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Synthesis, Characterization and Application Study of Two Glycosides as Flavour Precursors

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Two glycosides of benzyl alcohol and phenethyl alcohol as flavour precursors have been synthesized and their structures were characterized by IR, ^{1}H NMR spectra and ESI-MS. Their pyolysis products are investigated by the on-line pyrolysis gas chromatography-mass spectroscopy. Their transfer efficiency from tobacco to cigarette smoke was determined for evaluation of its possibility of application.

Key Words: Glycoside, Flavour precursor, Application.

INTRODUCTION

Glycosides as flavour precursors have extensive application prospect in tobacco flavour field due to their non-volatility and aroma-releasing stability[1,2]. Benzyl alcohol and phenethyl alcohol are common flavours but can hardly be applied in the tobacco flavour field for their lack of non-volatility. In this paper, we describe the synthesis of two glycosides of benzyl alcohol and phenethyl alcohol *i.e.*, benzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside and phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside[3-6]. Their pyrolysis products and transfer efficiency from cigarette to smoke was studied to explore their application prospect in the tobacco industry.

EXPERIMENTAL

All chemicals used were analytical grade. The solvents were purified by conventional methods. The molecular sieves (4A) were activated by microwave oven for 15 min before they were used.

Infrared spectra was obtained with a Nicolet 170SX. ^{1}H NMR spectra was recorded on a Jeol FX-90Q spectrometer. ESI-MS was determined by the Center of Structure and Elemental Analysis, University of Science and Technology of China.

Syntheses of α-acetobromoglucose (A): To 40 mL (0.424 mol) dry acetic anhydride, 0.24 mL 60 % perchloric acid and 10 g (0.056 mol) of powdered D- glucose were added. The mixture was refluxed with stirring for 2 h and cooled

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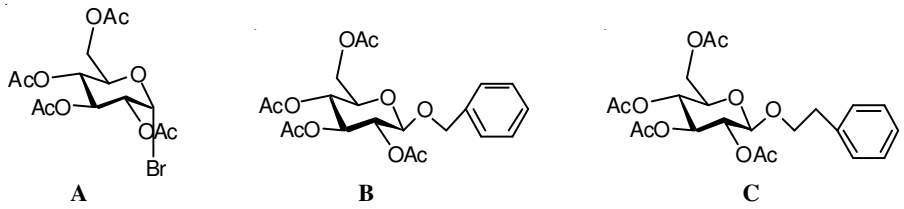
with the ice water. Pure of HBr gas was added to the mixture for 0.5 h very slowly and then the mixture was refluxed with stirring for 6 h. The α-acetobromoglucose was extracted by CH_2Cl_2 and recrystallized from dry ether with a yield of 90 %.

Syntheses of benzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (B): To 40 mL of dry CH_2Cl_2, 0.03 mol of benzyl alcohol in 10 mL dry CH_2Cl_2, 5 g activated molecular sieves (4A) and 8.3 g (0.03 mol) powdered silver carbonate were added. The mixture was refluxed with stirring for 2 h in dark environment. 12.3 g (0.03 mol) α-acetobromoglucose was added and then the mixture was refluxed with stirring for 24 h in darkness. The mixture was filtered through a thin layer of diatomaceous earth. After nearly complete evaporation of the solvent, the residue was purified through silica gel column chromatography and white powder of benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was obtained (yield *ca.* 94 %).



Structural formulae of α-acetobromoglucose (**A**), benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**B**) and phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**C**)

Syntheses of phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (B): To 40 mL of dry CH_2Cl_2, 0.06 mol of benzyl phenylethyl in 10 mL dry CH_2Cl_2, 5 g activated molecular sieves (4A) and 8.3 g (0.03 mol) powdered silver carbonate were added. The mixture was refluxed with stirring for 4 h in dark environment. 12.3 g (0.03 mol) of α-acetobromoglucose was added and then the mixture was refluxed with stirring for 24 h in darkness. The mixture was filtered through a thin layer of diatomaceous earth. After nearly complete evaporation of the solvent, the residue was purified through silica gel column chromatography and white powder of phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was obtained (yield *ca.* 87 %).

