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Analysis of wool fiber by alkali-catalyzed pyrolysis gas chromatography

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Abstract

Alkali-catalyzed pyrolysis gas chromatography (PyGC) has been used to identify minute samples of wool fiber. The wool sample to which aqueous sodium hydroxide was added was pyrolyzed in a Curie-point pyrolyzer attached to a gas chromatograph or a gas chromatograph-mass spectrometer. The addition of an aqueous solution of sodium hydroxide increased the production of specific volatile pyrolysis products from the constitutive amino acid residues of wool protein, i.e. acetaldehyde from alanine or proline, isobutyronitrile from valine, 2-methylbutyronitrile from isoleucine, isovaleronitrile from leucine and toluene from phenylalanine. Compared with conventional non-catalyzed PyGC, the alkali-catalyzed PyGC was found to greatly improve the detection limit of wool fiber and make it possible to analyze very minute samples. The alkali-catalyzed PyGC presented here has been shown to be applicable to minute thermally-denatured samples of wool fiber which cannot be identified successfully by morphological inspection using a microscope or by using Fourier-transform infrared microspectroscopy. Furthermore, the present PyGC method was successfully used for several protein samples and was shown to be useful for analysis of proteins other than wool fibers by using different special pyrograms reflecting different amino acid compositions. ©1997 Elsevier Science Ltd © 1997 Elsevier Science Ireland Ltd

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1. Introduction

A textile fiber sample collected from a crime scene is a commonly used item of evidence [1,2]. It will provide important information on the contact between victim(s) and suspect(s) as well as on the connection between the crime scene and the victim(s) and/or suspect(s).

In addition to the most popular morphological inspection using a microscope and testing by Fourier-transform infrared microspectroscopy, PyGC can be used as one of the methods for identifying textile fibers in forensic science [3], and can be especially useful in the case where other methods, such as those mentioned above, cannot be successfully used because samples may have been subjected to partial pyrolysis.

In the PyGC analysis of natural fibers such as cotton and wool, these fibers usually produce their volatile pyrolysis products in small quantities. Their detection limit in the PyGC is 50 μg . For such a minute single fiber sample that is collected from a crime scene, it is therefore difficult to detect the peaks of the specific pyrolysis products for identification of the fiber using conventional PyGC.

For PyGC analysis of cotton fiber, the use of the acid-catalyzed pyrolysis method using phosphoric or hydrochloric acid to produce specific volatile substances, such as furfural in sufficient quantity for detection has been reported in previous papers [4,5]. This made it possible to use PyGC for identifying minute cotton fiber samples. For PyGC analysis of protein fibers such as wool, the use of such acid-catalyzed methods was found insufficient to improve the detection limit for identifying minute samples of these fibers. Miki et al. [6,7] reported a version of PyGC for analysis of wool and other protein fibers, but this was not satisfactory from the point of the detection limit when used in the field of forensic science which requires the analysis of very minute fiber samples.

However, because wool is relatively resistant to acid, but labile to alkali, constitutive proteins of wool can be hydrolyzed catalytically by alkali in addition to acid. The present work has studied the method of alkali-catalyzed PyGC for analyzing wool fiber by using various kinds of alkalis. In addition, this method was also used in the analysis of silk, 6-nylon fiber and various kinds of proteins.

2. Materials and methods

2.1. Reagents and test materials

2.1.1. Major α -amino acids

Glycine, L-cysteine, L-threonine, L-histidine, L-arginine, and L-tyrosine (Nacalai Tesque, Inc.); L-glutamic acid, L-lysine, L-aspartic acid, L-cystine, L-tryptophan, L-methionine, L-serine, L-hydroxyproline, L-proline (Pro), L-leucine (Leu), L-isoleucine (Ileu), L-valine (Val), L-alanine (Ala) and L-phenylalanine (Phe) (Wako Pure Chemical Industries Co.). 2-Methylbutyronitrile, isobutyronitrile and isovaleronitrile (Tokyo-Kasei Organic Chemicals Co.), acetonitrile and toluene (Wako Pure Chemical Industries Co.).

2.1.2. Proteins

Human hemoglobin, bovine hemoglobin, human serum albumin, ovalbumin and casein (Tokyo-Kasei Organic Chemicals Co.).

