Communication

Photoionization Mass Spectrometry: A Useful Method to Evaluate the Pyrolysis Process of Glycoside Flavor Precursor

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The thermal decomposition behavior and the pyrolysis products of benzyl-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside (BGLU) were studied with synchrotron vacuum ultraviolet (VUV) photoionization mass spectrometry at temperatures of 300, 500 and 700 °C at 0.062 Pa. Several pyrolysis products and intermediates were identified by the measurement of photoionization mass spectra at different photon energies. The results indicated that the primary decomposition reaction was the cleavage of O-glycosidic bond of the glycoside at low temperature, proven by the discoveries of benzyloxy radical (m/z = 107) and glycon radical (m/z = 331) in mass spectra. As pyrolysis temperature increased from 300 to 700 °C, two possible pyrolytic modes were observed. This work reported an application of synchrotron VUV photoionization mass spectred to help understand the thermal decomposition mechanism of this type of compound. The possibility of this glycoside to be used as a flavor precursor in high temperature process was evaluated.

Keywords: Glycoside; Flavor precursor; Thermal decomposition; Synchrotron VUV photoionization; Mass spectrometry.

INTRODUCTION

Glycosides widely existing in nature have been extensively studied in recent decades, and interests usually focus on their role as flavor precursors.¹⁻³ Nowadays, it is well established that flavorless glycosides represent one accumulation form of aroma substances in fruits and other plant tissues.⁴ In general, biochemical aroma formation is thought to proceed by two different processes. One type follows normal biosynthetic routes and the other type involves the hydrolysis of glycosides having aroma compounds as their aglycons.^{5,6} Many studies on tea leaves and grape wine showed that a significant portion of volatile flavor compounds, such as linalool, geraniol, etc., were discovered to be present as glycosides and liberated from their glycosidic aroma precursors by the action of endogenous enzymes during the manufacturing process.⁷⁻⁹ As a matter of fact, glycosidic flavor precursors presented in many plant materials and played an important role in the flavor of the plants.

In tobacco leaves, there also a large number of flavor compounds are in glycosylated form.¹⁰ And it has been revealed that the glycoside in tobacco leaves could contribute to generating aroma and taste in the curing and aging processes of flue-cured tobacco.¹¹ However, there are also some gaps in our understanding of the process of aroma formation from this kind of flavor precursor during high temperature process, such as cigarette burning. In general, the cleavage of O-glycosidic bond could be achieved either by enzymatic/chemical hydrolysis or by pyrolysis.⁵ Previous studies on the glycoside flavor precursor were mainly focused on the enzymatic/chemical hydrolysis.^{12,13} The thermolysis study of this compound had been rarely reported. Until recently, Wancui Xie et al.¹⁴ studied the thermal decomposition of menthyl-glycoside by pyrolysis-gas

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chromatography-mass spectroscopy (Py-GC-MS) analysis.

However, it is known that above technique have some drawbacks in analyzing the thermal decomposition process of analyte, such as the problems in real-time analysis and secondary reaction. During Py-GC-MS analysis, the primary rupture of bonds is usually followed by a complex series of competitive reactions between the radicals and the fragment molecules produced in thermal decomposition process. These secondary reactions complicated the pyrolysis products and made the mechanism of decomposition process unclear.

Here, we reported an experimental study of thermal decomposition of synthetic benzyl-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside (BGLU) at low pressure by means of synchrotron vacuum ultraviolet (VUV) photoionization combined with molecular-beam mass spectrometry (MBMS), also called as synchrotron VUV photoionization mass spectrometry (SVUV-PIMS).¹⁵ The experimental apparatus exhibited some advantages in analyzing the pyrolytic process of glycoside flavor precursor, such as real-time analysis and less secondary reactions, compared with conventional method. Benefitted from the wide tunability and high energy resolution in VUV region, synchrotron VUV photoionization can minimize fragmentation and detect radicals¹⁶⁻¹⁸. Therefore, SVUV-PIMS can selectively detect most pyrolysis products in real time. The results observed in this work were significant to this type of flavor precursor to be used as flavor additive in high temperature process.

