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Enzymatic Synthesis of Aroma Compound Xylosides Using Transfer Reaction by *Trichoderma longibrachiatum* Xylanase

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Enzymatic synthesis of aroma compound xylosides was performed by *Trichoderma longibrachiatum* xylanase. Information concerning the nature of xylosides present in the reaction medium was obtained by GC-EI-MS, by GC-NCI-MS of TFA derivatives, and by positive FAB-MS of the reaction mixtures. Moreover, the structures of isolated benzyl β-D-xylopyranoside and 4-*O*-β-xylopyranosyl-β-D-xylopyranoside were established by ^{1}H and ^{13}C NMR and heteronuclear two-dimensional ($^{1}H-^{13}C$) chemical shift correlation. The results obtained for hexyl and benzyl alcohol xylosides indicated that a reaction implying a transfer of one to two or three xylose units from xylan was involved. The enzyme was able to recognize xylobiose, xylotriose, and xylan as xylose donors. Benzyl xyloside, produced independently of xylobioside and xylotrioside, was found as the major kinetic product of the reaction. Benzyl xyloside was produced in higher quantities and at a higher rate than that obtained for the di- and trixyloside derivatives. The maximum production for benzyl xyloside, 1.29 g/L, was obtained in the presence of hexane (50%) used as cosolvent. Xylosides and xylobiosides of several aroma compounds, (*Z*)-hex-3-en-1-ol, heptan-2-ol, geraniol, nerol, and citronellol, were synthesized in different amounts, from 850 mg/L for (*Z*)-hex-3-en-1-yl xylosides to 1.5 mg/L for citronellyl xylosides. No synthesis occurred when menthol, linalool, and eugenol were used as acceptors.

KEYWORDS: Enzymatic synthesis; xylanase; aroma compound xylosides; xylan; transfer reaction

INTRODUCTION

Aroma compounds present in food are characterized by a high volatility, a more or less important hydrophobicity, and an important heat, oxygen, water, and light instability, resulting in a reduced shelf life. It is necessary to protect these compounds for some applications such as hot beverages, products used after cooking or thawing in a microwave oven, and chewing gum or tobacco (*1*).

Several ways have been explored to protect aroma compounds or to impart a controlled release during processing, storage, or final preparation of food: adsorption on substrates such as lactose or salt; fixation in molten sugars; encapsulation; or formation of inclusion complexes with cyclodextrins. One other possibility is the formation of flavor precursors in which the aroma compounds are covalently bound to an auxiliary component (*1*). Such precursors, more particularly glycosidically bound derivatives, are present in nature, and the aglycon moieties are released by enzymatic or acid-catalyzed hydrolysis (*2*). These glycosides, identified in >170 different plants from 50 botanical families, are present in all plant organs, flowers, fruits, leaves, barks, and roots.

Glycosidically bound aroma compound could be obtained by chemical synthesis using the Koenigs−Knorr modified method. As an example, l-menthyl β-D-galactopyranoside and β-D-mannopyranoside have been obtained in good yield (*3*). Several primeverosides and vicianosides found in tea leaves were also synthesized by using this method (*4*). However, the derivatizated sugars required for synthesis are not always available or easily prepared. Moreover, the chemical synthesis is not generally stereospecific. The synthesis of glucoside acetals by acid-catalyzed addition of an aldehyde or its acetal to methyl α-glucopyranoside has also been studied (*1*).

The use of plant or microbial enzymes for the synthesis of alkyl glycosides with surfactant properties has been extensively studied (*5*−*16*). Reports on the enzymatic synthesis of glycosides corresponding to several aroma compound series are more scarce (*6*, *8*, *17*−*21*).

The aim of the present work was to investigate the enzymatic synthesis of aroma compound glycosides. The synthesis of hexyl and benzyl xylosides was chosen as a model, according to previous studies of the synthesis of alkyl xylosides (*5*, *6*, *8*−*11*, *15*), the general availability of xylan, and the possibility of having an industrial enzyme preparation of *Trichoderma longibrachiatum* containing an endoxylanase activity.

MATERIALS AND METHODS

Enzyme. Xylanase XL-200 from *T. longibrachiatum* was kindly supplied by Saf-Isis (Souston, France).

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Products. Hexane (95% purity) and ethyl acetate (99% purity) were from Prolabo (Paris, France), and acetonitrile (99.8% purity) was from SDS (Peypin, France).

