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Journal of the Korean Society of Clothing and Textiles
Vol. 34, No. 6 (2010) p.1042~1052

GC-MS Analysis of Amur Cork Tree Extract and Its Degradation Products

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Received June 21, 2010; Accepted June 27, 2010

Abstract

The Degradation of amur cork tree extract is investigated by GC-MS after treating the dye with three thermal degradation systems of, room temperature (RT), 4°C refrigeration (LT), 100°C oven (OV), and H_2O_2/UV/O_2 (PER) degradation system for 0-24 days. It was found that PER degradation system represented the highest intensity of degradation treatment followed by OV treatment among the four degradation parameters. The possible fingerprint products of amur cork tree dye, that yielded 68% (or higher) reliability in the NIST spectral match, were isobenzofuran-1,3-dione,4,5-dimethoxy- (8.37 min, PER only), 1,3-dioxolo[4,5-g]isoquinolin-5(6H)-one,7,8-dihydro (9.41 min, PER only), canthine-6-one (10.24 min, RT, LT, OV only), and dihydroberberine (15.05 min, RT, LT, OV, PER) in the order of higher to lower possibility of detection. Unknown products 7 (13.43 min) and 8 (16.35 min) are two other possible fingerprint products of amur cork tree dye that require future identification.

Key words: Amur cork tree, Berberine, Degradation, GC-MS, Dye identification

I. Introduction

This study is part of the long-term project of dye identification in the badly faded archaeological textiles excavated from the burial context. The goal of the long-term project is to establish the database of possible fingerprint degradation compounds of each natural dyes by degrading the dyes using selective laboratory treatments. In the previous paper the author investigated the degradation products of berberine dye, the major dye component of amur cork tree, using the GC-MS analysis. Applying H_2O_2/UV/O_2 and 110°C oven degradation, the study found four products- dihydroberberine, 2-pteridinamine, 6,7-dimethyl-N-[(trimethylsilyl)oxy]-, and 8-methoxy-11-[3-methylbutyl]-11H-indolo[3,2-c]-quinoline, 5-oxide- as the fingerprint products of berberine dye (Ahn, 2009b). While the previous paper dealt with the berberine chloride which was purchased from the chemical stock, whether the same degradation behavior is observed in the natural dye extract of amur cork tree is still in question. In this regards, this paper purports to examine the degradation products of the dye liquor extracted from the inner bark of amur cork tree and compare the result with the previous investigation on the berberine dye. The degradation of the dye extracted from amur cork tree was carried out by using the four different degradation parameters, three of which are thermal condition- room temperature, 4°C refrigeration, 110°C oven- and one which is the H_2O_2/UV/O_2 method. The goals of the present investigation were to identify the fingerprint products of amur cork tree extract after degrading it with the four treatments, to compare the result with the previous investigation on the stan-

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This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0068065). This work was supported by the University of Incheon Research Grant in 2009.

Fig. 1. Berberine (a), Berberine Chloride (b).

dard berberine degradation, and to compare the degradation behavior among the four degradation treatments.

Amur cork tree (*Phellodendron ammurense* R.) is native to northeast Asia and it has long been used as the source of medicine and also as a strong yellow dye. The dye is found in the stems or inner bark of amur cork tree and also in the roots of Rhizome Coptidis. The major chromophoric substance of amur cork tree is berberine (molecular weight: 336, $C_{20}H_{18}NO_4^+$, 5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a] quinolizinium), an isoquinoline akaloid, and it is the only cationic dye among the natural plant dyes (Fig. 1). The chemical standard of berberine is sold as berberine chloride by global chemical stores such as Sigma-Aldrich.

II. Theoretical Framework

While the degradation of most exhumed textiles is the result of long-term burial in the coffin, it is difficult to simulate the same condition due to the complex nature of the coffin environment. Studies which examined the degradation of textiles by actually burying them in the ground found out that the color of the textile darkened (Needles et al., 1986) and the strength decreased (Peacock, 1996). Although, burying the textile under the ground seems to be the closest treatment for simulating the burial environment, the typical underground environment is usually different from the coffin environment due to possible micro-interaction with the dead and the artifacts. Furthermore, the soil content is different across geography and depth, and a tremendous amount of experiment time would be required to simulate the lengthy burial. The uncontrollable nature of the underground condition also presents a serious drawback.

