosides except caffeic acid were lower than those of the respective free hydroxycinnamic acids. Fukumoto et al. suggested that the size of the steric hindrance of the sugar moiety reduced the antioxidant properties.[37] These results were in good agreement with their suggestion. There was no great difference in the DPPH· scavenging activities between *p*-coumaroyl β-D-glucopyranosides and β-D-xylopyranosides. The difference between acetoxy group (**1b**) and hydroxy group (**2b**) at C-4 position did not affect the DPPH· radical scavenging activities because DPPH· radical scavenging activities of *p*-coumaroyl β-D-xylopyranosides (**1b** and **2b**) were originally low. The activities of caffeoyl β-D-xylopyranosides (**1c** and **2c**) were almost the same compared with that of free caffeic acid. Glycosylation did not affect for DPPH· radical scavenging activity for caffeic acid. Both free caffeic acid and caffeoyl β-D-xylopyranosides (**1c** and **2c**) did not show concentration dependence. Additionally, deacetylation at C-4 position did not affect the activity. It was suggested that there was no effect on DPPH· radical scavenging activity because of the originally high activity of free caffeic acid. Feruloyl β-D-glucopyranosides (**1d** and **2d**) were more dependent on concentration than feruloylβ-D-glucopyranosides. Great reduction of DPPH· radical scavenging activity of feruloyl β-D-xylopyranosides (**1d** and **2d**) in 0.1 μmol/mL was observed. Although the concentration dependence of DPPH· radical scavenging activity was hardly seen in sinapoyl β-D-glucopyranosides, that in sinapoyl β-D-xylopyranosides (**1e** and **2e**) was slightly observed. Xylose moiety reduced the DPPH· radical scavenging activity compared with glucose moiety by above mentioned reason, and it seemed that the concentration dependency was expressed. Difference between acetoxy group (**1d**) and hydroxyl group (**2d**) of feruloyl β-D-xylopyranosides in C-4 position did not affect the DPPH· radical scavenging activity. However, DPPH· radical scavenging activity of **2e** was slightly increased compared with that of **1e**. It was assumed that DPPH· radical scavenging became easy by increase of water solubility.

3.7. Inhibitory effect on autoxidation of bulk methyl linoleate

Formation of hydroperoxides in bulk methyl linoleate at 40 °C was estimated based on the absorbance at 234 nm due to a conjugated methyl linoleate yielded during the initial oxidation stage. Figure 2 shows the inhibitory effect on autoxidation of bulk methyl linoliate using hydroxycinnamic acids and respective β-D-xylopyranosides. The activity decreased in the order caffeoyl β-D-xylopyranosides (**1c** ≈ **2c**) > caffeic acid ≈ sinapinic acid > sinapoyl β-D-xylopyranosides (**2e** > **1e**) > ferulic acid > feruloyl β-D-xylopyranosides (**1d** ≈ **2d**) > *p*-coumaric acid ≈ *p*-coumaroyl β-D-xylopyranosides (**1b** ≈ **2b**). Both caffeoyl β-D-xylopyranosides (**1c** and **2c**) showed the highest antioxidant capacities, which were higher than that of quercetin. Free caffeic acid showed the antioxidant activity similar to quercetin. Free sinapinic acid, sinapoyl β-D-xylopyranosides (**1e** and **2e**), and free ferulic acid showed a higher antioxidant capacity compared with α-tocopherol. Free caffeic acid and caffeoyl β-D-xylopyranosides (**1c** and **2c**) that had two phenolic hydroxyl groups showed the highest activities against autoxidation of methyl linoleate as well as DPPH· radical scavenging activity. Sinapinic acid showed the highest antioxidant capacity, and *p*-coumaric acid was the lowest antioxidant capacity by above mentioned reason. Slight decrease of antioxidant capacity in xylopyranosides was observed compared with that of glucopyranosides as with DPPH radical· scavenging activity. Glucopyranosides had acetoxy group or hydroxyl group at C-6 position. Xylopyranosides show comparatively high solubility for methyl linoleate compared with glucopyranosides. Porter et al. advanced the so-called 'polar paradox' to describe the observation that polar antioxidants are more effective in nonpolar lipids, whereas nonpolar antioxidants are more effective in polar lipid emulsions.[38] To explain this theory, a mechanism was previously postulated on the basis of interfacial properties of different antioxidants.[39] It was seemed that hydroxycinnamoyl β-D-xylopyranosides expressed low antioxidant capacity compared with hydroxycinnamoyl β-D-glucopyranosides for 'polar paradox' theory. Feruloyl and sinapoyl β-D-xylopyranosides showed low antioxidant capacity compared with the respective free carboxylic acids. Solubility of free hydroxycinnamic acids for methyl linoleate was low because of high polar carboxy group, and it was seemed that their antioxidant capacity was higher than that of the respective β-D-xylopyranosides. The antioxidant capacity of **2e** was higher than that of **1e**. Antioxidant capacity of **2e** increased because solubility for methyl linoleate reduced by deacetylation at C-4 position. These results were in good agreement with the 'Polar paradox'. Both caffeoyl β-D-xylopyranosides (**1c** and **2c**) showed higher antioxidant capacities compared with free caffeic acid. Solubility for methyl linoleate decreased compared with free caffeic acid because these caffeoyl β-D-xylopyranosides had high polar functional