Water, conductivity = 18. 2 MΩ·cm, was furnished by a Purelab Plus system (US Filter).

Aroma compounds used in the present work (purity = 97−100%) were from Fluka, Sigma, or Aldrich (St Quentin Fallavier, France) and Merck (Darmstadt, Germany).

Xylan, from birch wood, D-(+)-xylose and D-(+)-xylobiose (purity > 97%), and *N*-methylbis(trifluoroacetamide) reagent (TFA) were obtained from Sigma.

Xylobiose and xylotriose were also prepared by enzymatic hydrolysis of xylan. Three hundred milliliters of a xylan solution (10 g/L) in sodium acetate buffer, 0.1 M, pH 5, was incubated during 14 h at 40 °C under stirring in the presence of 0.3 mL (14×10^3 IU) of a crude enzymatic preparation of xylanase XL-200. After elimination of the excess of xylan by ethanol precipitation and centrifugation, the supernatant was concentrated to 5 mL, and xylobiose and xylotriose were separated by gel filtration on Bio-Gel P-2 (Bio-Rad, Hercules, CA) and identified by GC-MS after TFA derivatization.

Hydrolysis Activity Measurement. Hydrolysis was carried out by incubation of 250 μL of xylan solution (10 g/L) and 250 μL of diluted (1/1000) crude or partially purified xylanase XL-200 for a total volume of 0.5 mL at 40 °C during 10 min. The liberated reducing sugars were determined by using the Somogyi−Nelson method with xylose as standard. The activity was expressed in international units (IU). One IU is defined as the enzyme amount catalyzing the release of 1 μmol of equivalent xylose per minute.

Partial Purification of Endoxylanase. The industrial XL-200 enzyme preparation (10 mL) was treated by ultrafiltration using an Amicon model 52 cell fitted with a PM 10 membrane at 4 °C under 2×10^5 Pa nitrogen pressure. The preparation was washed with 40 mL of sodium acetate buffer, 20 mM, pH 5, and finally adjusted to 10 mL with the same buffer. The activity of this preparation, called crude enzyme, was 48×10^3 IU/mL.

This crude enzyme was partially purified to eliminate enzymatic activities such as glycosidases and exoxylanase by FPLC using an ÄKTA basic 900 unit (Amersham Pharmacia Biotech, Uppsala, Sweden), with a 20 cm × 5 cm i.d. XK 50/20 Pharmacia Biotech column filled with CM Sepharose LC-6B equilibrated with a sodium acetate buffer, 20 mM, pH 5. The protein elution was followed by spectrophotometry at 260 nm. Crude enzyme preparation (10 mL) was loaded on the column, and elution was performed with sodium acetate buffer at 2 mL/min. An elution gradient 0−40% (v/v) of 0.5 M NaCl was first applied, followed by an isocratic step at 40% of 0.5 M NaCl during 30 min and by a second 40−100% (v/v) NaCl gradient. Twenty milliliter fractions were collected, and the fractions containing the endoxylanase possessing a transfer activity as previously reported by Royer and Nakas (*22*) were pooled and concentrated by ultrafiltration to a final volume of 10 mL in 0.1 M, pH 5, sodium acetate buffer. The activity of this preparation, called partially purified endoxylanase, was 32×10^3 IU/mL.

Enzymatic Synthesis. In a typical experiment, a mixture of 50 mg of xylan, 9 mL of aroma compound (benzyl alcohol or hexanol), and 1 mL of xylanase XL-200 crude or partially purified enzymatic preparation was stirred under magnetic agitation (400 rpm) at 50 °C for 30 min−5 h in a tightly closed flask. The reaction was stopped by heating in boiling water for 10 min, the excess of aroma compound was then distilled at 90 °C under vacuum, and the residue was dissolved in 10 mL of water. The mixture was flash purified on an RP 18 cartridge equilibrated with water; 3 × 2 mL of water was used for washing, and the elution was performed using 3 × 2 mL of methanol. The methanol extract was trifluoroacetylated as indicated below.

