Aromatic Glycoside from Flue-Cured Tobacco (*Nicotiana tabacum*) Leaves and its Pyrolysis Behavior Studied by Pyrolysis-GC/MS

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The aromatic glycoside, named benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside, was isolated from the leaves of *Nicotiana tabacum* L. The structure of which was elucidated by analysis of its spectroscopic data. This compound's thermal behavior and pyrolysis products were also investigated by on-line pyrolysis-gas chromatography mass spectroscopy (Py-GC/MS) at 300, 500, 700, 900 °C. The results indicated that, due to the primary decomposition reaction of the O-glycosidic bound's cleavage, the characteristic and main pyrolysis product was the typical aroma constituent benzyl alcohol, suggesting that glycoside would contribute to produce specific aroma for tobacco products on heating as a novel aroma precursor.

Keywords: *Nicotiana tabacum*, Benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside, Pyrolysis, Py-GC/MS.

INTRODUCTION

Nicotiana tabacum L. is the most commonly grown of all plants in the *Nicotiana* genus of the family Solanaceae, whose leaves can be used as an insecticide and medicinally for anaesthetic¹, but especially for cigarettes and other tobacco products². The flue-cured tobacco (*Nicotiana tabacum* L.) var. honghuadajinyuan is one of the main tobacco cultivar in China, most planted in high altitude areas of Sichuan and Yunnan province, selected and improved from Virginia flue-cured tobacco about fifty years ago. It has draw much attention for its superior quality, strong resistance, low fertilizer requirement and eurytopicity³⁻⁵.

There has been consideration that the glycosidic components of tobacco leaves may contribute to generating aroma and taste in the curing and aging processes of flue-cured tobacco⁶ and several kind of glycosides were isolated from the leaves of flue-cured tobacco (*Nicotiana tabacum* L.)⁶⁻⁹. However, most of these reports were concentrated on the natural extraction, structure identification, synthesis and biological activities and the thermal behavior of these glycosides were seldom studied. In the course of investigation on the potential aroma precursors of *N. tabacum* L. var. honghuadajinyuan, a known aromatic glycoside¹⁰⁻¹⁸, named benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside (BAG) was separated, whose presence is reported in *Nicotiana* species for the first time.

To further explore the possibility of benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside (BAG) as aroma precursor, on-line pyrolysis-gas chromatography mass spectroscopy (Py-GC/MS) was used to study its decomposition process and pyrolysis products.

EXPERIMENTAL

The leaves of the flue-cured tobacco (*Nicotiana tabacum* L.) var. honghuadajinyuan are collected in Xichang region, Sichuan Province, China, in September 2010 and were identified by Prof. Y.L. Peng, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (zr2011081001) was deposited at the Herbarium of Chengdu Institute of Biology, CAS. All solvents including petroleum ether (60-90 °C) were distilled prior to use.

NMR data were obtained on a Bruker Avance 600 spectrometer. ESI-MS and HR-ESI-MS were performed on a BioTOF-Q mass spectrometer. Silica gel (200-300 mesh) for column chromatography and silica gel GF254 (10-40 mm) for thin layer chromatography (TLC) were purchased from Qingdao Haiyang Chemical Company, China. Semi-preparative HPLC was performed on a Waters 2605 LC system with a Waters RP-18 column (20 × 250 mm², 3 mL min⁻¹).

Extraction and isolation procedure: The air-dried powder of the leaves of *N. tabacum* L. cultivar honghuadajinyuan (5 kg) were extracted with 95 % ethanol (45 L) for three times