2.1.3. Textile fibers

Wool, silk and 6-nylon fibers (standard adjacent fabrics for staining of color-fastness test specified by JIS).

2.2. Instruments for PyGC and PyGC-MS and their operational conditions

In Table 1 the instruments for PyGC and PyGC-MS and their operational conditions are shown.

Two types of Curie-point pyrolyzers, Models JHP-2 and JHP-3 (Japan Analytical Industry Co.), were used, directly connecting to a FID gas chromatograph (Shimadzu GC-7AG) or a gas chromatograph-mass spectrometer unit (Shimadzu QP-1000).

A DB-5 fused-silica capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm; J&W Co., CA) was used.

Table 1
PyGC and PyGC-MS conditions

Pyrolyzer	
Instrument	Japan analytical industry Curie point Model JHP-2 (GC), JHP-3 (GC-MS)
Pyrolysis	590°C; 3 s
Oven temperature	120°C
Pipe temperature	250°C
GC	
Instrument	Shimadzu GC-7AG
Detector	FID
Column	J&W DB-5 (0.25 mm i.d.×30 m), film thickness 0.25 μm
Column temperature	60°C (8 min)–230°C (10°C/min)
Injection temperature	230°C
Detector temperature	230°C
Carrier gas	Nitrogen, flow rate 1.0 ml/min, split 30:1
Make up gas	Nitrogen, flow rate 50 ml/min
GC-MS	
Instrument	Shimadzu QP-1000
Column	J&W DB-5 (0.25 mm i.d.×30 m), film thickness 0.25 μm
Column temperature	60°C (8 min)–230°C, 10°C/min
Injection temperature	230°C
Carrier gas	Helium, flow rate 1.0 ml/min
Make up gas	Helium, flow rate 40 ml/min
Ion source energy	70 eV(EI), 20 eV(CI)
Reaction gas	Isobutane

The operational conditions of PyGC were as follows:

- The column oven temperature was initially kept at 60°C for 8 min and then increased to 230°C at a constant rate of 10°C/min.
- The injector and detector were held at 230°C. The carrier gas was nitrogen, flowing at a rate of 1.0 ml/min.
- The split ratio was 30:1. The pipe and oven of the pyrolyzer were maintained at 250 and 120°C, respectively.
- A pyrofoil for 590°C applications was used, and pyrolysis was performed at this temperature.

The identification of peak substances was carried out using GC-MS under the conditions described below.

The ionization energy was used at levels of 70 eV (EI method) and 20 eV (CI method), and the ionization current at the levels of 60 μA (EI method) and 200 μA (CI method). The reaction gas for CI method was isobutane.

2.3. Procedures for alkali-catalyzed PyGC

A sample (approx. 20 μg) consisting of four single wool fibers each approx. 10 mm in length was placed on a piece of pyrofoil. After adding 0.5 μl of 25% aqueous sodium hydroxide, the sample was wrapped in pyrofoil for use in PyGC.

3. Results and discussion

3.1. Alkali and its concentration to be used for alkali-catalyzed PyGC of wool fiber

According to the above procedures for alkali-catalyzed PyGC, the following alkali aqueous solutions were examined as catalysts for alkali-catalyzed PyGC of wool fiber: sodium hydroxide aqueous solutions at concentrations of 2.5–50%, sodium carbonate aqueous solutions at concentrations of 2.5–25%, and sodium hydrogencarbonate aqueous solutions at concentrations of 2.5–10%.

Fig. 1 shows a comparison using the pyrogram obtained by the alkali-catalyzed PyGC with the addition of 25% aqueous sodium hydroxide and that by the conventional non-catalyzed PyGC. It could be clearly seen from Fig. 1 that the presence of aqueous sodium hydroxide during pyrolysis caused a remarkable increase in the production of specific pyrolysis products, i.e. peak 1 (acetonitrile), peak 2 (isobutyronitrile), peak 3 (2-methylbutyronitrile), peak 4 (isovaleronitrile) and peak 5 (toluene) which appeared at the points of retention time (t_R) of 3.0, 3.5, 4.5, 4.6 and 5.3 min, respectively. This indicates that the addition of aqueous sodium hydroxide accelerated the pyrolysis and, at the same time, suggests that the hydrolysis of protein to amino acids was promoted by the catalytic action of sodium hydroxide.