RESULTS AND DISCUSSION

Sample characterization

Some techniques such as IR, ¹HNMR and EI-MS were adopted to identify the structure of the glycoside. From the results of the ¹HNMR and IR, we can see that the data were perfectly corresponding to the molecular structure of BGLU (see Table 1). The purity of the product can be evaluated from the ¹HNMR spectra data. There was no impurity peak in ¹HNMR spectra (see supporting information S-Figure 1). The data of MS spectra showed that the molecular ion peak was absent, possibly because the energy of EI source was too high (see Fig. 1). The yield of the product was about 35%.

Identification of the thermal decomposition products

As for BGLU to be used as flavor precursor, the py-

 Table 1.
 ¹HNMR, IR, EI-MS identification of the synthetic BGLY

IR (cm ⁻¹ , KBr micropollets)	¹ HNMR δ (in CDCl ₃)	EI-MS (M/Z)
2960	1.99-2.10 (12H, C-2,3,4,6-Acetyl)	331
1753	3.68 (1H, C-5)	245
1499	4.16-4.28 (2H, C-6)	139
1453	4.53-4.64 (2H, C-1')	97
1428	4.90 (1H, C-1)	91
	5.03-5.20 (3H, C-2,3,4)	43
	7.27-7.37 (5H, Benzene ring)	

rolysis product we concerned was aromatic benzyl alcohol. In our study, since the photon energy was tunable, the identification of pyrolysis products of glycoside, such as aromatic alcohol, could be deduced from the measurements of photoionization mass spectra. With the variation of photon energy, a series of mass spectra could be taken at the fixed temperature for identification of pyrolysis products, based mainly on the comparison of appearance energy region and known ionization energies (IEs) of pyrolysis products. For example, benzyl alcohol (C_7H_8O , m/z = 108) is very important decomposition product in our study. However, there are several possible isomers of C_7H_8O (m/z = 108), such as benzyl alcohol (IE = 9.53 eV)¹⁹ and methyl-phenol isomers (IEs below 8.5 eV).²⁰⁻²¹ In our study, no signal could be observed at the photon energy below 9.00 eV, which excluded the existence of phenolic compounds; and thus the signal of m/z = 108 could be attributed to the substance of benzyl alcohol. Some other products and intermediates could be identified by the same way as above.

Fig. 2 shows the mass spectra at the temperature of 700 °C and with four photon energies labeled on the figure. The selection of photon energy in our experiment was important. The final photonionization energy was selected as



Fig. 1. The EI spectrum of BGLU.

11.5 eV, at which most of the pyrolysis intermediates and products could be ionized and detected.

Possible pyrolysis mechanism of BGLU

The studies on components of pyrolysis products were important for the potential application of the glycoside as flavor additives in high temperature process. Different temperature can usually result to the difference in pyrolysis products. The thermal decomposition of glycoside starts at about 250-300 °C. Fig. 3 shows the mass spectra recorded for BGLU with various temperatures of 300, 500, 700 °C. As shown in Fig. 3, the number of pyrolysis products and intermediates increased with increasing temperature, which was in good agreement with previous Py-GC-MS studies.¹⁴ At low temperature (300 °C), only a small



Fig. 2. Photoionization mass spectra of the thermal decomposition products of BGLU at the temperature of 700 °C and various photon energies: (a) 14.5 eV, (b) 11.5 eV, (c) 10.0 eV and (d) 9.0 eV.



Fig. 3. Photoionization mass spectra of the thermal decomposition products of BGLU at the photon energy of 11.5 eV and various temperatures: (a) 300 °C, (b) 500 °C, and (c) 700 °C.

number of pyrolysis products at m/z from 59 to 331 were detected. As the temperature increased, it can be seen that more pyrolysis products and intermediates ion signals ranging from m/z = 43 to 347 were observed in our study.

From the products and intermediates study, we conclude that the cleavage of glycosidic bond can be carried out by two possible modes in the pyrolysis process of BGLU. As shown in Scheme I, the intermediates of benzyloxy radical (m/z = 107) and glycon moiety radical (m/z = 107)331) would be observed in mass spectra by Route 1. As compared with Route 1, Route 2 directly resulted into the formation of glycon moiety radical (m/z = 347) and benzyl radical (m/z = 91). From Fig. 3, we can see that the cleavage of glycoside was carried out by Route 1 mode at low temperature (300 °C). Because there was no presence of the signal at m/z = 347. When the temperature increased to 500 and 700 °C, Route 2 was becoming an alternative mode in the pyrolysis process of BGLU. The formation of benzyl alcohol from benzyloxy radical should be easier than benzyl radical in gas phase. For the H radical was easier to be captured than OH radical in gas phase. The former was just via H addition reaction and the latter needed OH addition reaction. It was possibly the reason why the aromatic flavor component would dramatically decrease at high temperature according to previous Py-GC-MS analysis.^{22,23}