When the cosolvent effect was studied, for a constant total volume of 10 mL, either 1−7 mL of benzyl alcohol and 2−8 mL of hexane were used or 4 mL of benzyl alcohol and 5 mL of ethyl acetate or acetonitrile were reacted. A reaction time of 3 h was used for the synthesis of other aroma compound glycosides: (*Z*)-hex-3-en-1-ol, heptan-2-ol, geraniol, nerol, citronellol, linalool, menthol, or eugenol.

Separation of Benzyl Xyloside and Benzyl Xylobioside. The distillation residue, used without flash purification, was concentrated, and 10 mL of a solution containing ~500 mg/L of benzyl xylosides was evaporated under a smooth flow of nitrogen and then suspended in 1 mL of chloroform. This suspension was applied on a 22 × 4.6 cm, 5 μm, Kromasil (silica 5 Å) column (Touzart and Matignon, Paris, France) equilibrated with methanol. The mobile phase, at 1 mL/min, was a chloroform/methanol gradient, 3−8% of methanol during 5 min and then 8−20% of methanol during 10 min. The xyloside elution was followed by spectrophotometry at 260 nm, and purity was checked by GC after trifluoroacetylation.

Trifluoroacetylation. An aliquot of the methanolic solution obtained after elution of the RP 18 column was concentrated to dryness in a vial at 60 °C under a stream of nitrogen. Anhydrous pyridine (20 μL) and 20 μL of trifluoroacetylating (TFA) reagent, *N*-methylbis(trifluoroacetamide) were added. The vial was tightly closed, stirred, heated at 60 °C for 20 min, and then allowed to cool to room temperature (*23*).

Gas Chromatography (GC) Analysis. A 30 m × 0.25 mm i.d., 0.25 μm, bonded phase DB-5MS fused silica capillary column (J&W Scientific, Folsom, CA) was used. The column temperature was isothermal at 125 °C during 5 min, then raised from 125 to 220 °C at 3 °C /min, and increased to 280 °C at 6 °C /min; injector and detector temperatures were 280 and 300 °C, respectively. The flow rate for the carrier gas, hydrogen, was 1.8 mL/min. Split mode injection with a 1/10 ratio and a makeup of 30 mL/min of nitrogen were used. Tentative identification and quantification were made on the basis of retention time relative to phenyl glucoside, used as internal standard.

Gas Chromatography—Mass Spectrometry (GC-MS) Analysis. EI-MS spectra were recorded by coupling a Varian 3400 (Walnut Creek, CA) gas chromatograph, equipped with the same DB-5MS fused silica capillary as that used for GC analysis, to an Automass 020 (Unicam, Argenteuil, France) mass spectrometer. The transfer line was maintained at 290 °C, and the injector temperature was 280 °C. Injections were ~1 μL. The column temperature was programmed as indicated for GC analysis. Helium, at 1.2 mL/min, was the carrier gas. The source temperature was 200 °C, and mass spectra were scanned at 70 eV in the *m*/*z* range of 60−600 mass units.

NCI-MS of trifluoroacetylated glycosides was performed using an HP 5890 (Palo Alto, CA) gas chromatograph fitted with the DB-5MS column and coupled to an HP 5989 mass spectrometer, under the conditions previously described by Chassagne et al. (*23*). The operating conditions were as follows: emission current, 350 μA; energy of the electrons, 200 eV; temperatures of the source and quadrupole, 200 and 120 °C, respectively; reactant gas, methane, at 80 Pa as measured at the source ion gauge. The ion source tuning was carried out in the positive ion mode by using perfluorotributylamine. Mass spectra were scanned in the range *m*/*z* of 100−1400 at 500 ms intervals with a repeller potential of 7 V. The mass spectra reported were recorded when the abundance of pseudo-molecular ions maximized.

Positive Fast Atom Bombardment (FAB). Positive FAB spectra were obtained, from the mixture resulting from enzyme biosynthesis, using a JEOL DX 300 mass spectrometer (Laboratoire de mesures physiques, Université de Montpellier 2, Montpellier, France). Xenon was the inert gas, and nitrobenzyl alcohol was used as matrix.

Nuclear Magnetic Resonance (NMR). Spectra were recorded with a multinuclear Bruker Advance DRX 400 spectrometer (Wissenbourg, France) operating at 400 MHz for ^{1}H, from 0 to 10 ppm, and at 100 MHz for ^{13}C, from 0 to 200 ppm. The solvent was deuterated water, and the chemical shifts were given relative to tetramethylsilane (TMS) used as internal standard in both measurements. For ^{13}C NMR, total irradiation of protons was operated and 6144 scans were accumulated. For the heteronuclear two-dimensional (^{1}H−^{13}C) chemical shift correlation experiment, 0−200 ppm for ^{13}C and 0−7.5 ppm for ^{1}H were scanned.