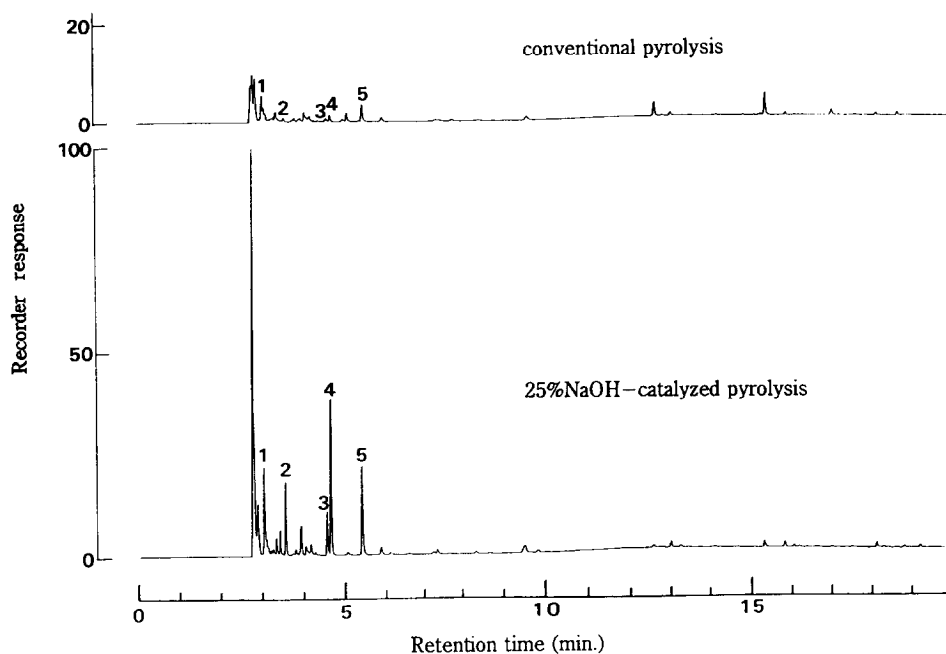


Fig. 1. Pyrograms of wool obtained by conventional pyrolysis and 25% NaOH-catalyzed pyrolysis. peak 1: acetonitrile; peak 2: isobutyronitrile; peak 3: 2-methylbutyronitrile; peak 4: isovaleronitrile; peak 5: toluene.

Fig. 2 shows EI and CI mass spectra used for identifying these pyrolysis products. These mass spectra were correlated to those of the known substances.

The addition of an aqueous sodium carbonate as well as sodium hydrogencarbonate did not provide such a remarkable increase in the production of specific pyrolysis products.

It can be concluded from these results that aqueous sodium hydroxide should be used for alkali-catalyzed PyGC of wool fiber.

Fig. 3 shows the results of the investigation of the concentration of aqueous sodium hydroxide to be used. Compared with the case of conventional non-catalyzed PyGC, the use of 2.5% aqueous sodium hydroxide did not show any remarkable increase in the production of all five specific pyrolysis products, i.e. acetonitrile, isobutyronitrile, 2-methylbutyronitrile, isovaleronitrile and toluene. The latter four pyrolysis products, i.e. isobutyronitrile, 2-methylbutyronitrile, isovaleronitrile and toluene could be observed as increasing production with an increase in the concentration of the alkali solution up to 25% and, at 25%, they were found at levels of 7.6, 8.2, 13.5 and 3.5 times those in the case of non-catalyzed PyGC, respectively. Between concentrations of 25 and 50%, however, there were no differences in their production.

As regards acetonitrile, however, it was found that there was a maximum production at 5% concentration, which was 2.4 times that of the case of non-catalyzed PyGC. After that, its production was seen as gradually decreasing.

Based on the above results, it was decided to use aqueous sodium hydroxide at a concentration of 25%.