at 65 °C and condensed. The concrete was suspended in H₂O (12.5 L) at 65 °C. The aqueous phase was obtained by removing the insoluble constitution and partitioned successively with petroleum ether, ethyl acetate and *n*-butanol. Only the *n*-butanol extract was applied, considering it contains most of the glycosidic components with strong polarity and relatively higher hydrophilicity. The aqueous phase obtained after repeatedly dispersion and filtration of the *n*-butanol extract (750 g) in H₂O (6.5 L) at 65 °C was subjected to macroporous resin D101 column, sequentially eluted with water, 20 % ethanol, 40 % ethanol, 60 % ethanol, 80 % ethanol and ethanol. According to the results of silica gel column chromatography, no glycosides existed in the eluates of 60 % ethanol, 80 % ethanol and ethanol. The eluates of 20 % ethanol and 40 % ethanol with similar TLC profile were combined and then crystallized for several times to yield Fraction A (1050 g). Upon further silica gel column chromatography (CHCl₃/MeOH 15:1 to 0:1), fractions were collected and combined based on TLC, giving nine subfractions AA, AB, AC, AD, AE, AF, AG, AH, AI. After repeated purification of AI (140 g) by molecular sieve, the compound BAG (0.02 g) was obtained by separation of this part with preparative HPLC (25 % MeOH-H₂O) at the flow rate of 3 mL min⁻¹.

Benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside (BAG), white powder, ¹H NMR (C₅D₅N, 600 MHz): δ 3.26 (1H, m, H'-2), 3.37-3.38 (2H, m), 3.44-3.61 (4H, m), 3.75 (1H, dd, *J* = 11.4, 5.8 Hz, H'-6), 3.85 (2H), 4.11 (1H, dd, *J* = 11.5, 2.0 Hz, H'-6), 4.33 (1H, d, *J* = 6.8 Hz, H''-1), 4.36 (1H, d, *J* = 7.9 Hz, H'-1), 4.65 (1H, d, *J* = 11.8 Hz, H-7), 4.90 (1H, d, *J* = 11.8 Hz, H-7), 7.25-7.41 (5H, m, H-2-6); ¹³C NMR (C₅D₅N, 150 MHz), aglycone moiety: δ 139.2 (C-1), 129.4 (C-2), 129.3 (C-3), 128.8 (C-4), 129.3 (C-5), 129.4 (C-6), 72.5 (C-7); sugar moiety: δ 103.5 (C-1'), 75.2 (C-2'), 78.1 (C-3'), 70.8 (C-4'), 77.1 (C-5'), 69.6 (C-6'), 105.3 (C-1''), 72.0 (C-2''), 74.3 (C-3''), 69.7 (C-4''), 66.8 (C-5'').

Pyrolysis-GC/MS procedure: The online Py-GC/MS analysis was carried out with an American CDS5200 Pyroprobe coupled directly to a PerkinElmer Clarus 600 GCMA. Pyrolysis temperature was set up at 300, 500, 700 and 900 °C, respectively, heating from 50 °C at the rate of 10 °C min⁻¹ for 15 s. GC qualitative analysis was conducted with a DB-5 fused silica capillary column (30 m $\times$ 0.25 mm i.d., film thickness 0.25 μ m); flow rate of He was 1 mL min⁻¹; column temperature was held at 50 °C for 1 min, then raised to 260 °C at the rate of 5 °C min⁻¹; split ratio 50:1; injector temperature 250 °C; ion source temperature 230 °C; inlet line temperature 250 °C; EI-MS scan range 50-450 amu; EI ionization energy 70 eV; solvent delay 2 min. The pyrolysis products were identified on the basis of their high resolution mass spectra.

RESULTS AND DISCUSSION

Identification of benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside: The compound BAG (Fig. 1), a white powder, revealed a m.f. C₁₈H₂₆O₁₀, based on the molecular ion at *m/z* 425.1413 ([M + Na]⁺, calc. 425.3878) in the HR-ESI-MS and the 18 carbon signals showed by the ¹³C NMR spectrum, indicating six degrees of unsaturation. Its optical rotation was [α]_D²⁵ -39.7° (c 0.10, H₂O). The ¹H NMR signals at δ _H 7.41 (2H,

