Biotransformation of Benzaldehyde-Type and Acetophenone-Type Derivatives by *Pharbitis nil* Hairy Roots

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The glucosylation of some coumarin and flavone derivatives on incubation with the hairy roots of morning glory (*Pharbitis nil*) was previously reported. We further studied the biotransformation of benzaldehyde- and acetophenone-type derivatives. Vanillin and isovanillin were reduced to alcoholic derivatives and glucosylated at the phenolic and the alcoholic hydroxyl groups. In the case of 3,4-dihydroxybenzaldehyde, the formyl group was reduced and the 3-hydroxyl or 4-hydroxyl groups were glucosylated to give monoglucosides. The 3-hydroxyl group was predominantly glucosylated to the 4-hydroxyl group. 4-β-D-Glucopyranosyloxy-3-methoxybenzylalcohol was obtained in low yield. In time-course experiments with vanillin, it was found that the high-level reduction of the formyl group and glucosylation of the phenolic hydroxyl group occurred, and finally 4-*O*-β-D-glucopyranosylvanillylalcohol was obtained as the main product. In the case of 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, and salicylaldehyde, the formyl groups were reduced, and then the hydroxyl groups at the benyl position were glucosylated to give alcoholic glucosides in relatively high yields. In 4-hydroxy-3-methoxyacetophenone, the 4-hydroxyl group was glucosylated and two dimerized glucosides, biphenyl and biphenylether types, were obtained in low yields. In acetophenone, 1-β-D-glucopyranosyloxy-1-phenylethane and 2-β-D-glucopyranosyloxyacetophenone were obtained. As mentioned above *P. nil* hairy roots showed various biotransformative activities including glucosylation of phenolic and benzylic hydroxyl groups, reduction of the formyl group near the benzene ring, and phenol oxidation dimerization. The glucosylation reaction was especially interesting for the production of valuable glucosides.

Key words *Pharbitis nil* hairy root; glucosylation; reduction; benzaldehyde derivative; acetophenone deivative

Hairy roots induced by infection with *Agrobacterium rhizogenes* are differentiated cells capable of the biosynthesis of secondary metabolites.[1—3] Hairy roots also have a high rate of proliferation. They are therefore expected to become widely used culture systems for the production of useful secondary metabolites[4] and for studies on the production of biologically active compounds and transformation of organic substrates.[5] We reported the production of a stress compound, umbelliferone, upon treatment of morning glory (*Pharbitis nil*) hairy roots with $CuSO_4$ and that umbelliferone was highly glucosylated to a glucoside, skimmin, by *P. nil* hairy roots.[6] We further reported the glucosylation of phenolic compounds, such as coumarin and flavone derivatives by the hairy roots.[7] Many types of secondary metabolites exist as glycosides such as saponines,[8] terpene glycosides,[9] flavone glycosides,[10] and other phenolic glycosides. Some glycosides exist as prebioactive specimens,[11] detoxicated specimens,[12] and bioactive specimens. Thus the biotransformation of various organic substrates to glycosides by plant cell cultures should be useful for supplying glucosides. We are also interested in the glucosylation of other simple phenolic compounds. Vanillin is a common natural product with a benzaldehyde skeleton. The 3- and 4-positions of benzaldehyde and acetophenone derivatives correspond to the 6- and 7-positions of coumarin derivatives. Thus the biotransformation of benzaldehyde- and acetophenone-type derivatives were carried out using *P. nil* hairy roots.

This report deals with the glucosylation of benzaldehyde- and acetophenone-type derivatives having phenolic hydroxyl groups or not, and the reduction of a formyl and a carbonyl group of these compounds.

Results and Discussion

Benzaldehyde-Type Compounds One of the most popular benzaldehyde derivatives, vanillin (**1**) (100 mg), was incubated with the hairy root culture (5 flasks with 200 ml of 1/2 MS medium) for 5 d and gave the products **2** (3 mg), **3** (25 mg), and **5** (8 mg) (obtained as pure compounds after purification with preparative TLC and HPLC). Vanilyl alcohol (**4**) (100 mg) was also incubated with the hairy root culture to give **3** (28 mg) and **5** (6 mg). Isovanillin (**6**) gave **7** (23 mg). Isovanillyl alcohol (**8**) gave **7** (28 mg) and **9** (5 mg). 3,4-Dihydroxybenzaldehyde (**10**) gave **11** (26 mg), **12** (3 mg), and **3** (2 mg). 3,4-Dimethoxybenzaldehyde (**13**) gave **14** (22 mg) and **15** (67 mg). 3,4,6-Trimehoxybenzaldehyde (**16**) gave **17** (56 mg). Salicylaldehyde (**18**) gave **19** (35 mg) and **20** (50 mg).

The fast-atom bombardment (FAB)-MS, ^{1}H- and ^{13}C-NMR spectral data (see Experimental) showed that **2**, **3**, **5**, **7**, and **9** were monoglucosides, an that **2** had a formyl group but **3**, **5**, **7** and **9** had oxymethyl groups. These results indicated that **2** was vanillin-4-*O*-β-D-glucopyranoside and **3**, **5**, **7**, and **9** were glucosides of which the formyl groups were reduced. The position of the glucopyranosyl groups was determined from the heteronuclear multiple-bond correlation (HMBC) spectra. The structures of **3**, **5**, **7**, and **9** were therefore determined to be vanillyl alcohol-4-*O*-β-D-glucopyranoside, vanillyl alcohol-7-*O*-β-D-glucopyraniside, isovanillyl alcohol-3-*O*-β-D-glucopyranoside, and isovanillyl alcohol-7-*O*-β-D-glucopyranoside, respectively.