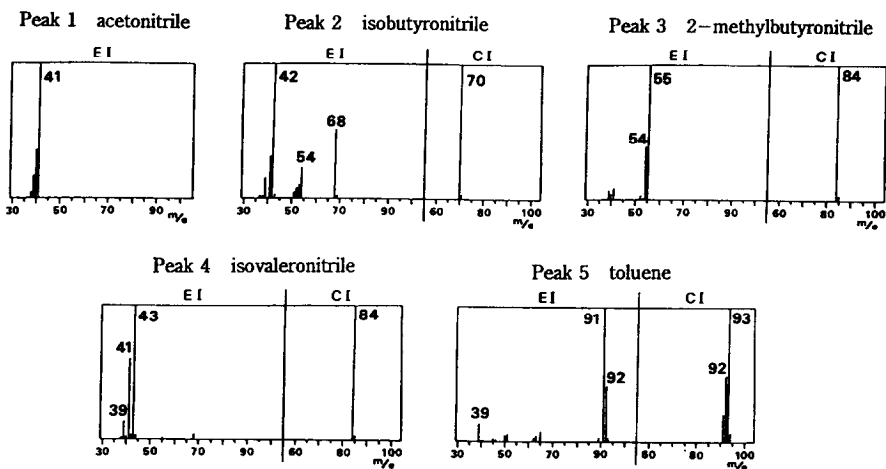


Fig. 2. Mass spectra of major degradation products in alkali-catalyzed pyrolysis of wool using EI and CI ionization.

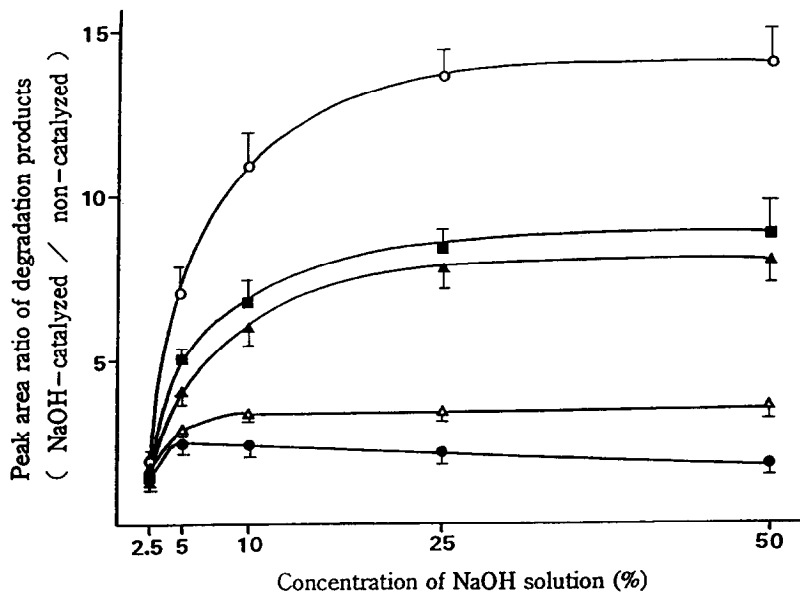


Fig. 3. Effect of concentration of NaOH solution on yields of degradation products in wool pyrolysis. Data presented: mean \pm S.D. of five samples. ●: acetonitrile; ▲: isobutyronitrile; ■: 2-methylbutyronitrile; ○: isovaleronitrile; △: toluene.

3.2. Investigation of column to be used

For use in the present alkali-catalyzed PyGC of wool fiber the following four types of fused-silica capillary columns were examined: DB-1, DB-5 and DB-17 (30 m×0.25 mm i.d., film thickness 0.25 μm) supplied by J&W Co., CA; and SP-WAX (30 m×0.25 mm i.d., film thickness 0.25 μm) supplied by Sigma Aldrich Japan Co. Ltd. They were all tested under the same conditions regarding temperature, etc.


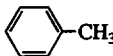
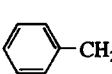
DB-1 was unable to carry out the separation of 2-methylbutyronitrile and isovaleronitrile. DB-17 had difficulty in carrying out the separation of isovaleronitrile and toluene. SP-WAX had difficulties in separating acetonitrile and isobutyronitrile. Only DB-5 was able to carry out the separation of all five specific pyrolysis products.

It was decided from these results to use DB-5 in the present alkali-catalyzed PyGC.

3.3. Assignment of the five major peaks

Each of the major α-amino acids mentioned above was prepared in the form of an aqueous solution, dropped on a piece of pyrofoil so as to weigh 20 μg, dried on a hot plate at approx. 40°C, added with 0.5 μl of 25% aqueous sodium hydroxide, and then wrapped in the pyrofoil for use in PyGC.