RESULTS AND DISCUSSION

Benzyl and Hexyl Xylosides Synthesis. GC analysis of TFA derivatives of compounds obtained after 5 h of reaction time, from hexanol or benzyl alcohol and xylan in the presence of crude enzyme, indicated the presence of two and three compounds, respectively. These compounds had retention times

Table 1. Identification of Benzyl and Hexyl Xylosides by GC-MS in Negative Chemical Ionization (NCI) of Their Trifluoroacetyl Derivatives and in Positive Fast Atom Bombardment (FAB) of Glycoside Mixtures

	NCI			FAB		
compd	$[M-113]^-$	M^-	$[M+113]^-$	M	$(M+1)^+$	$(M+Na)^+$
benzyl xyloside	415	528	641	240	241	263
benzyl xylobioside	nd[a]	nd	nd	372	373	395
benzyl xylotrioside	nd	nd	nd	504	505	527
hexyl xyloside	409	522	635	234	235	257
hexyl xylobioside	733	846	959	366	367	389

[a] nd, not detected.

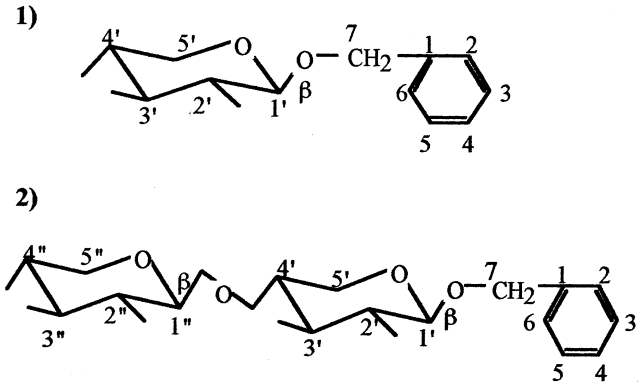

Figure 1. Structures of compound **1**, identified as benzyl β-D-xylopyranoside, and compound **2**, identified as 4-*O*-β-xylopyranosyl-β-D-xylopyranoside.

comparable with those of TFA glycosides previously studied in this laboratory. The EI mass spectra obtained by GC-MS were identical for the three benzyl derivatives or for the two hexyl derivatives. They were characterized by the presence of peaks corresponding to the aglycon moiety of the molecule, *m*/*z* 57 and 85 for hexyl xylosides and *m*/*z* 91 for benzyl xylosides, and peaks at *m*/*z* 193, 278, 307, and 421 characteristic of the xylose moiety of these compounds. These results, as well as the endoxylanase activity of the enzyme preparation, were in agreement with the enzymatic synthesis of two and three compounds, tentatively identified as hexyl and benzyl xylosides.

The presence in the reaction media of hexyl and benzyl xylosides and xylobiosides and benzyl xylotrioside was confirmed by negative chemical ionization (NCI) of their TFA derivatives and/or positive FAB of the glycoside mixture (**Table 1**). NCI mass spectra were characterized by the presence of a molecular ion M^- and fragment at $[M + 113]^-$ resulting from the formation of an adduct with the radical TFA and a fragment at $[M - 113]^-$ formed from M by the release of a radical TFA (*23*). Positive FAB gave pseudo-molecular ions $[M + 1]^+$ and $[M + Na]^+$ (*24*).

Identification of Benzyl Xylosides. The separation of benzyl xyloside, **1**, and benzyl xylobioside, **2** (**Figure 1**), was achieved by column chromatography; 25 and 15 mg of these two products were obtained, respectively. The quantity of xylotrioside synthesized was not sufficient to be recovered. The purity of the two compounds isolated, checked by GC after trifluoroacetylation, was 99%.