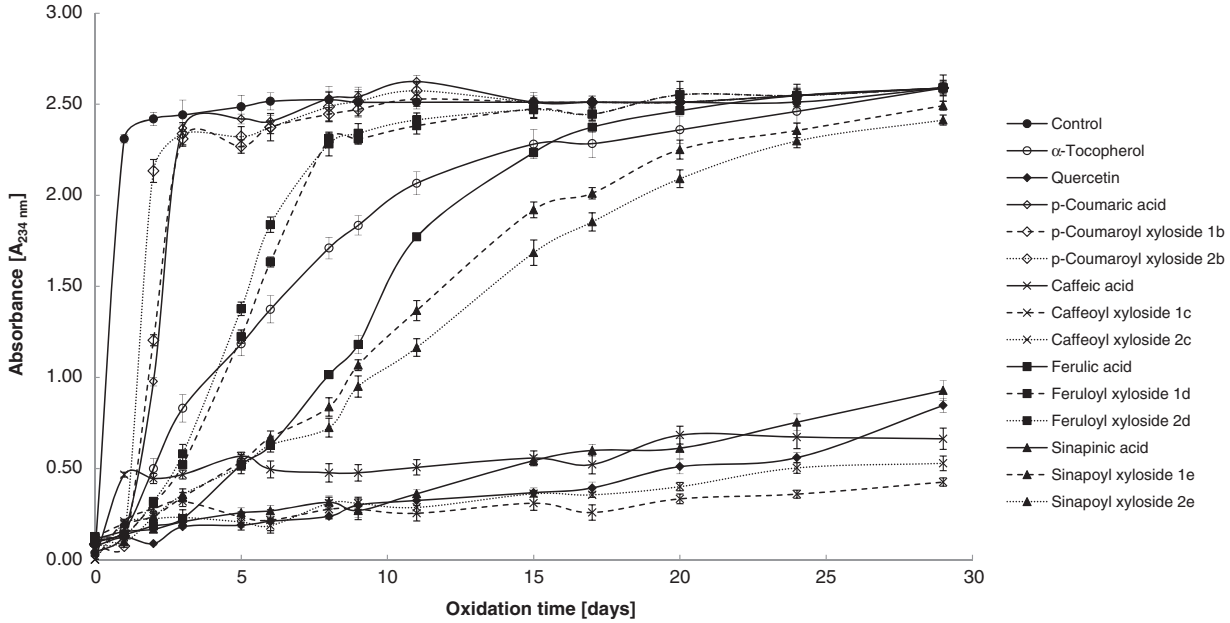


Figure 2. Inhibition of the formation of hydroperoxide in bulk methyl linoleate by hydroxycinnamic acids and its β-D-xylopyranosides.

groups such as acetoxyl and hydroxyl groups. The degree of autoxidation of methyl linoleate in the case of adding caffeoyl β-D-xylopyranosides (**1c** and **2c**) was smaller compared with that of caffeic acid. Especially, the degree was noticeable from 11 to 28 day. Caffeoyl β-D-xylopyranosides can expect further prolonged antioxidative potency. These results showed that xylose moiety was important.

4. Conclusion

Hydroxycinnamoyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides were effectively prepared from various hydroxycinnamic acids except caffeic acid in about 70% yields. Acetoxy group of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides was transesterified at C-4 position regioselectively by Novozym 435. The regioselective deacetylation of only acetoxyl group at C-4 position in these compounds with Novozym 435 is primarily interesting. The antioxidant properties of hydroxycinnamic acids and the respective hydroxycinnamoyl β-D-xylopyranosides were investigated. Caffeoyl β-D-xylopyranosides (**1c** and **2c**) showed the highest DPPH· radical scavenging activities in all hydroxycinnamoyl β-D-xylopyranosides, and its activities were almost the same as quercetin. Feruloyl (**1d** and **2d**) and sinapoyl (**1e** and **2e**) β-D-xylopyranosides showed high DPPH· radical scavenging activity as same as α-tocopherol. DPPH· radical scavenging activity of hydroxycinnamoyl β-D-xylopyranosides except caffeic acid and *p*-coumaric acid derivatives slightly decreased compared with the respective free carboxylic acids. In the case of sinapoyl β-D-xylopyranosides (**1e** and **2e**), the activity of **2e** deacetylated was higher than that of **1e**. Caffeic acid and caffeoyl β-D-xylopyranosides (**1c** and **2c**) had the most effective antioxidant activity among tested hydroxycinnamic acid derivatives against autoxidation of methyl linoleate. Caffeoyl β-D-xylopyranosides (**1c** and **2c**) had high antioxidant activity and long durability of antioxidative potency. The importance of xylose moiety was confirmed from these results. The antioxidant activities of **1c** and **2c** were almost the same as quercetin and higher than that of α-tochopherol. When xylosyl bromide was used instead of glucosyl bromide, the corresponding hydroxycinnamoyl β-D-xylopyranosides was able to be synthesized with high yield. The availability of amine-promoted glycosylation without heavy metal catalyst was shown from these results. It expects to be used by many researchers as environmentally friendly glycosylation method.

Acknowledgement

We are grateful to the Novozymes A/S for the generous gift of Novozym 435.

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