Table 2
Degradation products identified on the alkali-catalyzed pyrolysis of wool

Peak No. ^{a)}	Degradation products	Amino acid residues assigned
1	CH ₃ -CN (Acetonitrile)	$\begin{array}{c} \text{NH-} \\ \\ \text{CH}_3\text{-CH-CO- (Alanine)} \end{array}$ <p style="text-align: center;">or</p>  CO- (Proline)
2	$\begin{array}{c} \text{CH}_3\text{-CH-CN (Isobutyronitrile)} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{NH-} \\ \\ \text{CH}_3\text{-CH-CH-CO- (Valine)} \\ \\ \text{CH}_3 \end{array}$
3	$\begin{array}{c} \text{CH}_3\text{-CH}_2\text{-CH-CN (2-Methylbutyronitrile)} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{NH-} \\ \\ \text{CH}_3\text{-CH}_2\text{-CH-CH-CO- (Isoleucine)} \\ \\ \text{CH}_3 \end{array}$
4	$\begin{array}{c} \text{CH}_3\text{-CH-CH}_2\text{-CN (Isovaleronitrile)} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{NH-} \\ \\ \text{CH}_3\text{-CH-CH}_2\text{-CH-CO- (Leucine)} \\ \\ \text{CH}_3 \end{array}$
5	 (Toluene)	 (Phenylalanine)

a) Peak numbers correspond to the pyrogram of wool (Fig.1).

As a major pyrolysis product of these amino acids, acetonitrile was produced from Ala and Pro, isobutyronitrile from Val, 2-methylbutyronitrile from Ileu, isovaleronitrile from Leu, and toluene from Phe, respectively.

As shown in Table 2, it can be concluded from these results that the five major specific pyrolysis products of the wool fiber mentioned above resulted from the amino acid residues of wool protein as follows: acetonitrile resulted from Ala and Pro, isobutyronitrile from Val, 2-methylbutyronitrile from Ileu, isovaleronitrile from Leu, and toluene from Phe.

There are some reports [8–10] that the conventional non-catalyzed PyGC of amino acids and proteins detected amines and aldehydes as the major pyrolysates. In the present alkali-catalyzed PyGC, these amines and aldehydes could not be observed.

3.4. Analysis of wool fiber subjected to thermal denaturation

Wool fiber is morphological, so that, when kept in its normal condition without showing any heat denaturation, it can be identified by inspection using a microscope and material testing using Fourier-transform infrared microspectroscopy. However, a wool

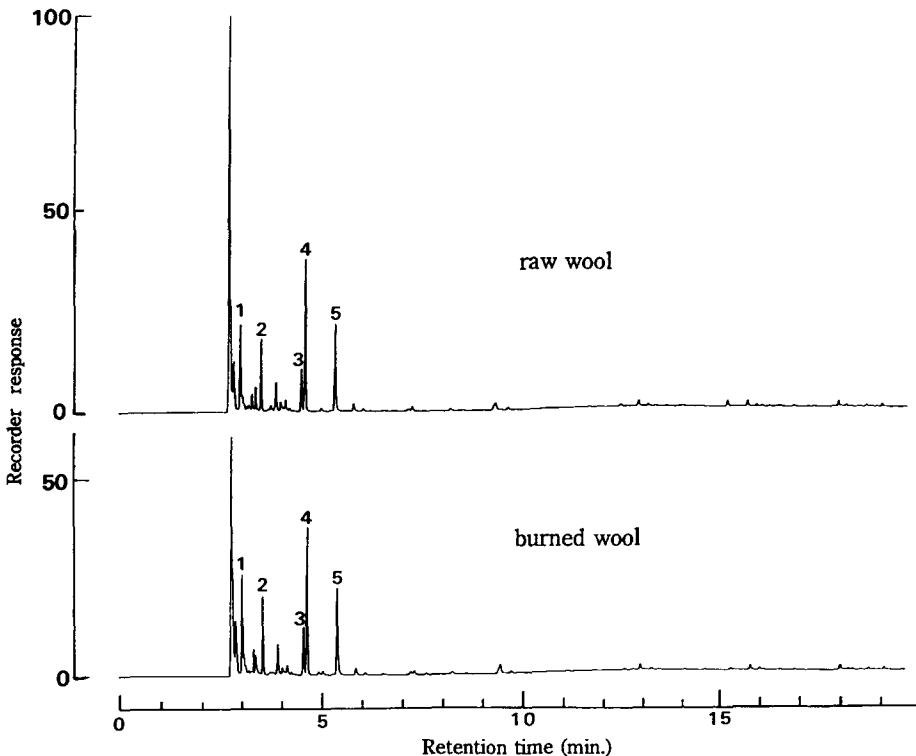


Fig. 4. Pyrograms of raw and burned wool obtained by alkali-catalyzed pyrolysis.

fiber sample subjected to thermal denaturation is difficult to identify using these methods.