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The FAB-MS, ^{1}H- and ^{13}C-NMR spectral data (see the Experimental) indicated that **11**, **12**, **15**, **17**, **19**, and **20** were monoglucosides with oxymethyl groups. Their glucopyranoyl group positions were determined from the HMBC spectrum. The structures of **11**, **12**, **15**, **17**, **19**, and **20** were therefore determined to be 3,4-dihydroxybenzyl alcohol-3-*O*-*β*-D-glucopyranoside, 3,4-dihydroxybenzyl alcohol-4-*O*-*β*-D-glucopyranoside, 3,4-dimethoxybenzyl alcohol-7-*O*-*β*-D-glucopyranoside, 3,4,5-trimethoxybenzyl alcohol-7-*O*-*β*-D-glucopyranoside, salicyl alcohol-2-*O*-*β*-D-glucopyranoside, and salicyl alcohol-7-*O*-*β*-D-glucopyrsnoside, respectively. The structure of **14** was determined to be 3,4-dimethoxybenzyl alcohol based on the spectral data (see Experimental).

The hairy roots had good ability to reduce a formyl group of benzaldehyde derivatives to a hydroxymethyl group. The glucosylation of phenolic hydroxyl groups of benzaldehyde derivatives and reduced benzyl alcohol derivatives was also easily carried out, but the primary alcoholic hydroxyl groups were only slightly glucosylated by the hairy roots. Thus, in the case of benzaldehyde with phenolic hydroxyl groups, the final main product is benzyl alcohol derivatives with *O*-*β*-D-glucopyranosyl groups at a phenolic hydroxyl group. But in the case of benzaldehyde derivatives lacking a phenolic hydroxyl group, the main product is a benzyl alcohol derivative with *O*-*β*-D-glucopyranosyl groups at the alcoholic hydroxyl groups. Transformation of benzaldehyde-type derivatives and isolation yields of products are shown in Fig. 1.

Acetophenone Derivatives 4-Hydroxy-3-methoxyacetophenone (**21**) gave **22** (12 mg), **23** (3 mg), and **24** (3 mg). Acetophenone (**25**) gave **26** (9 mg) and **27** (5 mg). The spectral data of **22** showed that it is a monoglucoside of **21**. Thus the structure of **22** was determined to be 4-hydroxy-3-methoxyacetophenone-4-*O*-*β*-D-glucopyranoside.

The FAB-MS of **23** showed a pseudomolecular ion at *m*/*z* 515 $[M+Na]^+$ for $C_{24}H_{28}O_{11}Na$, and *m*/*z* 493 $[M+H]^+$ for $C_{24}H_{29}O_{11}$. The ^{1}H-NMR spectrum of **23** showed the presence of two acetyl groups [δ 2.47 (3H, s), 2.54 (3H, s)], two methoxyl groups [δ 3.89 (3H, s), 3.92 (3H, s)], five aromatic H [δ 6.90 (1H, d, J=8.5 Hz), 7.16 (1H, d, J=2.0 Hz), 7.44 (1H, d, J=2.0 Hz), 7.57 (1H, dd, J=8.5, 2.0 Hz), 7.63 (1H, d, J=2.0 Hz)], and an *β*-D-glucopyranosyl moiety [δ 3.55 (1H, dd, J=11.5, 5.5 Hz, H-6″), 3.67 (1H, dd, J=11.5, 2.5 Hz, H-6″), 5.17 (1H, d, J=7.5 Hz, H-1″)]. The ^{13}C-NMR data of **23** also showed the presence of two acetyl groups (δ 26.4, 26.5, 198.6, 199.2), two methoxyl groups (δ 56.6, 57.0), 12 aromatic carbons (δ 109.6, 113.0, 114.4, 118.9, 124.0, 134.3, 134.4, 142.4, 150.2, 151.4, 151.8, 154.9), and a glucopyranosyl moiety (δ 62.4, 71.2, 75.6, 77.7, 78.3, 103.9). These data indicated that **23** was a dimeric glucoside derivative of **21**. The coupling patterns of the aromatic H showed the presence of 1,2,4-trisubstituted and 1,2,3,5-tetrasubstituted benzene rings and a biphenyl ether structure. Dimeric and glucosylated positions of **23** were determined from the HMBC experiments of **23** (see Fig. 2). The structure of **23** was therefore determined to be 5,4′-diacetyl-3,2′-dimethoxy-2-*β*-D-glucopyranosyloxybiphenyl ether.

The FAB-MS of **24** showed a pseudo molecular ion at *m*/*z* 515 $[M+Na]^+$ $C_{24}H_{28}O_{11}Na$, and *m*/*z* 493 $[M+H]^+$ for $C_{24}H_{29}O_{11}$. The ^{1}H- and ^{13}C-NMR spectral data (see Experimental) for **24** showed that **24** is also a dimeric glucoside of **21**. Product **24** showed the presence of two pairs of *m*-coupled aromatic H at δ 7.49 (1H, d, J=2.0 Hz), 7.53 (1H, d, J=2.0 Hz), 7.41 (1H, d, J=2.0 Hz), and 7.71 (1H, d, J=2.0 Hz). This indicated that **24** had a biphenyl structure from the dimerization of **21**. The dimeric and glucosy positions of **24** were determined from the HMBC experiments (see Fig. 2). The structure of **24** was therefore determined to be 5,5′-diacetyl-3,3′-dimethoxy-2-*β*-D-glucopuranosyloxy-2′-hydroxybiphenyl.