Positive FAB mass spectrometric data of the isolated compounds are given **Table 2**. Compound **1** had pseudo-molecular ions at *m*/*z* 241 $[M + 1]^+$ and 263 $[M + Na]^+$ together with a fragment at *m*/*z* 133 characteristic of $[Xyl + H]^+$. For compound **2** pseudo-molecular ions at *m*/*z* 373 $[M + 1]^+$ and 395 $[M + Na]^+$ and a fragment at *m*/*z* 265 $[Xyl - Xyl + H]^+$ were detected; a fragment at *m*/*z* 109 $[AH]2^+$ resulting from the cleavage of the glycosidic linkage (*25*) was also present. Other fragments at *m*/*z* 108, 109, 91, 89, 79, and 51 characteristic of the aglycon moiety were detected in the spectra of the two compounds. These results agree with the identification of compound **1** as benzyl xyloside M = 240 and with the identification of compound **2** as benzyl xylobioside M = 372.

Table 2. Benzyl Xyloside, **1**, and Benzyl Xylobioside, **2**, Positive FAB Data

	m/*z*			
compd	M + 1	M + 23	saccharidic moiety	aglycon moiety
1	241	263	133 $(Xyl + H)^+$	108, 109, 91, 89, 79, 51
2	373	395	295 $(Xyl - Xyl + H)^+$ 109 $(AH)2^+$	108, 109, 91, 89, 79, 51

Table 3. Benzyl Xyloside and Benzyl Xylobioside ^1H NMR and ^{13}C NMR Data in D_2O at 400 and 100 MHz, Respectively

position ^1H (**Figure 1**)	chemical shift (ppm)	proton no.	multiplicity[a]	position ^{13}C (**Figure 1**)	chemical shift (ppm)
			Benzyl Xyloside		
1′	4.37	1	d (J = 7.90 Hz)	1′	102.4
2′, 5′a	3.20	2	m	2′	73.3
3′	3.31	1	m	3′	76.1
4′	3.52	1	m	4′	69.5
5′b	3.85	1	m	5′	65.5
7a	4.61	1	d (J = 11.6 Hz)	7	71.9
7b	4.77	1	d (J = 11.6 Hz)		
			Benzyl Xylobioside		
1′	4.44	1	d (J = 7.80 Hz)	1′	102.3
2′, 5′a 2″, 5′a	3.15–3.26	4	br	2′	73.2
3′	3.44	1	m	3′	74.1
4′	3.68	1	m	4′	76.7
5′b	4.0	1	m	5′	63.3
1″	4.35	1	d (J = 7.86 Hz)	1″	102.2
				2″	73.1
3″	3.32	1	m	3″	75.9
4″	3.53	1	m	4″	69.5
5″b	3.85	1	m	5″	65.5
7a	4.61	1	d (J = 11.6 Hz)	7	72.0
7b	4.77	1	d (J = 11.6 Hz)		

[a] d, doublet; m, multiplet; br, broad peak.

The ^1H NMR spectrum of **1** in D_2O showed signals at 7.51 ppm (5H) corresponding to the monosubstituted aromatic ring and two methylene protons at 4.61 and 4.77 ppm (J = 11.6 Hz). Chemical shifts of H-1′ to H-5′ (**Table 3**) were in agreement with those reported by Drouet et al. (*8*) for ethyl, propyl, isopropyl butyl, and isobutyl β-D-xylosides. The ^{13}C NMR chemical shifts were close to those determined in $CDCl_3$ for octyl β-D-xyloside (*9*). These results and two-dimensional NMR data (not shown) corroborated the occurrence of benzyl xyloside.

The chemical shifts of the anomeric C-1′ at 102.4 and of H-1′ at 4.37 ppm, as well as the coupling constant value measured for H-1′, J = 7.9 Hz, indicated a β-linkage between the xylose unit and benzyl alcohol. The values reported for this coupling constant were 7.8−7.9 Hz for several alkyl β-D-xylosides (*8*). These data clearly demonstrated that compound **1** obtained by transglycosylation from xylan and benzyl alcohol catalyzed by *T. longibrachiatum* endoxylanase could be identified as benzyl β-D-xylopyranoside.

