Pergamon

PII: S0031-9422(97)01016-9

Phytochemistry, Vol. 49, No. 1, pp. 109–111, 1998

Printed in Great Britain
0031-9422/98/$–see front matter

BIOTRANSFORMATION OF *p*-AMINOBENZOIC ACID BY CULTURED CELLS OF *EUCALYPTUS PERRINIANA*

TSUTOMU FURUYA,* YOSHIHISA ASADA,† SATOSHI MIZOBATA,‡ YOUICHI MATSUURA‡ and HIROKI HAMADA

Department of Applied Science, Faculty of Science, Okayama University of Science, Ridai-cho, Okayama 700, Japan; † School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan; ‡ Omnitech Corporation, Kita-ku, Osaka 530, Japan

(*Received in revised form* 27 *October* 1997)

Key Word Index—*Eucalyptus perriniana*; Myrtaceae; cell culture; biotransformation; *p*-aminobenzoyl β-D-glucopyranoside; *p*-(*N*-β-D-glucopyranosylamino) benzoyl β-D-glucopyranoside; glucosylation; ^{1}H NMR; ^{13}C NMR.

Abstract—Two new biotransformation products, *p*-aminobenzoyl β-D-glucopyranoside and *p*-(*N*-β-D-glucopyranosylamino) benzoyl β-D-glucopyranoside, were isolated from cell suspension culture of *Eucalyptus perriniana* following administration of *p*-aminobenzoic acid. The chemical structures of these products were determined by ^{1}H and ^{13}C NMR and MS spectra. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Biotransformation reactions in cell cultures are usually stereospecific, and involve such processes as oxidation, reduction, hydroxylation, methylation, demethylation, glycosylation, esterification and saponification [1–4]. We have recently reported that cultured cells of eucalyptus (*Eucalyptus perriniana*) are able to biotransform three aromatics, e.g. eugenol [5], isoeugenol [5] and β-thujaplicin (hinokitiol) [6] into *O*-glycosides. In continued studies on biotransformation of aromatics, we were interested in *p*-aminobenzoic acid, as an aromatic compound having two functional groups, namely a carboxyl and an amino group.

p-Aminobenzoic acid is widely distributed in nature as a vitamin B complex factor. It absorbs ultraviolet light, 290 to 320 nm (the UV-B region), and is used as a cosmetic sunscreen. However, its ultraviolet absorption in polar solvents is decreased, owing to a hypsochromic shift caused by the formation of an amphoteric ion. Furthermore, as a consequence of its water insolubility, the use of *p*-aminobenzoic acid is limited. In order to overcome some of these undesirable properties,we have investigated the biotransformation of *p*-aminobenzoic acid into glycosylation products by cultured cells of *Eucalyptus perriniana*. In this paper, we describe the isolation and structure elucidation of the biotransformation products.

RESULTS AND DISCUSSION

A eucalyptus cell suspension was incubated into 500 ml flasks containing MS and BA1 medium for 3 weeks. *p*-Aminobenzoic acid (**1**) was fed and the culture maintained for an additional 7 days. The cells were harvested and extracted as described in the Experimental. The biotransformation products **2** and **3** were obtained (Fig. 1). Enzymatic hydrolysis of **2** and **3** afforded *p*-aminobenzoic acid as the aglycone.

In the FAB-MS spectrum, **2** showed a pseudomolecular ion peak at *m/z* 300 $[M+H]^+$. The A_2X_2-type aromatic proton signals were observed at δ 6.63 (2H, *d*, $J = 9.0$ Hz) and 7.81 (2H, *d*, $J = 9.0$ Hz) in the ^{1}H NMR spectrum of **2**. By analysis of the ^{1}H–^{1}H COSY spectrum, these signals were assigned to H-4, 6 and H-3, 7, respectively. Furthermore, an anomeric proton signal was observed at δ 5.65 (1H, *d*, $J = 8.0$ Hz), indicating the β-configuration. The proton signals due to the sugar moiety were observed at δ 3.39 ~ 5.65. In the ^{13}C NMR spectrum, thirteen carbon signals were observed. On the basis of the ^{13}C–^{1}H COSY spectrum, six signals at δ 62.4 ~ 95.8 and seven signals at δ 114.3 ~ 167.4 were assigned to carbons due to β-glucopyranoside and aglycone moieties, respectively. In addition, an anomeric carbon signal at δ 95.8 suggested that **2** was glucosyl ester. Therefore, **2** is *p*-amino-benzoyl β-D-glucopyranoside.