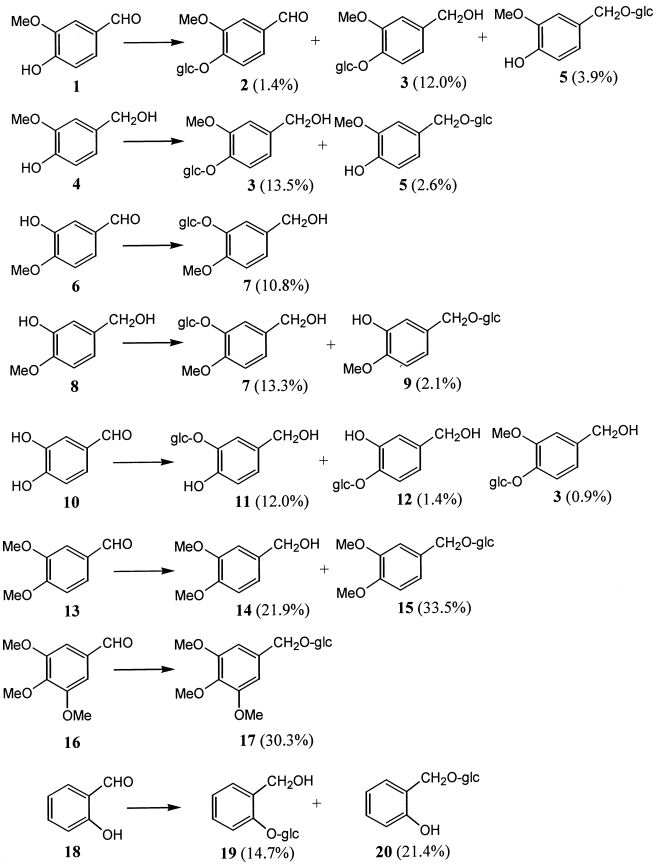

Fig. 1. Transformation of Benzaldehyde-Type Derivatives by *Pharbitis nil* Hairy Roots and Isolation Yields of Products

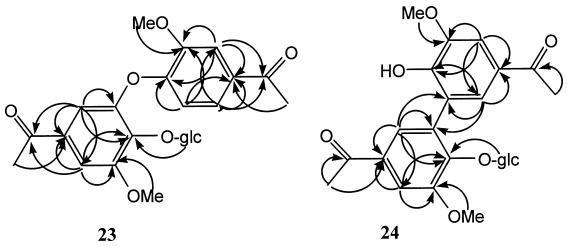

Fig. 2. Significant HMBC Correlations of Dimeric Derivatives **23** and **24**

The dimeric products **23** and **24** should be produced from **21** through a phenol oxidative coupling reaction[13] followed by glucosylation. The dimeric positions are consistent with a phenol oxidative coupling reaction. The phenol oxidative coupling reaction is important for the formation of some types of natural products,[14] such as lignins, lignans, tannins, stilbene polymers, *etc.* These reactions are catalyzed by oxidative enzymes, such as catalase, peroxidase, laccase, and tyrosinase, and some types of nonenzymatic oxidation. Compound **21** was not dimerized and recovered from the medium after incubation of **21** with the medium without the hairy

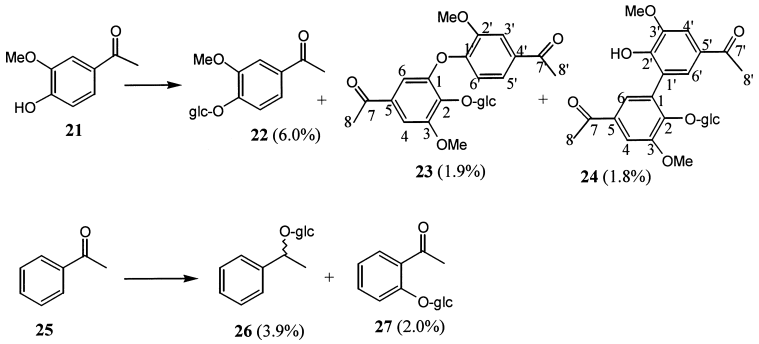

Fig. 3. Transformation of Acetophenone-Type Derivatives by *Pharbitis nil* Hairy Roots and Isolation Yields of Products

roots. Thus the formation of **23** and **24** from **21** should be carried out through a biologic process *via* dimerization in the presence of hairy roots in the culture medium.

FAB-MS, ^{1}H-, and ^{13}C-NMR data for **26** showed that **26** is a glucopyranoside of the reduced product of **25**. The structure of **26** was therefore determined to be 1-*β*-D-glucopyranosyloxy-1-phenylethane. The structure of **27** was assumed to be 2-*β*-D-glucopyranosyloxyacetophenone based on the ^{1}H- and ^{13}C-NMR data and comparison with the authentic data.[7] Transformation of acetophenone-type derivatives and isolation yields of products are shown in Fig. 3.

Time Course of Vanillin (1) Transformatioin by *P. nil* Hairy Roots The amount of vanillin (**1**) incubated with the hairy root culture decreased to zero in a short time (in 6 h) in the medium and vanillyl alcohol (**4**) appeared a few hours after the addition of **1** to the hairy root culture. The level of **4** in the medium reached a maximum at 12 h and decreased thereafter. In the hairy roots, the level of vanillin-4-*O*-*β*-D-glucopyranoside (**2**) increased, peaking 6 h after the addition, and then decreased. On the other hand, the level of vanillyl alcohol-4-*O*-*β*-D-glucopyranoside (**3**) in the hairy roots increased with the the decrease in the level of **2** and **4**. The level of vanillyl alcohol-7-*O*-*β*-D-glucopyranoside (**5**) also changed the same as that of **3** (see Fig. 4). Glucosides, **2**, **3**, and **5** were not identified in the medium. These results indicate that the reduction of the formyl group and glucosylation at C-4 of **1** were carried out to give **4** and **2** first. *O*-Glucosylation of **4** and reduction of **2** continuously gave **3** as the main product. Some of **4** was glucosylated at C-7 to give **5**. Aglycons **1** and **4** were transferred through the cell membrane but glucosides **2**, **3**, and **5** were not transferred through the cell membrane from the hairy roots to the medium. The reduction of a formyl group of **1** was carried out at the same level as glucosylation at C-4 of **1** (see Fig. 5). In the time-course experiment, production of **4** was observed, but **4** could not be isolated from the incubation experiment on **1** because of overlaping with constituents of the hairy roots in a preparative TLC.

