Pergamon

PII: S0031-9422(96)00693-0

Phytochemistry, Vol. 44, No. 8, pp. 1483–1484, 1997

0031-9422/97 $17.00+0.00

1-*O*-NICOTINOYL-β-D-GLUCOPYRANOSE IN CULTURED TOBACCO CELLS

NOBUKO OHSHIMA, HIROSHI NISHITANI,* SHIRO NISHIKAWA, KATSUZUMI OKUMURA and HIROSHI TAGUCHI†

Laboratory of Biological Chemistry, Faculty of Bioresources, Mie University, Tsu, Mie 514, Japan; *R & D Center, Terumo Corporation, 1500 Inokuchi, Nakai-machi, Ashigarakami-gun, Kanagawa 259-01, Japan

(*Received in revised form* 11 *September* 1996)

Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; 1-*O*-nicotinoyl-β-D-glucopyranose; cultured cells; niacin.

Abstract—The structure of a new niacin metabolite in cultured tobacco cells grown in a high concentration of niacin (1 mM) was confirmed to be 1-*O*-nicotinoyl-β-D-glucopyranose. This glucoside is thought to be one of the detoxification forms of niacin in plants. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In our studies on the metabolism of niacin in tobacco cells cultured with niacin, i.e. nicotinic acid (NiA) or nicotinamide (NAm) at 1 mM, growth was inhibited and several novel niacin metabolites were detected in the cells [1, 2]. These compounds did not coincide with well-known intermediates in NAD biosynthesis or metabolites of NAD with respect to their retention times by HPLC analyses. A major compound has been previously identified as *N*-(β-D-glucopyranosyl) nicotinic acid [2].

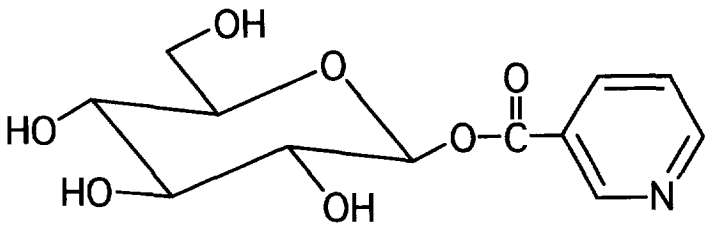
In the present work we looked at another niacin metabolite, NM-3, which was extracted with 80% methanol. After purification, it was confirmed by spectral data that this compound is 1-*O*-nicotinoyl-β-D-glucopyranose (**1**). As far as we know, this is a novel niacin metabolite.

RESULTS AND DISCUSSION

In a microbioassay using *Lactobacillus plantarum* ATCC 8014 after acid hydrolysis, NM-3 was found to have niacin-like vitamin activity [3]. One of the degradation products of NM-3 treated with 1 N HCl was identified as NiA by comparison with authentic NiA by HPLC. The hydrolysate of NM-3 was positive in the phenol–H_2SO_4 test, indicating that it contained sugar. R_f values of the sugar moiety in NM-3 coincided with those of glucose in five different TLC solvent systems. The observed coupling constant (6.7 Hz) of the anomeric proton of NM-3 was different from those of α- and β-glucofuranose pentabenzoate, as well as α-glucopyranose pentaacetate, but close to that of the β-isomer of the latter [4, 5], indicating that NM-3 has a pyranose ring with the β-cofiguration. The UV absorption maximum of NM-3 was not changed on addition of $NaBH_4$; this indicated that NM-3 is not a pyridinium salt [6, 7]. In order to determine the position of the hydroxyl group bound to NiA, NM-3 was treated with hydrolytic enzymes. It was degraded by β-glucosidase and esterase but not by α-glucosidase, suggesting that an ester bond is formed between the β-hydroxyl group at C-1 of the glucose and the carboxyl group of NiA. From the data described above, NM-3 was finally determined to be 1-*O*-nicotinoyl-β-D-glucopyranose. Schwenen *et al.* [8] have suggested that this compound was present in cultured cells and seedlings of parsley in their study on the metabolism of NiA, but without identifing it by instrumental analyses.

NAm incorporated in cultured cells of tobacco may be rapidly converted into NiA by the action of nicotinamidase (EC 3.5.1.19.) [1]. Therefore, NAm is equivalent to NiA as a precursor of NM-3 in cultured tobacco cells. Some of the NiA formed may then be converted into nicotinic acid mononucleotide as an intermediate of NAD biosynthesis by nicotinate phosphoribosyltransferase (EC 2.4.2.11), and trigonelline by nicotinate methyltransferase (EC 2.1.1.7). Since NiA inhibited the growth of cultured tobacco cells at 1 mM [1], excess NiA may be converted into *N*-(β-D-glucopyranosyl)nicotinic acid [2] and 1-*O*-nicotinoyl-β-D-glucopyranose so as to detoxify NiA. Glucose is the most common conjugating moiety of primary and secondary constituents for detoxification or storage

†Author to whom correspondence should be addressed.



form in plants. It is postulated that UDP-glucose (nicotinic acid–*N*-glucosyltransferase, EC 2.4.1.196) provides free NiA from *N*-(*β*-D-glucopyranosyl)nicotinic acid for the synthesis of NAD or catabolism of NiA in cultured parsley cells [9]. Since 1-*O*-nicotinoyl-*β*-D-glucopyranose was detected in tobacco cells only when cultured with niacin at high concentrations (such as 1 mM), it is thought to be another detoxification form of niacin.

EXPERIMENTAL

Cell cultures. Tobacco cells from *Nicotiana tabacum* XD-6 were cultured with NiA or NAm at 1 mM as described previously [1].