On-line pyrolysis gas chromatography-mass spectroscopy: The temperature of pyrolysis was 250 ºC and pyrolysis time was 1 min. A HP-5MS column (30 m × 0.25 mm × 0.25 μm) was used for the gas chromatography. The temperature of the HP-5MS column kept 2 min at 50 ºC and then rised up to 250 ºC at a rate of 5 ºC/min. The sample-input temperature was 250 ºC. The speed of the carrier gas was 1 mL/min and its split ratio was 30:1. The ionization energy of the mass spectroscopy was 70 eV and its scaning range was m/z 30-550[7-10].

Determination of transfer efficiency from tobacco to cigarette smoke: Four solutions of the benzyl alcohol, phenethyl alcohol, benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside and phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

were made to 8 mg/mL in the solvent of propylene glycol. Each cigarette was injected 10 μL and every 20 cigarettes made up a unit. The cigarette smoke particulates were captured by the cambridge filter on the smoking machine and extracted by CH_2Cl_2.

RESULTS AND DISCUSSION

IR, ^{1}H NMR spectra and ESI-MS

Benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside: IR (KBr, ν_{max}, cm^{-1}): 3010, 2870, 1748, 2870, 1748, 1370, 1196, 1117, 1040. ^{1}H NMR (400 MHz, $CDCl_3$, ppm, *J* in Hz): δ: 1.99-2.12 (12H, 4s, H of acetate CH_3), 3.67 (1H, ddd, Glc-H-5), 4.16 (1H, dd, *J* = 2.3, 12.2, Glc-H-6), 4.28 (1H, dd, *J* = 4.5, 12.2, Glc-H-6), 4.54 (1H, d, *J* = 7.9, Glc-H-1), 5.07 (1H, dd, *J* = 7.9, 9.4, Glc-H-2), 5.11 (1H, dd, *J* = 9.4, 9.4, Glc-H-4), 5.16 (1H, dd, *J* = 9.4, 9.4, Glc-H-3), 7.28-7.37 (5H, m, aromatic protons) ESI-MS (in solvent of methanol): $[M + H_2O]^+$ 456.1, $[M + Na]^+$ 461.12, $[M + K]^+$ 476.1, $[2M + Na]^+$ 898.7, $[2M + K]^+$ 914.5.

Phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside: IR (KBr, ν_{max}, cm^{-1}): 3005, 2875, 1757, 1368, 1195, 1111, 1042. ^{1}H NMR (400 MHz, $CDCl_3$, ppm, *J* in Hz): 1.89-2.16 (12H, 4s, H of acetate CH_3), 2.88-2.93 (2H, m, H_2C2),3.68(1H, m,HaC1), 3.89 (1H, ddd, *J* = 7.0, 6.5, 1.0, HC5'), 4.13 (1H, dd, *J* = 11.0, 7.0, HaC6'), 4.14 (1H, m, HbC1), 4.19 (1H, dd, *J* = 11.0, 6.5, HbC6'), 4.45 (1H, d, *J* = 8.0, HC1'), 4.98 (1H, dd, *J* = 10.5, 3.5, HC3'), 5.21 (1H, dd, *J* = 10.5, 8.0, HC2'), 5.38 (1H, dd, *J* = 3.5, 1.0, HC') 7.18-7.30 (5H, m, aromatic protons). ESI-MS (in solvent of methanol)[11,12]: $[M + H_2O]^+$ 470.1, $[M + Na]^+$ 475.2, $[M + K]^+$ 491.1, $[2M + Na]^+$ 926.7, $[2M + K]^+$ 942.6.

Determination of transfer efficiency from cigarette to smoke

Determination of transfer efficiency from cigarette to smoke of benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside: A series of benzyl alcohol solutions in the solvent of propylene glycol were determinated first to show the relationship between concentrations of the benzyl alcohol and its peak area. The result is shown in Table-1.