P. nil hairy roots showed not only a potent ability to glucosylate the phenolic hydroxyl group but also to reduce the formyl group on the aromatic ring along with an ability to glucosylate the alcoholic hydroxyl group at the benzyl position, methylation of the phenolic hydroxyl group, and an oxidative phenol coupling reaction. In the case of acetophenone, reduction of the carbonyl group and hydroxylation at the neighboring position of the acetyl group were observed. Of these various reactions in which the hairy roots were involved, glucosylation was especially interesting for the biotransformation of phenolic compounds to new types of glucosides. Isolation yields of the glucosides should be improved by optimization of the incubation conditions and isolation procedurs. The substrate specificity of the biotransformation of further phenolic compounds must be investigated for actual application. We are studying cloning of glucosyltransferase genes and their expression to clarify the substrate specificity of the enzyme.

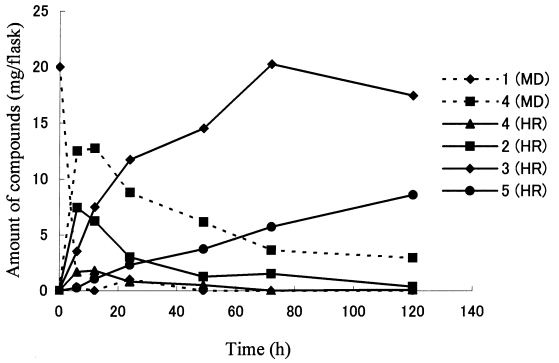

Fig. 4. Time-Course of Transformation of Vanillin (**1**) by *Pharbitis nil* Hairy Root Culture

After incubation of **1** with the hairy root culture, amounts of transformed products, vanillyl alcohol (**4**), vanillin 4-*O*-*β*-D-glucopyranoside (**2**), vanillyl alcohol 4-*O*-*β*-D-glucopyranoside (**3**), vanillyl alcohol 7-*O*-*β*-D-glucopyranoside (**5**), and **1** in the medium (MD) and the hairy roots (HR) were determined. Glucosides, **2**, **3**, and **5**, were not identified in the medium and **1** was not identified in the hairy roots on TLC detection. Trace **2** was identified in the medium 120 h after incubation.

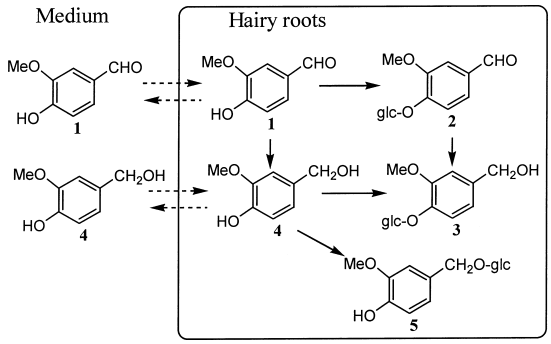

Fig. 5. Biotransformation of Vanillin (**1**) by *Pharbits nil* Hairy Roots *via* Vanillyl Alcohol (**4**) and Vanillin 4-*O*-*β*-D-Glucopyranoside (**2**)

Reduction and glucosilation reactions of **1** were took place in similar level by incubation with the hairy roots. Aglycones **1** and **4** were transferred through the cell membrane but glucosides **2**, **3**, and **5** were not.

Experimental

General Experimental Procedures FAB-MS was measured on a JEOL JMS-AX505F mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL α-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR spectrometer including NOE, HMQC, and HMBC experiments. Chemical shifts are give as δ-values (ppm) with tetramethylsilane (TMS) as an internal standard. Analytical and semipreparative HPLC was carried out using a reverse-phase (ODS, Mightysil RP-18 GP packed column, Kanto Chemical Co.) column. TLC was carried out using precoated silica gel 60F_{254} (Merck) 200×200×0.25-mm plates for analysis and 200×200×0.5-mm plates for the preparation of products.

Chemicals Vanillin was purchased from Kanto Chemical Co. isovanillin, vanillyl alcohol, isovanillyl alcohol, and salicylaldehyde were obtained from Aldrich Chemical. 3,4,5-Trimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, acetophenone, and 4-hydroxy-3-methoxyacetophenone were purchased from Tokyo Kasei Kogyo Co.

Hairy Root Culture, Incubation of Substrates, and Purification of Products The induction of hairy roots of *P. nil* by inoculation of *A. rhizogenes* (MAFF 03-1725) was described in a previous paper.[6] The hairy roots were subcultured every 4 weeks in 1/2 MS medium, supplemented with 3% sucrose. A 100-mg aliquot of each substrate was incubated with the 3-week-old hairy root culture (five flasks including 200 ml of 1/2 MS medium and 20 mg of substrate/flask) for 5 d. After incubation, the culture medium was filtered to give the hairy roots and medium. The hairy roots were lyophilized, mashed, and extracted with boiling water. The extract was passed through a HP 20 (Mitsubishi Chemical) column, washed thoroughly with water, and eluted with MeOH to give a MeOH eluate. The MeOH eluate was analyzed using TLC and HPLC and purified by means of semipreparative HPLC and TLC to give glucosylated products.

The culture medium was passed through an HP 20 column, washed thoroughly with water, and eluted with MeOH to give the MeOH eluate. The MeOH eluate was analyzed for the presence of residual substrate and products.