Product **3** revealed a pseudomolecular ion peak at *m/z* 484 $[M+H]^+$ which is larger by 162 mass units than that of **2**. In the ^{1}H NMR spectrum of **3**, four assignable aromatic proton signals at δ 6.81 (2H, *d*, $J = 9.0$ Hz, H-4 and 6) and 7.90 (2H, *d*, $J = 9.0$ Hz, H-3 and 7) were similar to those of **2**, suggesting that

* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data of biotransformation products in C_5D_5N

Product		2	3
Aglycone	1	167.4	167.2
	2	117.6	119.5
	3	133.1	132.8
	4	114.3	114.0
	5	155.3	153.4
	6	114.3	114.0
	7	133.1	132.8
Glc	1′	95.8	95.9
	2′	74.1	74.1
	3′	78.2	78.2
	4′	71.2	71.2
	5′	78.8	78.8
	6′	62.4	62.4
	1″		85.6
	2″		74.5
	3″		78.6
	4″		71.7
	5″		79.2
	6″		62.7

2 and **3** have the same aglycone. Furthermore, two anomeric proton signals were observed at δ 4.63 (1H, *d*, J = 8.0 Hz) and 5.66 (1H, *d*, J = 8.0 Hz), suggesting β-configuration. In the ^{13}C NMR spectrum, **3** showed nineteen carbon signals (Table 1). The carbon signals at δ 85.6 and 95.9 were assigned to anomeric carbons by analysis of the ^{13}C–^{1}H COSY spectrum. In the comparison of the ^{13}C NMR spectra of **2** and **3**, the carbon signals due to an additional glucopyranosyl moiety [7], attached to the amino group were observed in **3**. Consequently, the structure of **3** is *p*-(*N*-glucopyranosylamino) benzoyl β-D-glucopyranoside.

Based on the structures of biotransformation products **2** and **3**, it was considered that **3** was formed by glucosylation of the amino group in **2**. It was also observed that the solubilities of **2** and **3** in water were about 10 times and more than 100 times that of **1**, respectively. Thus the water solubilities of these compounds are proportional to the number of glucose units. The UV maximum of **1** in water was 273.5 nm, but those of **2** and **3** were 295.0 and 297.5 nm. The bathochromic shift is due to the inhibition of the formation of an amphoteric ion. Furthermore, the molar absorptivities of **2** and **3** corresponding to their absorption maxima were higher than that of **1**. Therefore, we expect that harmful effects of **1** to the human body is decreased by glycosylation of carboxyl and amino groups. The two biotransformation products **2** and **3** are new. This is the first report of glycosylation of an aromatic primary amine in biotransformation using cultured plant cells, although glycosylation of a heterocyclic tertiary amine is known [8].

EXPERIMENTAL

Cell line

The cultured cells of *Eucalyptus perriniana* used in this investigation were derived from young stems of *E. perriniana* in 1980 and maintained on BA1 agar medium [Murashige and Skoog (MS) medium solidified with agar (9 gl^{-1}) and supplemented with sucrose (30 gl^{-1}) and 6-benzylaminopurine (1 $mg\,l^{-1}$)].

Culture conditions and substrate feeding experiments

A cell suspension culture was initiated from static cultured callus in 500 ml flasks each containing 250 ml BA1 liquid medium (BA1 agar medium without agar). After 3 weeks for preculture on a rotary shaker (100 rotary min^{-1}) at 25° in the dark, a soln of substrate (25 mg *p*-aminobenzoic acid in 1 ml EtOH) was administered to each flask through a membrane filter and the cultures returned to the shaker for a further 7 days.

Extraction and purification of biotransformation products

The cells and medium were sepd by filtration with suction. The cells were extracted (×3) by homogenization with MeOH at room temp., and the extract was concd under red. pres. The residue was partitioned between H_2O and Et_2O followed by H_2O and EtOAc. The H_2O layer was applied to a Diaion HP-20 column and the column was washed with H_2O followed by elution with 20% MeOH. The 20% MeOH eluate was chromatographed on ODS-silica gel column and the biotransformation products were

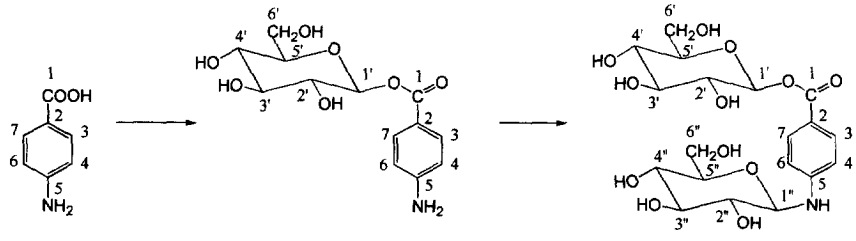

Fig. 1. Biotransformation of *p*-aminobenzoic acid (1) by cultured cells of *E. perriniana*.