For compound **2**, complete assignment of ^1H and ^{13}C resonances (**Table 3**) was made by the use of the heteronuclear chemical shift correlation diagram. As for benzyl β-D-xyloside, signals at 7.35 ppm (5H) corresponded to a monosubstituted

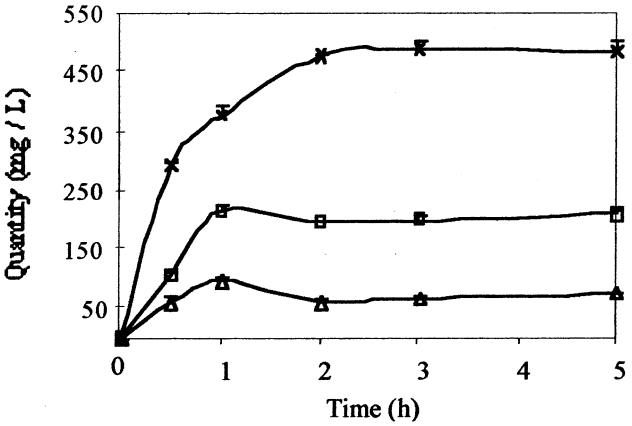

Figure 2. Kinetics of benzyl xyloside formation at 50 °C for 0–5 h, from xylan, 5 g/L, benzyl alcohol 90% (v/v), and xylanase XL-200 preparation: ×, benzyl xyloside; □, benzyl xylobioside; △, benzyl xylotrioside. Concentrations are expressed as grams of product per liter of reaction medium.

aromatic ring, and two protons at 4.61 and 4.77 ppm ($J = 11.6$ Hz) were characteristic of the methylene group of benzyl alcohol. Chemical shifts of H-1′ to H-5′ and H-1″ to H-5″ were similar to those given for benzyl β-D-xylopyranoside (this work) and geranyl β-D-xylopyranosyl β-D-glucopyranoside or geranyl primeveroside (*26*).

The ^{13}C NMR chemical shifts were in agreement with those reported by Matsumura et al. (*9*, *10*) for alkyl β-D-xylosides, in $CDCl_3$. The values obtained for the chemical shifts of the xylose unit in the terminal position, C-1″−C-5″, are close to those obtained in D_2O for the xylose unit of geranyl, linalyl, and *trans*- and *cis*-linalyl-3,6-oxide primeverosides isolated from tea leaves (*27*, *28*). The results obtained were in agreement with a benzyl group linked to two xylose units.

The chemical shifts of the anomeric C-1′ and C-1″ at 102.3 and 102.2 ppm were reasonably similar to those previously reported (*9*, *28*) for β-anomeric C-1′ and C-1″ of the xylopyranose ring. Moreover, the chemical shifts of H-1′ at 4.44 ppm and H-1″ at 4.35 ppm with coupling constants $J = 7.8$ and 7.86 Hz were also in agreement with those given for decyl β-D-xylobioside (*8*) and indicated β-linkages between the xylose moiety and benzyl alcohol and between the two xylose units. Therefore, we concluded that compound **2** was benzyl 4-*O*-β-xylopyranosyl-β-D-xylopyranoside.

Kinetic Study. The kinetics of benzyl xyloside synthesis, from xylan and benzyl alcohol catalyzed by the crude enzyme, was performed under the conditions described under Materials and Methods during 0−5 h (**Figure 2**). Three compounds were produced in the early stages of the reaction; the observed rate of formation of xyloside was more significant than the rates obtained for xylobioside and xylotrioside formation. Xylobioside and xylotrioside amounts reached a maximum after 1 h; beyond this time no more apparent synthesis occurred. Benzyl xyloside, the major product of the reaction, reached a maximum after 2−3 h, its amount remaining relatively constant thereafter. The glycoside amounts remained constant until 15 h of reaction time.

From the kinetic data it could be postulated that the synthesis of benzyl xyloside was independent of xylobioside and xylotrioside formation and that transfer of one to three xylose units from xylan to benzyl alcohol was possible.

Effect of the Nature of the Xylose Donor. To confirm the hypothesis relative to the transfer of one to three xylose units, the reaction was carried out in the presence of xylose, xylobiose, xylotriose, or xylan as donor. As indicated in **Figure 3**,