The sodium hydroxide alkali-catalyzed PyGC was successfully applied to a wool fiber sample subjected to thermal denaturation, with the sample being prepared in the following way: a set (approx. 100 μg) of 20 pieces of wool fiber each 10 mm in length was burned using a small flame.

As shown in Fig. 4, a pyrogram was obtained that showed a pattern of peaks quite similar to that obtained from a raw wool fiber sample. It can be concluded from this that the sodium hydroxide alkali-catalyzed PyGC makes it possible to identify a minute wool fiber sample subjected to extreme thermal denaturation.

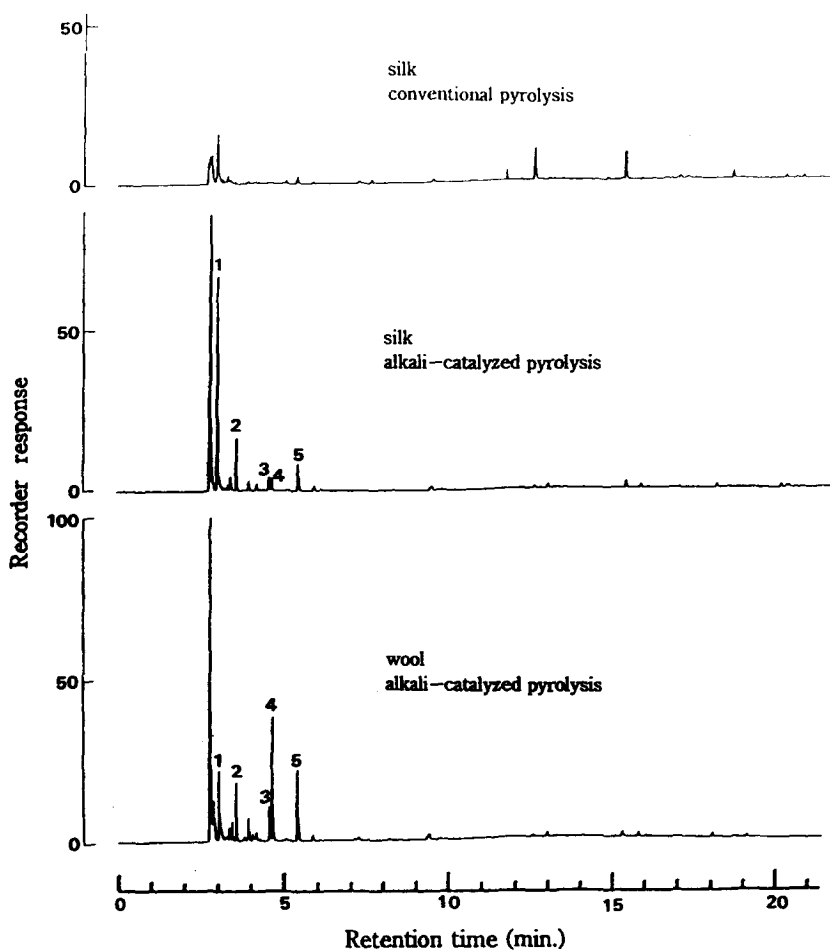


Fig. 5. Pyrograms of silk obtained by conventional pyrolysis and alkali-catalyzed pyrolysis and wool obtained by alkali-catalyzed pyrolysis.

3.5. Application to other textile fiber samples

The present sodium hydroxide alkali-catalyzed PyGC was applied to silk and 6-nylon fiber samples in the form of an 8-mm twisted yarn in length for the former (approx. 20 μg) and in the form of a set of five pieces of fiber each 10 mm in length for the latter (approx. 20 μg): silk was also a typical protein fiber and 6-nylon fiber was a typical synthetic fiber having acid–amide bonds in its molecule similar to a protein.