Incubation of Vanillin (1) The MeOH eluate from the hairy roots incubated with 100 mg of **1** gave pure products **2** (3 mg), **3** (25 mg), and **5** (8 mg). **2**: FAB-MS: *m/z* 337 $[M+Na]^+$ $C_{14}H_{18}O_9Na$, *m/z* 315 $[M+H]^+$ $C_{14}H_{19}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.40 (1H, dd, J=9.5, 9.0 Hz, H-4′), 3.48 (1H, t, J=9.5 Hz, H-3′), 3.48 (1H, ddd, J=9.0, 6.0, 2.0 Hz, H-5′), 3.54 (1H, dd, J=9.5, 8.0 Hz, H-2′), 3.69 (1H, dd, J=11.5, 6.0 Hz, H-6′), 3.88 (1H, dd, J=11.5, 2.0 Hz, H-6′), 3.91 (3H, s, MeO), 5.06 (1H, d, J=7.5 Hz, H-1′), 7.31 (1H, d, J=8.5 Hz, H-5), 7.50 (1H, d, J=1.5 Hz, H-2), 7.52 (1H, dd, J=8.5, 1.5 Hz, H-6), 9.83 (1H, s, H-7). ^{13}C-NMR (CD_3OD) δ: 56.6 (MeO), 62.4 (C-6′), 71.2 (C-4′), 74.7 (C-2′), 77.8 (C-3′), 78.3 (C-5′), 101.7 (C-1′), 116.5 (C-5), 111.7 (C-2), 126.9 (C-6), 132.8 (C-1), 151.2 (C-4), 153.5 (C-3), 192.9 (C-7). **3**: FAB-MS: *m/z* 339 $[M+Na]^+$ $C_{14}H_{20}O_8Na$, *m/z* 317 $[M+H]^+$ $C_{14}H_{21}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.38 (2H, overlap, H-2′, 5′), 3.45 (1H, t, J=9.0 Hz, H-3′), 3.48 (1H, t, J=9.0 Hz, H-2′), 3.68 (1H, dd, J=12.0, 4.0 Hz, H-6′), 3.86 (3H, s, MeO), 3.86 (1H, dd, J=12.0, 1.5 Hz, H-6′), 4.53 (2H, s, H-7), 4.86 (1H, d, J=7.5 Hz, H-1′), 6.87 (1H, dd, J=8.0, 2.0 Hz, H-6), 7.02 (1H, d, J=2.0 Hz, H-2), 7.12 (1H, d, J=8.0 Hz, H-5). ^{13}C-NMR (CD_3OD) δ: 56.6 (MeO), 62.5 (C-6′), 64.9 (C-7), 71.3 (C-4′), 74.9 (C-2′), 77.8 (C-3′), 78.1 (C-5′), 102.9 (C-1′), 112.6 (C-2), 117.9 (C-5), 120.7 (C-6), 137.7 (C-1), 147.2 (C-4), 150.8 (C-3). **5**: FAB-MS: *m/z* 339 $[M+Na]^+$ $C_{14}H_{20}O_8Na$, *m/z* 317 $[M+H]^+$ $C_{14}H_{21}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.22 (1H, dd, J=9.0, 8.0 Hz, H-4′), 3.25 (1H, overlap, H-5′), 3.27 (1H, t, J=9.0 Hz, H-3′), 3.30 (1H, overlap, H-2′), 3.68 (1H, dd, J=12.0, 5.5 Hz, H-6′), 3.85 (3H, s, MeO), 3.89 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.30 (1H, d, J=8.0 Hz, H-1′), 4.58 (1H, d, J=11.3 Hz, H-7), 4.81 (1H, d, J=11.3 Hz, H-7), 6.74 (1H, d, J=8.0 Hz, H-5), 6.82 (1H, dd, J=8.0, 2.0 Hz, H-6), 7.04 (1H, d, J=2.0 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 56.4 (MeO), 62.9 (C-6′), 71.7 (C-7 or C-4′), 71.8 (C-4′ or C-7), 75.1 (C-2′), 78.0 (C-3′), 78.1 (C-5′), 102.7 (C-1′), 113.3 (C-2), 115.8 (C-5), 122.5 (C-6), 130.3 (C-1), 147.4 (C-4), 148.9 (C-3).

Incubation of Vanillyl Alcohol (4) The MeOH eluate from the *P. nil* hairy roots incubated with 100 mg of **4** gave **3** (28 mg) and **5** (6 mg).

Incubation of Isovanillin (6) The MeOH eluate from the hairy roots incubated with 100 mg of **6** gave **7** (23 mg). FAB-MS: *m/z* 339 $[M+Na]^+$ $C_{14}H_{20}O_8Na$, *m/z* 317 $[M+H]^+$ $C_{14}H_{21}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.36 (1H, d, J=9.5, 9.0 Hz, H-4′), 3.41 (1H, ddd, J=9.0, 5.5, 2.0 Hz, H-5′), 3.46 (1H, t, J=9.0 Hz, H-3′), 3.49 (1H, t, J=9.5 Hz, H-2′), 3.68 (1H, dd, J=12.0, 5.5 Hz, H-6′), 3.84 (3H, s, MeO), 3.88 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.51 (2H, s, H-7), 4.90 (1H, d, J=7.5 Hz, H-1′), 6.96 (1H, d, J=8.0 Hz, H-5), 6.98 (1H, dd, J=8.0, 2.0 Hz, H-6), 7.17 (1H, d, J=2.0 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 56.8 (MeO), 62.6 (C-6′), 65.0 (C-7), 71.4 (C-4′), 74.9 (C-2′), 77.8 (C-3′), 78.3 (C-5′), 102.8 (C-1′), 113.5 (C-5), 117.2 (C-2), 122.7 (C-6), 135.8 (C-1), 147.9 (C-3), 150.1 (C-4).

Incubation of Isovanillyl Alcohol (8) The MeOH eluate from the *P. nil* hairy roots incubated with 100 mg of **8** gave **7** (28 mg) and **9** (5 mg). FAB-MS: *m/z*, 339 $[M+Na]^+$ $C_{14}H_{20}O_8Na$, *m/z* 317 $[M+H]^+$ $C_{14}H_{21}O_8$, *m/z* 316 $[M]^+$ $C_{14}H_{20}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.21 (1H, dd, J=9.0, 8.5 Hz, H-4′), 3.24 (1H, overlap, H-5′), 3.28 (1H, t, J=8.0 Hz, H-3′), 3.32 (1H, t, J=9.0 Hz, H-2′), 3.68 (1H, dd, J=12.0, 5.5 Hz, H-6′), 3.83 (3H, s, MeO), 3.88 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.31 (1H, d, J=8.0 Hz, H-1′), 4.54 (1H, d, J=11.0 Hz, H-7), 4.78 (1H, d, J=11.0 Hz, H-7), 6.84 (1H, dd, J=8.0, 1.5 Hz, H-6), 6.87 (1H, d, J=8.0 Hz, H-5), 6.88 (1H, d, J=1.5 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 56.3 (MeO), 62.8 (C-6′), 71.5 (C-4′), 71.7 (C-7), 75.1 (C-2′), 77.9 (C-3′), 78.1 (C-5′), 102.8 (C-1′), 112.3 (C-5), 116.6 (C-2), 121.0 (C-6), 131.7 (C-1), 147.4 (C-3), 148.7 (C-4).