d, *J* = 7.2 Hz, H-3,5), 7.32 (2H, *t*, *J* = 7.2 Hz, H-2,6) and 7.25 (1H, *t*, *J* = 7.2 Hz, H-4) suggested a monosubstituted benzene ring, following a chiral methylene before oxygen at δ _H 4.65 (1H, *d*, *J* = 11.8 Hz, H-7) and 4.90 (1H, *t*, *J* = 11.8 Hz, H-7). The ¹³C NMR spectrum disclosed the presence of two sugar group (δ _C 103.5 and 105.3) and glucosyl group (δ _C 75.2, 78.1, 70.8, 77.1 and 69.6). To further confirm the structure of dual-glycoside, the acid hydrolysis reaction was conducted by adding BAG (5 mg) into 2N HCl (2 mL) solution and reflux heating for 0.5 h. The aqueous solution left after ethyl acetate extraction of the reaction mixture, was analysed by TLC, and the results proved the existence of both D-glucose and L-arabinose in BAG's hydrolyzate. Above all the information mentioned, compound BAG was characterized as benzyl alcohol bioside, more precisely as benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside by comparison with previously published data¹⁷.

Fig. 1. Structure of benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside

Pyrolysis-GC/MS analysis of BAG: The temperature chosen to carry out the on-line pyrolysis-gas chromatography mass spectroscopy (Py-GC/MS) were 300, 500, 700 and 900 °C. The pyrolysis data, including retention time (*t_R*), molecular weight (MW), assigned formula and relative peak area proportions for each identified pyroproducts of compound BAG are listed in Table-1.

According to the results, no compounds could be detected at 300 °C, suggesting BAG's certain feature of heat-resistance. At 500, 700 and 900 °C, benzyl alcohol was obviously the most favorable product, with the relative intensity of 66.45, 75.65 and 62.07 %, respectively. Along with benzyl alcohol, there were also 3,4-altrosan, D-glucuronic acid and D-allose, with the relative intensity lower than 10 %, which derived from the disaccharide part of BAG. These pyroproducts proved that the rupture of the O-glycosidic bound was the primary decomposition reaction during the pyrolysis procedure of BAG and the α -glycosidic bound between benzylidene and L-arabinose was more likely to cleave than the β 1-6 glycosidic bound between L-arabinose and D-glucose, generating more benzyl alcohol.

Compared with the pyroproducts at lower temperatures (500 and 700 °C), benzene, toluene, ethylbenzene and benzaldehyde could be observed at 900 °C, which were the by-products of benzylalcohol following further degradation.

The Py-GC/MS results indicated that, BAG had the highest pyrolysis efficiency at 700 °C, producing the largest amount of benzyl alcohol and it could be considered as a novel aroma precursor in tobacco products, because during high temperature

TABLE-1
 PYROLYSIS-GC/MS RESULTS OF BAG AT DIFFERENT TEMPERATURES

t_R (min)	Compounds of pyrolysis	m.f.	Area (%)				
			MW	300 °C	500 °C	700 °C	900 °C
2.53	Benzene	C ₆ H ₆	78	nd	nd	nd	3.66
3.68	Toluene	C ₇ H ₈	92	nd	nd	nd	4.41
5.39	Ethyl benzene	C ₈ H ₁₀	106	nd	nd	nd	2.98
9.66	Benzaldehyde	C ₇ H ₆ O	106	nd	nd	nd	2.59
11.62	Benzyl alcohol	C ₇ H ₈ O	108	nd	66.45	75.65	62.07
14.52	3,4-Altrosan	C ₆ H ₁₀ O ₅	162	nd	7.67	5.40	6.20
17.19	D-Glucuronic acid	C ₆ H ₁₀ O ₇	196	nd	15.92	9.34	11.80
26.19	D-Allose	C ₆ H ₁₂ O ₆	180	nd	9.95	9.62	6.30

nd: not detected, below the limit of detection

treatment, it would release a significant amount of benzyl alcohol, which was one of the most dominant aroma constituents in many ways.

Conclusion

In continuation of our project to search for possible aroma precursors in flue-cured tobacco (*Nicotiana tabacum* L.) var. honghuadajinyuan, hereby we have isolated and identified the benzyl-6-O- α -L-arabinopyranosyl(1-6)- β -D-glucopyranoside from *Nicotiana tabacum* L. The pyrolysis-GC/MS analysis showed the compound could regenerate typical aroma components on heating, suggesting its further application to be used as additive aroma precursor of tobacco products.

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