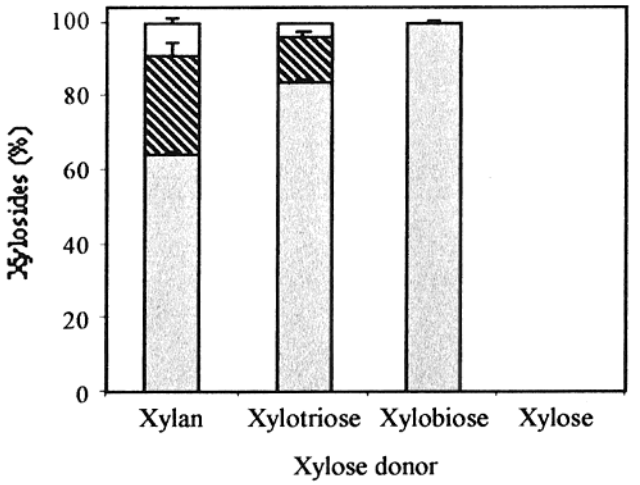

Figure 3. Relative percent of benzyl xyloside, xylobioside, and xylotrioside synthesized for 3 h from several xylose unit donors (xylane, xylotriose, xylobiose, or xylose and benzyl alcohol 90% (v/v) in the presence of xylanase XL-200 preparation): ■, benzyl xyloside; ▨, benzyl xylobioside; □, benzyl xylotrioside.

Table 4. Enzymatic Synthesis for 3 h at 50 °C of Benzyl Xylosides Using Crude and Partially Purified *T. longibrachiatum* Endoxylanase (Results Expressed in Relative Percent)

enzyme	xyloside	xylobioside	xylotrioside
crude	64.6	26.8	8.6
partially purified	40.6	43.1	16.3

xylobiose and xylotriose were, like xylan, substrates for the reaction, whereas xylose was not a substrate. However, when xylobiose was used as donor, only benzyl xyloside was detected.

Transfer reactions by xylanase preparations from *Aureobasidium pullulans* xylanase were previously reported by Matsumura et al. (*4*, *9*−*11*). However, alkyl xylosides were only obtained when the donor was at least a xylotriose unit (*11*). In contrast, the results reported in the present work indicated that only two xylose units were required when *T. longibrachiatum* endoxylanase was used.

Synthesis of benzyl glucoside and benzyl maltoside by transglycosylation catalyzed by α-amylase of *Aspergillus oryzae* was previously reported by Park et al. (*29*).

Effect of Enzyme Purification Degree. The results obtained for enzymatic synthesis of benzyl xylosides with crude and partially purified xylanase are given **Table 4**. The relative percents of xylobiosides and xylotriosides were more important in the presence of partially purified enzyme, whereas the xyloside was quantitatively more important when crude enzyme was used as catalyst. These data were in favor of the existence of a hydrolysis reaction catalyzed by glycosidase activities of the crude preparation (*22*). Such simultaneous hydrolysis was previously reported during the enzymatic synthesis of glucosides or maltosides (*29*).

Cosolvent Effects. To increase the xyloside production, the transglycosylation reaction was performed in the presence of water miscible or water nonmiscible solvents. The effect of organic cosolvents, hexane, ethyl acetate, and acetonitrile (50% v/v), on benzyl xyloside synthesis in the presence of crude enzyme is reported **Figure 4**. When ethyl acetate or acetonitrile was present in the reaction medium, lesser amounts of the three xylosides were synthesized relative to those obtained in the absence of cosolvent.

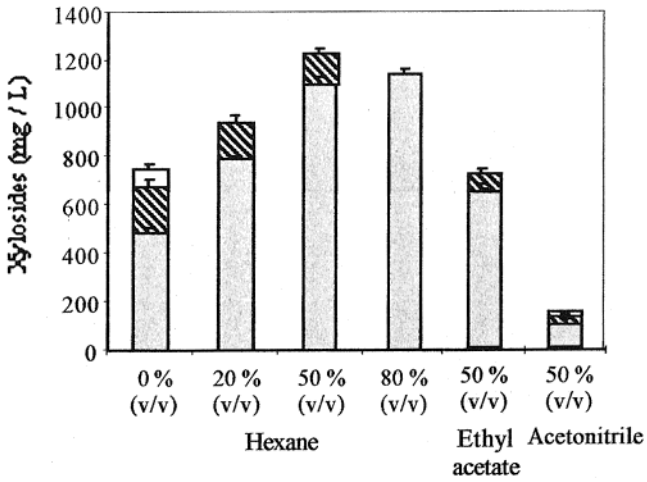

Figure 4. Quantity, in grams of product per liter of reaction medium, of benzyl xyloside, xylobioside, and xylotrioside synthesized from xylan, 5 g/L, benzyl alcool 50% (v/v), and xylanase XL-200 preparation during 3 h at 50 °C in the presence of organic solvent [hexane 0, 20, 50, and 80% (v/v), ethyl acetate, and acetonitrile 50% (v/v)]: ■, benzyl xyloside; ▨, benzyl xylobioside; □, benzyl xylotrioside.