Scheme I The schematic diagram of possible pyrolysis pathways of BGLU



Scheme II shows the principal pyrolysis products of BGLU observed in PIMS study. The thermal decomposition of glycon moiety was almost the same both by Route 1 and Route 2 mode. Some intermediates and radicals can be observed by this technique. In comparison, from previous Py-GC-MS study on menthyl-glycoside we couldn't observe some useful information about the pyrolysis process of glycon moiety. The major pyrolysis products for menthyl-glycoside identified by Py-GC-MS were methanol, ethanol, menthol, acetic acid, dodecanal, geraniol, 1,6-anhydro-triacetate- β -D-glucose and 1,6-anhydro- β -D-glucose. There were seldom pyrolysis intermediates and radicals identified.¹⁴

Scheme II Proposed pyrolysis scheme of BGLU



As a comparison with PIMS method, the pyrolysis results of BGLU produced by Py-GC-MS under high temperature was also obtained in our study. Fig. 4 shows the total ion chromatogram of BGLU obtained by Py-GC-MS at the temperature of 700 °C. Major pyrolysis products were listed in Table 2. From the results we can see that there were many derivatives (mainly include some benzene derivatives) detected by this technique.

EXPERIMENTAL

General

The Koenigs-Knorr method for the synthesis of glycosides probably represents the most widely used procedure in the chemistry of carbohydrate derivatives. In our work, BGLU was synthesized by the modified Koenigs-Knorr reaction²⁴ under strictly anhydrous condition. The product

Table 2.	Py-GC-MS result of BGLU at the temperature of 700
	°C

Retention Time (min)	Compounds of pyrolysis	Area (%)
3.52	Toluene	14.385
4.68	Ethylbenzene	8.161
5.12	Styrene	8.098
6.56	Benzaldehyde	8.648
7.11	2-Furanmethanol, acetate	1.100
8.31	Benzyl Alcohol	6.437
9.40	Formic acid, phenylmethyl ester	11.935
10.16	Phenylethyl Alcohol	1.073
10.68	4-Methyl-Benzenemethanol	1.279
11.02	2-Methylindene	0.527
11.42	Acetic acid, phenylmethyl ester	1.148
11.80	Formic acid, 2-phenylethyl ester	1.760
11.98	Naphthalene	1.117
12.54	1-(Bromomethyl)-4-methyl-Benzene	0.870
15.37	5-Acetoxymethyl-2-furaldehyde	2.359
17.04	Biphenyl	0.945
17.10	1,2-Benzenediol, diacetate	0.575
20.69	Bibenzyl	6.198
21.32	1,1'-(1-methyl-1,2-ethanediyl)bis-	0.571
	benzene	
24.56	Heptadecane	0.278
24.83	(E)-Stilbene	0.506
31.70	α -D-Glucopyranose, pentaacetate	0.384
33.22	Hexanoic acid, 2-phenylethyl ester	1.337
36.46	3-Methyl-butanoic acid, 2-phenylethyl	0.736
	ester	

was separated by using a column chromatography and then the pyrolysis was carried out. All reagents used were of analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

Synthetic process

Acetobromo-a-D-glucose (2,3,4,6-tetra-O-acetyl-



Fig. 4. Total ion chromatogram of BGLU produced by Py-GC-MS at the temperature of 700 °C.

 α -D-glucopyranosyl bromide, III in Scheme III) was prepared in 40 mL acetic anhydride via successive addition of 10 g dry glucose, 3.1 g red phosphorous, 6 mL liquid bromine and 3.6 mL water. At the end of the reaction, 30 mL dichloromethane and 2×80 mL of ice water were added into the mixture, respectively. The mixture was vigorously shaken and the phases were allowed to separate. The upper aqueous phase was discarded. And then, 80 mL saturated sodium bicarbonate solution was added and the same extraction process was performed as above. The acetobromo- α -D-glucose was eventually retained in dichloromethane. Then, 1.62 g benzyl alcohol and 5 g 4 Å molecular sieve were mixed with acetobromo- α -D-glucose dispersed in dichloromethane. In the end, 4.14 g freshly prepared, dried silver carbonate was added into the mixture. The reaction mixture was stirred free of light for about 48 H. On the completion of the reaction, the mixture was filtered and condensed and separated on the silica gel column chromatography.