further purified by HPLC [column; Waters μ Bondasphere C18–100 Å (19 × 150 mm), solvent; MeCN–H_2O (1/32–1/4 gradient), detection by UV (254 nm) absorption]. From these eluates, **2** and **3** were obtained. The production ratio of **2** and **3** were 12.6% and 10.4%, respectively.

p-*Aminobenzoyl β-D-glucopyranoside* (**2**). Colorless needles, mp 201–202°. $[\alpha]_D^{15}$ −17.9° (*c* 0.1, MeOH); UV $\lambda_{max}^{H_2O}$ nm (log ε): 295 (4.15), λ_{max}^{EtOH} nm (log ε): 299 (4.30); solubility 5.9 mg/ml in H_2O; Positive FAB-MS (glycerol + thioglycerol) *m/z*: 322 $[M+Na]^+$, 300 $[M+H]^+$; ^1H NMR (CD_3OD) δ_H (*J* in Hz): 3.39 ~ 3.45 (2H, *m*, *J* = 7.0, H′-4 and H′-5), 3.45 ~ 3.51 (2H, *m*, H′-2 and H′-3), 3.69 (1H, *dd*, *J* = 12.0, 5.0, H′-6a), 3.85 (1H, *dd*, *J* = 12.0, 2.0, H′-6b), 5.65 (1H, *d*, *J* = 8.0, H′-1), 6.63 (2H, *d*, *J* = 9.0, H-4 and H-6), 7.81 (2H, *d*, *J* = 9.0, H-3 and H-7).

p-(N-*β-D-Glucopyranosylamino*) *benzoyl β-D-glucopyranoside* (**3**). Amorphous solid. $[\alpha]_D^{32}$ −46.8° (*c* 0.15, MeOH); UV $\lambda_{max}^{H_2O}$ nm (log ε): 297.5 (4.40), λ_{max}^{EtOH} nm (log ε): 296.5 (4.23); solubility more than 590 mg/ml in H_2O; positive FAB-MS (glycerol + thioglycerol) *m/z*: 484 $[M+Na]^+$, 462 $[M+H]^+$; ^1H NMR (CD_3OD) δ_H (*J* in Hz): 3.36 (1H, *dd*, *J* = 8.0, 8.0, H″-2), 3.47 (1H, *dd*, *J* = 8.0, 8.0, H′-2), 3.35 ~ 3.50 (6H, *m*, H′-3, 4, 5, and H″-3, 4, 5), 3.67 (1H, *dd*, *J* = 12.0, 5.0, H′-6a or H″-6a), 3.70 (1H, *dd*, *J* = 12.0, 5.0 H′-6a or H″-6a), 3.86 (1H, *dd*, *J* = 12.0, 2.0, H′-6b or H″-6b), 3.86 (1H, *dd*, *J* = 12.0, 2.0, H′-6b or H″-6b), 4.63 (1H, *d*, *J* = 8.0, H″-1), 5.66 (1H, *d*, *J* = 8.0, H′-1), 6.81 (2H, *d*, *J* = 9.0, H-4 and 6), 7.90 (2H, *d*, *J* = 9.0, H-3 and 7).

Acknowledgements—The authors thank President M. Murata, Omnitech Corporation.

REFERENCES

1. Furuya, T., in *Frontiers of Plant Tissue Culture*, 1978, ed. T. A. Thorpe. The International Association for Plant Tissue Culture, University of Calgary, Calgary, 1979, p. 191.
2. Reinhard, E. and Alfermann, A. W., in *Advances in Biochemical Engineering*, Vol. 16., ed. A. Fiechter. Springer, Berlin, 1980, p. 49.
3. Suga, T. and Hirata, T., *Phytochemistry*, 1990, **29**, 2393.
4. Hamada, H. and Furuya, T., *Plant Tissue Cultures and Biotechnology*, 1996, **2**, 52.
5. Orihara, Y., Furuya, T., Hashimoto, N., Deguchi, Y., Tokoro, K. and Kanisawa, T., *Phytochemistry*, 1992, **31**, 827.
6. Furuya, T., Asada, Y., Matsuura, Y., Mizobata, S. and Hamada, H., *Phytochemistry*, 1997, **46**, 1355.
7. Shibata, S. and Nakanishi, H., *Carbohydrate Research*, 1980, **86**, 316.