Their pyrograms thus obtained are shown in Fig. 5 Fig. 6 in comparison with those obtained using conventional non-catalyzed PyGC and that for wool.

It can be clearly seen that the sodium hydroxide alkali-catalyzed PyGC provided specific pyrograms for silk and 6-nylon fiber different from that for wool fiber, leading to the conclusion that this method can adequately identify both of these fibers.

For the case of the silk sample, compared with the non-catalyzed PyGC, this alkali-catalyzed method provided increased production of the five specific pyrolysis

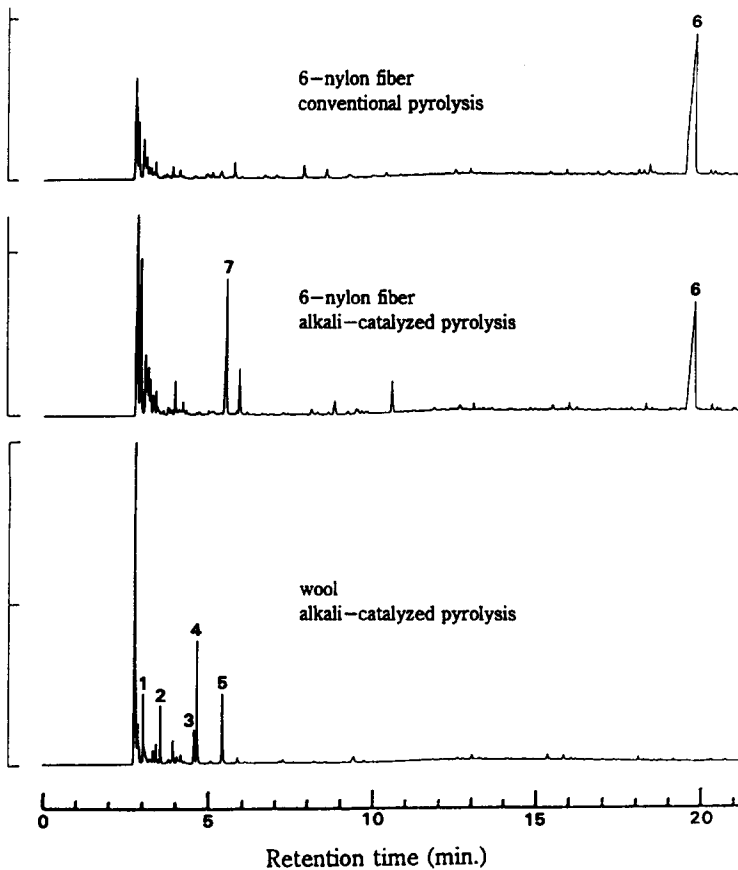


Fig. 6. Pyrograms of 6-nylon fiber obtained by conventional pyrolysis and alkali-catalyzed pyrolysis and wool obtained by alkali-catalyzed pyrolysis. Peak 6: ϵ -caprolactam; Peak 7: pentanenitrile.

Table 3
Peak intensity of major degradation products derived from each amino acid residue on the alkali-catalyzed pyrolysis of seven protein samples and their amino acid contents

Sample	Peak intensity ^{a,b} (amino acid residue ^c)				
	1 (Ala+Pro)	2 (Val)	3 (Ileu)	4 (Leu)	5 (Phe)
Wool	1.56±0.12 (2.09+3.77)	0.74±0.070 (1.82)	0.55±0.040 (1.25)	1.76±0.091 (2.70)	1.00 (1.00)
Silk	7.33±0.25 (47.63+0.63)	1.36±0.11 (3.63)	0.42±0.017 (0.88)	0.41±0.010 (1.13)	1.00 (1.00)
Haemoglobin (human)	0.67±0.012 (2.40+0.93)	0.71±0.022 (2.07)	0.00 (0.00)	1.54±0.043 (2.40)	1.00 (1.00)
Haemoglobin (bovine)	0.63±0.019 (2.12+0.59)	0.57±0.011 (1.79)	0.00 (0.00)	1.37±0.013 (2.18)	1.00 (1.00)
Albumin (human)	0.64±0.024 (2.00+0.77)	0.51±0.018 (1.32)	0.16±0.009 (0.26)	1.31±0.034 (1.97)	1.00 (1.00)
Albumin (egg white)	0.91±0.061 (1.33+0.00)	0.54±0.008 (1.33)	0.46±0.021 (1.24)	0.98±0.016 (1.57)	1.00 (1.00)
Casein (milk)	1.14±0.077 (1.20+3.07)	0.67±0.006 (2.03)	0.61±0.009 (1.57)	1.41±0.022 (2.33)	1.00 (1.00)

^aRelative peak intensity of degradation products derived from each amino acid residue against to the peak intensity of toluene (1.00) derived from Phe.