Incubation of 3,4-Dihydroxybenzaldehyde (10) The MeOH eluate from the *P. nil* hairy roots incubated with 100 mg of **10** gave **11** (26 mg), **12** (3 mg), and **3** (2 mg). **11**: FAB-MS: *m/z* 325 $[M+Na]^+$ $C_{13}H_{18}O_8Na$. ^{1}H-NMR (CD_3OD) δ: 3.40 (1H, t, J=9.5 Hz, H-4′), 3.41 (1H, over lap, H-5′), 3.46 (1H, t, J=9.0 Hz, H-3′), 3.49 (1H, t, J=9.0 Hz, H-2′), 3.72 (1H, dd, J=2.5, 5.0 Hz, H-6′), 3.90 (1H, dd, J=12.5, 2.0 Hz, H-6′), 4.48 (2H, s, H-7), 4.77 (1H, d, J=7.5 Hz, H-1′), 6.81 (1H, d, J=7.5 Hz, H-5), 6.91 (1H, dd, J=7.5, 2.0 Hz, H-6), 7.20 (1H, d, J=2.0 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 62.6 (C-6′), 65.1 (C-7), 71.4 (C-4′), 75.0 (C-2′), 77.7 (C-3′), 78.4 (C-5′), 104.5 (C-1′), 117.0 (C-5), 118.2 (C-2), 123.8 (C-6), 134.5 (C-1), 146.7 (C-3), 147.9 (C-4). **12**: FAB-MS: *m/z* 325 $[M+Na]^+$ $C_{13}H_{18}O_8Na$, *m/z* 303 $[M+H]^+$ $C_{13}H_{19}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.39 (2H, overlap, H-4′, 5′), 3.45 (1H, t, J=9.0 Hz, H-3′), 3.48 (1H, J=9.0 Hz, H-2′), 3.72 (1H, dd, J=12.0, 5.0 Hz, H-6′), 3.89 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.73 (1H, d, J=8.5 Hz, H-1′), 4.48 (2H, s, H-7), 6.75 (1H, dd, J=8.5, 2.0 Hz, H-6), 6.85 (1H, d, J=2.0 Hz, H-2), 7.14 (1H, d, J=8.5 Hz, H-5). ^{13}C-NMR (CD_3OD) δ: 62.5 (C-6′), 65.0 (C-7), 71.4 (C-4′), 75.0 (C-2′), 77.7 (C-3′), 78.4 (C-5′), 104.7 (C-1′), 116.1 (C-2), 118.9 (C-5), 119.4 (C-6), 138.6 (C-1), 146.2 (C-4), 148.8 (C-3).

Incubation of 3,4-Dimethoxybenzaldehyde (13) The MeOH eluate from the *P. nil* hairy roots incubated with 100 mg of **13** gave **14** (22 mg) and **15** (67 mg). **14**: FAB-MS: *m/z* 169 $[M+H]^+$ $C_9H_{13}O_5$, *m/z* 168 $[M]^+$ $C_9H_{12}O_3$. ^{1}H-NMR (CD_3OD) δ: 3.80 (3H, s, MeO), 3.83 (3H, s, MeO), 4.52 (2H, s, H-7), 6.87 (1H, dd, J=8.0, 1.5 Hz, H-6), 6.90 (1H, d, J=8.0 Hz, H-5), 6.97 (1H, d, J=1.5 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 56.4 (MeO), 56.5 (MeO), 65.1 (C-7), 112.2 (C-5), 112.8 (C-2), 120.7 (C-6), 135.6 (C-1), 149.8 (C-4), 150.5 (C-3). **15**: FAB-MS: *m/z* 330 $[M]^+$ $C_{15}H_{22}O_8$. ^{1}H-NMR (CD_3OD) δ: 3.22 (1H, dd, J=9.0, 8.0 Hz, H-4′), 3.24 (1H, over lap, H-5′), 3.27 (1H, t, J=8.5 Hz, H-3′), 3.32 (1H, t, J=8.0 Hz, H-2′), 3.67 (1H, dd, J=12.0, 6.0 Hz, H-6′), 3.81 (3H, s, MeO), 3.83 (3H, s, MeO), 3.89 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.30 (1H, d, J=7.5 Hz, H-1′), 4.61 (1H, d, J=11.5 Hz, H-7), 4.84 (1H, d, J=11.5 Hz, H-7), 6.89 (1H, d, J=8.0 Hz, H-6), 6.93 (1H, dd, J=8.0, 2.0 Hz, H-6), 7.08 (1H, d, J=2.0 Hz, H-2). ^{13}C-NMR (CD_3OD) δ: 56.4 (6H, s, MeO×2), 62.8 (C-6′), 71.5 (C-7), 71.7 (C-4′), 75.1 (C-2′), 78.0 (C-3′), 78.1 (C-5′), 102.8 (C-1′), 112.5 (C-5), 113.3 (C-2), 122.0 (C-6), 131.7 (C-1), 150.1 (C-4), 150.4 (C-3).