Purification of NM-3. Cells cultured with NAm at 1 mM for 5 days at 25° were extracted with 2 vols of 80% MeOH and centrifuged at 10000 *g* for 10 min. The supernatant was concd *in vacuo* at 30°, extracted × 3 with petrol to give a residue, which was dissolved in a small vol. of MeOH and applied to a silica gel column (15 × 200 mm). The compound was eluted with $CHCl_3$–MeOH (3:1). After drying the combined frs, the residue was dissolved in a small vol. of MeOH. Attempts to recrystallize this from several solvents failed. Therefore, it was obtained as a hygroscopic powder after freeze drying. However, when the purity of NM-3 was checked by HPLC using 5 mobile phases, only a single peak was detected on each chromatogram.

Hydrolysis of NM-3. To 0.5 ml of a soln containing purified NM-3, excess 1 N HCl was added and the mixt. heated at 100° for 30 min. After cooling, it was neutralized and the total vol. made up to 5 ml by adding dist. H_2O. This soln was analysed by HPLC and TLC. α-Glucosidase (from baker's yeast), *β*-glucosidase (from almonds) or esterase (from hog liver) was used for enzymic hydrolysis of NM-3. After incubation, the supernatant of the reaction mixt. was analysed by HPLC.

Microbiological assay. Niacin activity of NM-3 was assayed using *Lactobacillus plantarum* ATCC 8014 after hydrolysis [3].

HPLC. The sample was analysed using a UV monitor and a column of Tosoh TSK-gel Super-ODS (4.6 × 50 mm) or TSK-gel ODS-80TM (4.6 × 150 mm). The mobile phases used were H_2O–MeCN (49:1), H_2O–MeCN (97:3), 20 mM NaOAc–MeCN (49:1), 10 mM KH_2PO_4 (pH 3)–MeCN (49:1), 10 mM KH_2PO_4 (pH 7)–MeCN (49:1), 10 mM KH_2PO_4 (pH 3)–MeCN (49:1) containing 5 mM sodium 1-pentanesulphonate and 10 mM NaH_2PO_4 (pH 7)–MeCN (49:1) with 5 mM tetra-*n*-butylammoniumbromide. The flow rate was 1 ml min^{-1}.

TLC. Sugar in the hydrolysate of NM-3 was analysed on silica gel using $CHCl_3$–MeOH (3:2), Me_2CO–H_2O (9:1), Me_2CO–$CHCl_3$–MeOH–H_2O (15:2:2:1), EtOAc–HOAc–MeOH–H_2O (12:3:3:2) and PrOH–H_2O (17:3) to compare the chromatographic behaviours of glucose, fructose, galactose and mannose. The sugars were detected using $AgNO_3$–NaOH.

Instrumental analyses. Microanalysis. Found: C, 49.00; H, 5.48; N, 5.06. $C_{12}H_{15}NO_7$ requires: C, 50.53; H, 5.30; N, 4.91%. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COO), 1385 (C=N), 1280 (C–O–C) and 1070 (C–O). ^1H NMR (500 MHz, D_2O): δ 8.98 (1H, *s*, H-2′), 8.58 (1H, *d*, J = 5.0, H-6′), 8.30 (1H, *d*, J = 8.2, H-4′), 7.42 (1H, *dd*, J = 5.2, 7.9, H-5′), 5.67 (1H, *d*, J = 6.7, H-1), 3.95–3.30 (6H, H-2, H-3, H-4, H-5, H-6). ^{13}C NMR (125 MHz, D_2O): δ 165.9 (C=O), 154.1 (C-2′), 150.7 (C-6′), 139.4 (C-5′), 125.8 (C-3′), 125.1 (C-4′), 95.5 (C-1), 77.6, 76.2, 72.7, 69.8, 61.0 (C-2, C-3, C-4, C-5, C-6). SI-MS: *m/z* (rel. int.): 125 (100), 154 (28), 186 (100), 216 (18), 256 (18), 287 (26, $M^+ + H$) and 379 (6, M^+ + glycerol + H). UV $\lambda_{max}^{H_2O}$ nm (log ε): 263 (3.22).

REFERENCES

1. Taguchi, H., Yamada, Y., Nishitani, H., Okumura, K. and Shimabayashi, Y., *Vitamins (Japan)*, 1993, **67**, 67.
2. Nishitani, H., Yamada, Y., Ohshima, N., Okumura, K. and Taguchi, H., *Bioscience, Biotechnology and Biochemistry*, 1995, **59**, 1336.
3. Snell, E. E. and Wright, L. D., *Journal of Biological Chemistry*, 1941, **139**, 675.
4. Horton, D., Hughes, J. B., Jewell, J. S., Philips, K. D. and Turner, W. N., *Journal of Organic Chemistry*, 1967, **32**, 1073.
5. Bock, K. and Pedersen, C., *Acta Chemica Scandinavica*, 1972, **26**, 2360.
6. Karrer, P., Schwarzenbach, G., Benz, F. and Solmssen, U., *Helvetica Chimica Acta*, 1936, **19**, 811.
7. Karrer, P., Ringier, B. H., Bünchi, J., Fritzsche, H. and Solmssen, U., *Helvetica Chimica Acta*, 1937, **20**, 55.
8. Schwenen, L., Komossa, D. and Barz, W., *Zeitschrift für Naturforschung, Teil c*, 1986, **41**, 148.
9. Upmeier, B., Thomzik, J. E. and Barz, W., *Zeitschrift für Naturforschung, Teil c*, 1988, **43**, 835.