^bData presented: mean±S.D., n=5.

^cRatio of the number of each amino acid residue in molecule to the number of Phe (1.00).

products, i.e. acetonitrile, isobutyronitrile, 2-methylbutyronitrile, isovaleronitrile and toluene, as major peaks just like those from a wool fiber sample. The patterns of relative intensity of these peaks, however, were found to be different between the cases of silk and wool, showing a much stronger intensity for acetonitrile peak than for the four other peaks in the case of the former. This probably arose from the fact that silk protein contains glycine and Ala as the main amino acid residues in much larger quantities than the other amino acid residues such as Val, Ileu, Leu and Phe.

For the case of the 6-nylon fiber sample, a remarkable peak at $t_R=5.3$ min, which was assigned to pentanenitrile, was observed in addition to the peak of ϵ -caprolactam, the monomer of 6-nylon that was detected as a main pyrolysis product due to the non-catalyzed PyGC.

3.6. Application to other various proteins

Aqueous solutions of human hemoglobin, bovine hemoglobin, human serum albumin, ovalbumin and casein were prepared. Each of these solutions was allowed to drip onto a piece of pyrofoil so as to weigh 20 μg when dried on a hot plate at 40°C, 0.5 μl of 25% aqueous sodium hydroxide was added, and wrapped in the pyrofoil for use in PyGC.

Table 3 shows the test results in terms of the contents of amino acid residues of wool, silk and these proteins and the peak intensities of their pyrolysis products both in their relative values, whereby the relative values for respective amino acid contents are expressed on the basis of 1.00 for Phe which is universally contained in proteins in general, and those for peak intensities on the basis of 1.00 for toluene which is the main pyrolysis product of Phe. The pattern of peaks of pyrolysis products was found clearly different in intensity between pyrograms obtained, depending on the differences in the contents of amino acid residues between proteins. This leads to the conclusion that identification of these proteins is possible.

In the case of human hemoglobin and bovine hemoglobin, 2-methylbutyronitrile could not be detected. This was probably due to the fact that these hemoglobins do not contain Ileu as a protein constitutive amino acid residue.

In the case of ovalbumin, the peak of acetonitrile appeared in intensity relatively stronger than expected from the contents of Ala and Pro residues in this protein. This results from the fact that ovalbumin is a glycoprotein: some pyrolysis products of its carbohydrate residue would have disturbed the correct measurement of the acetonitrile peak.

4. Conclusion

Using of various alkali compounds the usefulness of alkali-catalyzed pyrolysis gas chromatography was examined for identifying wool fiber samples of an extremely minute quantity.

The presence of sodium hydroxide during pyrolysis was proven to cause an increase in the production of specific volatile pyrolysis products from respective amino acid residues of wool protein, i.e. acetonitrile from Ala or Pro, isobutyronitrile from Val,

2-methylbutyronitrile from Ileu, isovaleronitrile from Leu and toluene from Phe. The sodium hydroxide alkali-catalyzed PyGC was found to provide a much better detection limit for wool fiber compared with conventional non-catalyzed PyGC, and was thus concluded suitable for identifying extremely minute fiber samples.

The sodium hydroxide alkali-catalyzed PyGC was proven applicable for identifying minute wool fiber samples subjected to thermal denaturation that were difficult to identify by the morphological inspection using a microscope and by the use of Fourier-transform infrared microspectroscopy.

In addition, this PyGC method has been successfully applied to minute samples of several protein substances other than wool, giving different pyrograms reflecting their different amino acid compositions.

It can be concluded from these results that the sodium hydroxide alkali-catalyzed PyGC is very useful from the viewpoint of forensic science.

This PyGC method operates in the same manner as the conventional non-catalyzed PyGC, except for the addition of aqueous sodium hydroxide. Therefore this is an easy-to-operate method, useful for rapid analysis of minute samples of wool and other protein-related substances.

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