Table 5. Production of Xylosides of Several Aroma Compounds in the Absence or Presence of a Cosolvent (Hexane 50%, v/v) (Results Expressed in Milligrams per Liter of Reaction Medium)

	xyloside		xylobioside		xylotrioside	
	hexane 0%	hexane 50%	hexane 0%	hexane 50%	hexane 0%	hexane 50%
benzyl alcohol	488	1111	202	119	65	1.6
hexanol	382	118	80			
(*Z*)-hex-3-en-1-ol	747	99	71	1.3		
heptan-2-ol	153	20	2.5			
geraniol	11	2.0	tr[a]			
nerol	4.7	1.4	tr			
citronellol	1.5	1.0				

[a] tr, traces.

The effect of several hexane contents, from 20 to 80%, is shown **Figure 4**. The maximum production obtained for benzyl glycosides, 1.29 g/L, was achieved in the presence of 50% (v/v) hexane. The xylobioside and xylotrioside amounts in the reaction medium decreased in the presence of hexane, to completely disappear when 80% (v/v) of this solvent was used.

Synthesis of Primary and Secondary Alcohol Xylosides. Several aroma compound xylosides were obtained by enzymatic synthesis in the standard conditions using xylan as donor and primary or secondary alcohols as acceptors. Xylosides analyzed by GC and identified by positive FAB mass spectroscopy were (*Z*)-hex-3-en-1-yl xyloside, *m*/*z* 255 $[M + Na]^+$; (*Z*)-hex-3-en-1-yl xylobioside, *m*/*z* 387 $[M + Na]^+$; heptan-2-yl xyloside, *m*/*z* 271 $[M + Na]^+$; heptan-2-yl xylobioside, *m*/*z* 403 $[M + Na]^+$; geranyl xyloside, *m*/*z* 309 $[M + Na]^+$; geranyl xylobioside, *m*/*z* 441 $[M + Na]^+$; neryl xyloside, *m*/*z* 309 $[M + Na]^+$; neryl xylobioside, *m*/*z* 411 $[M + Na]^+$; and citronellyl xyloside, *m*/*z* 311 $[M + Na]^+$. No synthesis occurred when menthol, linalool, and eugenol were acceptors.

Quantitative data obtained in the absence or in the presence of 50% (v/v) hexane are given **Table 5**. They showed that for all of the compounds studied, except benzyl alcohol, the yields obtained in the presence of hexane in the reaction medium were lower than the yields obtained when no cosolvent was added. Moreover, if significant amounts of xylosides and xylobiosides were obtained for benzyl alcohol, hexanol, (*Z*)-hex-3-en-1-ol, and, to lesser degree heptan-2-ol, only small amounts were recovered when geraniol, nerol, and citronellol were used. These results were in good agreement with those reported by van Rantwijk et al. (*30*), which indicated that glycosidases exhibited a high degree of chemoselectivity for hydroxyl groups, with the following order of reactivity: primary alcohols, secondary alcohols, and phenols, tertiary alcohols being unreactive.

Xylobiosides were synthesized only when the aglycon was benzyl alcohol, hexanol, hex-3-en-1-ol, or heptan-2-ol; benzyl xylotrioside was the sole trioside detected. These results seemed to indicate that if the enzymatic synthesis of several aroma xylosides is possible, the enzymatic reaction conditions must be defined for each compound.

The results obtained in the present work indicated that enzymatic synthesis of aliphatic and aromatic xylosides was possible using xylobiose, xylotriose, or xylan as xylose donors. The use of an endoxylosidase preparation of *T. longibrachiatum* allowed the production of monoxyloside and several oligoxyloside derivatives. This product diversity could not be achieved when β-xylosidases were used. The fact that only one xylose unit could be transferred to produce benzyl xyloside in the early stage of the reaction indicated that the enzymatic synthesis described in the present paper is suitable for the production of aroma compound heteroglycosides. The enzymatic synthesis of primeverosides is currently being developed in our laboratory.

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