Incubation of 3,4,5-Trimethoxybenzaldehyde (16) The MeOH from the hairy roots incubated with 100 mg of **16** gave **17** (56 mg). FAB-MS: *m/z* 361 $[M+H]^+$ $C_{16}H_{15}O_9$, *m/z* 360 $[M]^+$ $C_{16}H_{14}O_9$. ^{1}H-NMR (CD_3OD) δ: 3.25 (1H, dd, J=9.0, 8.0 Hz, H-4′), 3.28 (1H, t, J=9.0 Hz, H-3′), 3.33 (1H, t, J=9.0 Hz, H-2′), 3.67 (1H, dd, J=11.5, 6.0 Hz, H-6′), 3.74 (3H, s, MeO at C-4), 3.83 (6H, s, MeO at C-3, 5), 3.89 (1H, dd, J=11.5, 2.0 Hz, H-6′), 6.75 (2H, s, H-2, 6). ^{13}C-NMR (CD_3OD) δ: 56.6 (MeO at C-3, 5), 61.1 (MeO at C-4), 62.8 (C-6′), 71.5 (C-7), 71.8 (C-4′), 75.2 (C-2′), 78.1 (C-3′, 5′), 103.0 (C-1′), 106.4 (C-2, 6), 135.1 (C-1), 138.4 (C-4), 154.5 (C-3, 5).

Incubation of Salicylaldehyde (18) The MeOH eluate from the hairy roots incubated with 100 mg of **18** gave **19** (35 mg) and **20** (50 mg). **19**: FAB-MS: *m/z* 309 $[M+Na]^+$ $C_{13}H_{18}O_7Na$. ^{1}H-NMR (CD_3OD) δ: 3.38 (1H, dd, J=9.5, 8.3 Hz, H-4′), 3.42 (1H, ddd, J=9.5, 5.2, 2.0 Hz, H-5′), 3.45 (1H, t, J=8.3 Hz, H-3′), 3.50 (1H, dd, J=9.2, 7.5 Hz, H-2′), 3.70 (1H, dd, J=12.0, 5.4 Hz, H-6′), 3.89 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.56 (1H, d, J=12.9 Hz, H-7), 4.77 (1H, d, J=12.9 Hz, H-7), 4.86 (1H, overlap, H-1′), 7.02 (1H, dt, J=7.5, 1.1 Hz, H-5), 7.20 (1H, dd, J=7.2, 1.1 Hz, H-3), 7.25 (1H, dt, J=7.2, 1.5 Hz, H-4), 7.32 (1H, dd, J=7.5, 1.5 Hz, H-6). ^{13}C-NMR (CD_3OD) δ: 61.0 (C-7), 62.5 (C-6′), 71.4 (C-4′), 75.1 (C-2′), 78.0 (C-3′), 78.3 (C-5′), 103.4 (C-1′), 117.0 (C-3), 123.7 (C-5), 129.9 (C-4 or 6), 130.0 (C-6 or 4), 167.2 (C-2). **20**: FAB-MS: *m/z* 309 $[M+Na]^+$ $C_{13}H_{18}O_7Na$, *m/z* 287 $[M+H]^+$ $C_{13}H_{19}O_7$. ^{1}H-NMR (CD_3OD) δ: 3.23 (1H, dd, J=8.6, 8.0 Hz, H-4′), 3.29 (2H, overlap, H-3′, 5′), 3.34 (1H, t, J=7.7 Hz, H-2′), 3.68 (1H, dd, J=12.0, 5.4 Hz, H-6′), 3.88 (1H, dd, J=12.0, 2.0 Hz, H-6′), 4.38 (1H, d, J=7.7 Hz, H-1′), 4.72 (1H, d, J=12.0 Hz, H-7), 4.92 (1H, d, J=12.0 Hz, H-

7), 6.77 (1H, dd, J=8.0, 1.0 Hz, H-3), 6.80 (1H, dt, J=7.4, 1.1 Hz, H-5), 7.11 (1H, dt, J=1.7, 8.0 Hz, H-4), 7.33 (1H, dd, J=7.4, 1.7 Hz, H-6). ^{13}C-NMR (CD_3OD) δ: 62.8 (C-6′), 67.8 (C-7), 71.7 (C-4′), 75.1 (C-2′), 78.1 (C-3), 103.5 (C-1′), 116.3 (C-3), 120.5 (C-5), 125.2 (C-1), 130.1 (C-4 or 6), 131.0 (C-6 or 4), 156.6 (C-2).