Considering such difficulties, a laboratory experimental design which can somewhat simulate the possible degradation conditions under controlled environment seems to be in need. In the previous research, the author developed certain experimental conditions with the above purpose and has been used to examine the degradation of several natural dyes. The method includes leaving the dye liquor or the textile under $H_2O_2/UV/O_2$ condition, 110°C oven temperature, 4°C refrigeration, and room temperature (Ahn & Obendorf, 2004, 2007).

The background for selecting the $H_2O_2/UV/O_2$ condition is as follows. Extensive researches have been conducted on the microbiological degradation of dyes to enhance the effectiveness of biodegradation of dye wastewater in the factories. White-rot fungi are known to be effective in degrading and decolorizing dye wastewater since they produce and emit peroxidase enzymes (Jarosz-Wilkolazka et al., 2002). Researches on the soil microbial environment indicate that such microbes are present in most soil types with differing population size (Child, 1995). On the other hand, hydrogen peroxide can be produced in the soil by itself even with the absence of white-rot fungi. According to Scheck and Frimmel (1995) the humic acid present in most soil types can be reduced to superoxide ion radical (O_2^-), and the radical reacts with water to produce either H_2O_2 or O_2. This theory was verified by Cooper and Lean (1989) who said that H_2O_2 concentration of a North American lake during a sunny day was 400×10^{-9}M. Based on the literature $H_2O_2/UV/O_2$ method was chosen as the experimental condition.

The background for selecting the thermal degradation conditions is as follows. The refrigeration at 4°C

was chosen based on the studies related to the temperature of soil and burial grounds. According to Rieger (1983), the mean soil temperature about 115cm below vegetation is near 5°C, and Child (1995) suggests that the mean soil temperature of mid-latitude region within a burial context is above 0°C. The thermal degradation in 110°C oven temperature was chosen based on the researches simulating natural ageing of dyed textiles via accelerated thermal treatment (Brushwood, 1988; Needles et al., 1986; Needles & Nowak, 1989). In addition to 4°C refrigeration and 110°C oven treatment, room temperature was selected for the non-degradation condition. The four degradation conditions are labeled PER (H_2O_2/UV/O_2), OV (110°C oven), LT (4°C refrigeration), and RT (room temperature).

III. Experimental

1. Material

The inner bark of amur cork tree was purchased from the traditional herbal market of Korea. Berberine sold commercially as berberine chloride was purchased from Sigma Aldrich. Methanol used was HPLC grade (Acros Organics, USA), H_2O_2 was a reagent grade purchased from J.T. Baker (Phillipsburg, NJ, USA). Glass vial with cap (Fisher Scientific cat # 03-339-21H, capacity 29.6ml, size 25×95mm, USA) was used for preparing the liquid degradation samples. Glass fiber reinforced 0.45um syringe filter(Alltech, Deerfield IL, USA) was used to prepare the GC-MS vials and all the glassware was rinsed using acetone before usage.

2. Extraction

The dry bark was washed, dried, and ground in a mill (Thomas Scientific Model 3388-L10, USA). 10g of powdered amur cork tree and 100ml methanol was thrown into 250ml Erlenmyer flask. The mixture was heated for 1 hour on the 50°C hotplate with constant shaking, and the extract was filtered under reduced pressure using büchner funnel. A 0.1% solution of berberine standard dye was prepared by dissolving berberine chloride in HPLC grade methanol.