Scheme III General synthetic process of BGLU



Product separation

Silica gel column chromatography was performed using a glass column (40 cm length, 3 cm i.d.) filled with silica gel (0.03-0.06 mm, Qingdao ocean chemistry factory, China), eluted with petroleum ether-ethyl acetate (7:1, v/v). The eluent was monitored by thin layer chromatography. The outflow containing BGLU was collected and evaporated to dryness and white solid BGLU was obtained, immediately.

Structural characterization

IR spectra was recorded with an EQUIVOX55 infrared spectrometer (Bruker, German), the sample was analyzed as KBr micropellets. ¹HNMR spectra data was obtained on a Bruker 300 MHz Spectrometer with CDCl₃ as solvent and TMS as internal reference standard. EI-MS data were obtained using a GCT mass spectrometer (Micormass, UK).

Pyrolysis

The experimental work was carried out at the National Synchrotron Radiation Laboratory (NSRL) in Hefei, China. A detailed description of the instrument setup has been reported elsewhere,¹⁵ and only a brief description is presented here. An undulator radiation from the 800 MeV electron storage ring dispersed by a 1m Seya-Namioka monochromator with a 1,500 grooves/mm grating was used in our study. The available photon energy range covers from 7.8 to 24 eV with an average photon flux exceeding 10^{13} photons/sec. A gas filter with Ne or Ar filled in the gas cell was used to avoid higher-order harmonic radiation.

The thermal decomposition apparatus is composed of a pyrolysis chamber with a Shimadzu pyrolyzer (PYR-2A, Nakagyo-ku, Kyoto, Japan) and a photoionization chamber with a reflectron time-of-flight mass spectrometer (RTOF-MS) (see Fig. 1). Argon was used as the carrier gas with a flow rate of 20.0 standard cubic centimeters per minute, which was controlled by a mass flow controller. In our work, a stainless steel pole with a diameter of 6 mm was used to put the sample into the middle of the furnace (seen



Fig. 5. The schematic diagram of the thermal decomposition apparatus with molecular-beam mass spectrometry.¹⁵ Parts I and II are the pyrolysis chamber and the photoionization chamber, respectively. The synchrotron VUV light crosses vertically with the molecular beam. and were then ionized. A homemade RTOF mass spectrom-

eter was used to detect the positive ions with an approxi-

mate mass resolving power of 1400. The ion signal was re-

corded by a multiscaler P7888 (FAST Comtec, Germany)

after it was amplified with a preamplifier VT120C (EG &

G, ORTEC, USA). The temperature of the furnace and the

pyrolyzer can be controlled by a temperature controller

(SKY Technology Development Co., Ltd., Shenyang,

from the amplified part in Fig. 1). Argon was used as the carrier gas. The pyrolysis gas passes through a nickel skimmer to enter the ionization chamber, where it intersects with the synchrotron VUV light perpendicularly. The diameter of the skimmer is about 1.25 mm. Species produced at thermal decomposition process absorbed a VUV photon,
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China). During the experiment, the furnace was heated to a specific temperature, and the sample of about 50 mg inside the scoop was then pushed into the furnace. A series of mass spectra was measured with variations of photon energy or temperature. In our work, the mass spectra were collected at the photon energies of 14.50, 11.50, 10.00, 9.00 eV at selected temperatures ranged from 300 to 700 °C. With this 'soft' tunable photoionization method all the plyrolysis products were detected as molecular ions, M⁺. In order to avoid any secondary reactions, the experiment was performed at low pressure (0.062 Pa).

CONCLUSION

The pyrolysis behavior and the pyrolysis products of synthetic BGLU were studied with synchrotron VUV photoionization mass spectrometry at the temperatures of 300, 500 and 700 °C. According to the results, it can be concluded that the primary decomposition reaction of the glycoside flavor precursor was the cleavage of O-glycosidic bond at low decomposition temperature. As temperature increased, two different pyrolysis modes were observed in our study for the first time. This study would be significant to glycoside flavor precursor to be used as flavor additive in high temperature process.

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