Incubation of 4-Hydroxy-3-methoxyacetophenone (21) The MeOH eluate from the hairy roots incubated with 100 mg of **21** gave **22** (12 mg), **23** (3 mg) and **24** (3 mg). **22**: FAB-MS: m/z 351 $[M+Na]^+$ $C_{15}H_{20}O_8Na$, m/z 329 $[M+H]^+$ $C_{15}H_{21}O_8$. ^{1}H-NMR (DMSO-d_6) δ: 2.53 (3H, s, Me-8), 3.27 (3H, overlap, H-2′, 3′, 4′), 3.34 (ddd, J=9.5, 5.5, 2.0 Hz, H-5″), 3.44 (1H, dt, J=12.0, 6.0 Hz, H-6′), 3.65 (1H, ddd, J=12.0, 5.0, 2.0 Hz, H-6′), 3.82 (3H, s, MeO), 4.52 (1H, t, J=5.5 Hz, OH at C-6′), 5.02 (1H, br d, J=5.0 Hz, OH), 5.05 (1H, d, J=7.5 Hz, H-1′), 5.31 (1H, br d, J=5.5 Hz, OH), 7.17 (1H, d, J=8.5 Hz, H-5), 7.46 (1H, d, J=2.0 Hz, H-2), 7.57 (1H, dd, J=8.5, 2.0 Hz, H-6), ^{13}C-NMR (DMSO-d_6) δ: 26.4 (C-8), 55.6 (MeO), 60.5 (C-6′), 69.5 (C-4′), 73.1 (C-2′), 76.8 (C-3′), 77.1 (C-5′), 99.4 (C-1′), 110.9 (C-5), 114.1 (C-2), 122.7 (C-6), 130.8 (C-1), 148.7 (C-4), 150.6 (C-3), 196.4 (C-7). **23**: FAB-MS: m/z 515 $[M+Na]^+$ $C_{24}H_{28}O_{11}Na$, m/z 493 $[M+H]^+$ $C_{24}H_{29}O_{11}$. ^{1}H-NMR (CD_3OD) δ: 2.47 (3H, s, Me-8), 2.54 (3H, s, Me-8′), 3.14 (1H, ddd, J=9.0, 5.0, 2.0 Hz, H-5″), 3.23 (1H, dd, J=9.5, 8.0 Hz, H-2″), 3.30 (1H, overlap, H-4″), 3.32 (1H, t, J=9.0 Hz, H-3″), 3.55 (1H, dd, J=11.5, 5.5 Hz, H-6″), 3.67 (1H, dd, J=11.5, 2.5 Hz, H-6″), 3.89 (3H, s, MeO at C-2′), 3.92 (3H, s, MeO at C-3), 5.17 (1H, d, J=7.5 Hz, H-1″), 6.90 (1H, d, J=8.5 Hz, H-6′), 7.16 (1H, d, J=2.0 Hz, H-6), 7.44 (1H, d, J=2.0 Hz, H-4), 7.57 (1H, dd, J=8.5, 2.0 Hz, H-5′), 7.63 (1H, d, J=2.0 Hz, H-3′). ^{13}C-NMR (CD_3OD): δ: 26.4 (C-8), 26.5 (C-8′), 56.6 (MeO at C-2′), 57.0 (MeO at C-3), 62,4 (C-6″), 71.2 (C-4″), 75.6 (C-2″), 77.7 (C-3″), 78.3 (C-5″), 103.9 (C-1″), 109.6 (C-4), 113.0 (C-3′), 114.4 (C-6), 118.9 (C-6′), 124.0 (C-5′), 134.3 (C-4′), 134.4 (C-5), 142.4 (C-2), 150.2 (C-1), 151.4 (C-2′), 151.8 (C-1′), 154.9 (C-3), 198.6 (C-7), 199.2 (C-7′). **24**: FAB-MS: m/z 515 $[M+Na]^+$ $C_{24}H_{28}O_{11}Na$, m/z 493 $[M+H]^+$ $C_{24}H_{29}O_{11}$. ^{1}H-NMR (DMSO-d_6) δ: 2.50 (3H, s, Me-8′), 2.53 (3H, s, Me-8), 2.91 (1H, dd, J=8.5, 8.0 Hz, H-2″), 3.01 (1H, ddd, J=9.0, 4.5, 1.5 Hz, H-5″), 3.05 (1H, t, J=9.5 Hz, H-4″), 3.12 (1H, br t, J=8.0 Hz, H-3″), 3.37 (1H, dd, J=12.0, 5.0 Hz, H-6″), 3.55 (1H, br dd, J=12.0, 1.5 Hz, H-6″), 3.88 (3H, s, MeO at C-3′), 3.90 (MeO at C-3), 5.27 (1H, d, J=7.5 Hz, H-1″), 7.41 (1H, d, J=2.0 Hz, H-4′), 7.49 (1H, d, J=2.0 Hz, H-6), 7.53 (1H, d, J=2.0 Hz, H-4), 7.71 (1H, d, J=2.0 Hz, H-6′). ^{13}C-NMR (DMSO-d_6) δ: 26.4 (C-8′), 26.5 (C-8), 55.9 (MeO at C-3′), 56.4 (MeO at C-3), 60.5 (C-6″), 69.7 (C-4″), 73.9 (C-2″), 76.5 (C-3″), 77.5 (C-5″), 101.1 (C-1″), 108.8 (C-4′), 111.6 (C-4), 124.2 (C-1′), 125.1 (C-6), 126.8 (C-5′), 126.8 (C-6′), 131.5 (C-1), 131.8 (C-5), 146.5 (C-2), 147.8 (C-3′), 149.5 (C-2′), 151.8 (C-3), 196.2 (C-7′), 196.7 (C-7).

Incubation of Acetophenone (25) The MeOH eluate from the hairy roots incubated with 100 mg of **25** gave **26** (9 mg) and **27** (5 mg). **26**: FAB-MS: m/z 307 $[M+Na]^+$ $C_{14}H_{20}O_6Na$, m/z 285 $[M+H]^+$ $C_{14}H_{21}O_6$. ^{1}H-NMR (pyridine-d_5) δ: 1.50 (3H, d, J=6.5 Hz, Me-8), 3.78 (1H, ddd, J=9.0, 5.0, 2.5 Hz, H-5′), 4.12 (2H, overlap, H-3′, 4′), 4.26 (1H, t, J=8.5 Hz, H-2′), 4.40 (1H, br d, J=11.5 Hz, H-6′), 4.56 (1H, br d, J=11.5 Hz, H-6′), 4.82 (1H, d, J=7.5 Hz, H-1′), 5.35 (1H, q, J=6.5 Hz, H-7), 7.27 (1H, t, J=7.5 Hz, H-4), 7.36 (2H, t, J=7.5 Hz, H-3, 5), 7.68 (2H, d, J=7.5 Hz, H-2, 6). ^{13}C-NMR (pyridine-d_5) δ: 25.0 (C-8), 62.9 (C-6′), 71.8 (C-4′), 74.8 (C-7), 75.5 (C-2′), 78.5×2 (C-3′, 5′), 101.6 (C-1′), 127.2 (C-3, 5), 127.7 (C-4), 128.8 (C-2, 6), 144.1 (C-1). Product **27** was in comparison with the authentic data of 2-β-D-glucopyranosyloxyacetophenone.[7]

Time-Course Experiment of Transformation of Vanillin (1) by *P. nil* Hairy Roots Vanillin (**1**) was added to each hairy root culture (200 ml of 1/2 MS liquid medium, 4 weeks old) and incubated. Sampling of the culture medium and hairy roots for analysis was carried out 0, 3, 6, 12, 24, 48, 72, and 144 h after adding **1** to the culture. Each hairy root obtained from the cultures was lyophilized and extracted with boiling water. The water solutions were passed through an HP 20 column, washed with water, and finally eluted with MeOH. The MeOH eluate was analyzed using HPLC. The culture medium was passed through a HP 20 column, washed with water and eluted with MeOH. The MeOH eluate was analyzed by HPLC.

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