TABLE-1
RELATIONSHIP BETWEEN CONCENTRATIONS AND PEAK AREA

Peak area of benzyl alcohol	Concentrations of the benzyl alcohol (mg/50 mL)
9.7	0.1035
18.4	0.2070
34.4	0.4140
53.4	0.6210
96.3	1.0350

Its linear fit equation is $y = 0.0107x + 0.0203$, $R^2 = 0.9959$, (x: peak area of benzyl alcohol, y: concentration of benzyl alcohol). The solutions of the benzyl alcohol and benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside were made to 8 mg/mL

in the solvent of propylene glycol. Each cigarette was injected 10 μL and every 20 cigarettes made up a unit. The cigarette smoke particulates were captured by the cambridge filter on the smoking machine and extracted by CH_2Cl_2. The results of the transfer efficiency from cigarette to smoke of the benzyl alcohol and benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside were shown in Table-2.

TABLE-2
TRANSFER EFFICIENCY FROM CIGARETTE TO SMOKE OF THE BENZYL ALCOHOL AND ITS GLYCOSIDE

Sample	Peak area	Concentrations (mg/50 mL)	Transfer efficiency
Benzyl alcohol 1	58.3	0.644	20.13
Benzyl alcohol 2	58.2	0.643	20.09
Glycoside 1	51.2	0.568	71.63
Glycoside 2	44.9	0.501	62.16

As shown above, the transfer efficiency from cigarette to smoke of benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside is 332 % of the transfer efficiency of benzyl alcohol. It shows that the benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside can keep and transfer the target flavour benzyl alcohol more efficiently than the benzyl alcohol.

Determination of transfer efficiency from cigarette to smoke of phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside: A series of phenethyl alcohol solutions in the solvent of propylene glvcol were determinated first to show the relationship between concentrations of the benzyl alcohol and its peak area. The result is shown in Table-3.

TABLE-3
RELATIONSHIP BETWEEN CONCENTRATIONS AND PEAK AREA

Peak area of phenethyl alcohol	Concentrations of the benzyl alcohol (mg/50 mL)
9.2	0.0945
17.2	0.1890
32.5	0.3780
52.0	0.5670
91.8	0.9450

Its linear fit equation is $y = 0.0102x + 0.0198$, $R^2 = 0.9959$ (x: peak area of phenethyl alcohol, y: concentration of phenethyl alcohol): The solutions of the phenethyl alcohol and phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside were made to 8 mg/mL in the solvent of propylene glvcol.

Each cigarette was injected 10 μL and every 20 cigarettes made up a unit. The cigarette smoke particulates were captured by the cambridge filter on the smoking machine and extracted by CH_2Cl_2. The results of the transfer efficiency from cigarette to smoke of the phenethyl alcohol and phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside were shown in Table-4.

TABLE-4
TRANSFER EFFICIENCY FROM CIGARETTE TO SMOKE OF THE PHENETHYL ALCOHOL AND ITS GLYCOSIDE

Sample	Peak area	Concentrations (mg/50 mL)	Transfer efficiency
Phenethyl alcohol 1	37.5	0.400	25.50
Phenethyl alcohol 2	32.9	0.360	22.50
Glycoside 1	24.0	0.265	62.16
Glycoside 2	23.8	0.263	61.59

As shown above, the transfer efficiency from cigarette to smoke of phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside is 259 % of the transfer efficiency of phenethyl alcohol. It shows that the phenylethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside can keep and transfer the target flavour phenethyl alcohol more efficiently than the phenethyl alcohol.

On-line pyrolysis gas chromatography-mass spectroscopy: The main pyrolysis products of the benzyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside at 250 ºC are acetic acid (38.5 %), benzaldehyde (1.1 %), benzyl alcohol (7 %), phenylmethyl acetate (4.7 %), methyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (4.1 %), ethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8.5 %), β-penta-acetyl-glucose (10.4 %), α-penta-acetyl-glucose (19.4 %).

The main pyrolysis products of the phenethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside are phenethyl alcohol (28.6 %), phenylethyl acetate (15.9 %), ethyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (34.9 %), propyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (10.1 %). β-penta-acetyl-glucose (4.2 %), α-penta-acetyl-glucose (6.3 %).

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