3. Degradation

For each degradation treatment, individual vial was prepared for each degradation time (0, 1, 2, 4, 7, 10, 14, 17, 21, 24 day). 2ml of the extracted dye was poured in each glass vial. RT sample was tightly capped, sealed with parafilm and put inside the fume hood in the laboratory with minimum light exposure. LT sample was tightly capped, sealed and put inside the 4°C refrigerator. OV sample was loosely capped and put inside the 110°C oven. For PER samples, 0.5ml of 30% H_2O_2 was added to the 2ml extract. PER vials were uncapped and put under 365nm UV lamp inside the fume hood. The UV lamp was positioned so that the distance between the lower edge of the lamp and the hood top was about 12.1cm. With this positioning, the distance between the bottom edge of the lamp and the top of the liquid was about 9.3cm.

4. GC-MS Analysis

For PER samples, the liquor was completely evaporated and the residue was dissolved with 1ml of HPLC grade methanol. For OV samples, the residue from the oven treatment was dissolved with 1ml of HPLC grade methanol. The so prepared PER OV samples and LT, RT samples from the degradation setting were filtered using a 0.45μm glass fiber enhanced syringe filter.

The GC-MS used was a Hewlett-Packard GC 6890 Series coupled to the Agilent Technologies 5973N MSD. The GC column was a Hewlett Packard 19091s-433 capillary column (HP-5MS, 30cm×250μm i.d.). Column temperature was initially 50°C, gradually increased to 210°C at a 23°C/min rate, finally increased to 305°C at 30°C/min rate, and held for 14 min. The initial temperature of the MSD system was 310°C and the mass spectra were recorded at scan range of 75-40m/z (Ahn, 2009a). The assignment of possible degradation products was based on the match with standard mass spectrum available in the GC-MS library database (Agilent Technologies, 2000).

IV. Results and Discussion

1. Identification of Fingerprint Products

The GC chromatogram of the dye extracted from amur cork tree before degradation treatment is presented in <Fig. 2>. <Fig. 3> illustrates the chromatogram of berberine standard dye. Upon a full survey of all the peaks appearing in the chromatogram of the amur cork tree extract, product assignment of a number of peaks was possible by comparing the MS spectrum of each unknown peak with the closest spectral match provided by the NIST library based on the ion fragmentation pattern (Agilent Technologies, 2000) (Table 1). Among the spectral match of each peak given by the NIST database library, only those yielding reliability higher than 68% are considered to be the signature product of amur cork tree and are listed in <Table 1>. The criteria of 68% reliability was chosen based on McLafferty et al. (1991). The two peaks with the highest intensity, peak at 9.64 min and 8.97 min, are excluded in <Table 1> despite the fact that they their ion fragmentation pattern was very close to the library with high reliability. According to the NIST database library (Agilent Technologies, 2000) the two peaks were identified as linoleic acid (9.64 min) and palmitic acid (8.97 min), which are the colorless fatty acids naturally occurring in plants. Although the two peaks have the highest intensities in the chromatogram of amur cork tree

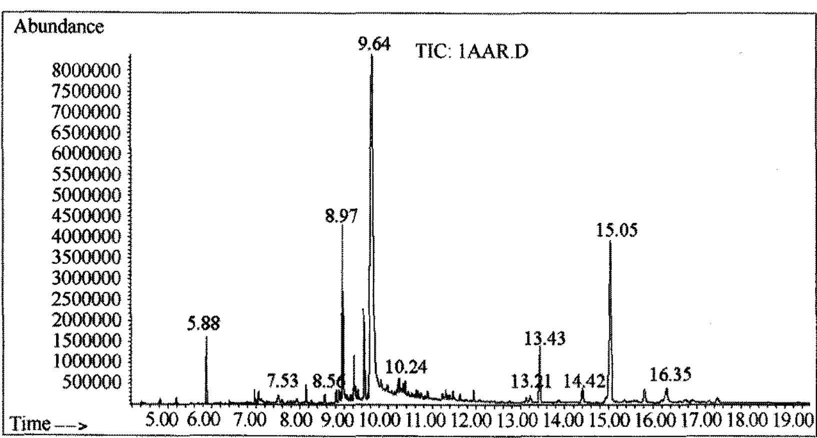

Fig. 2. GC chromatogram of the dye extracted from amur cork tree, before degradation treatment.

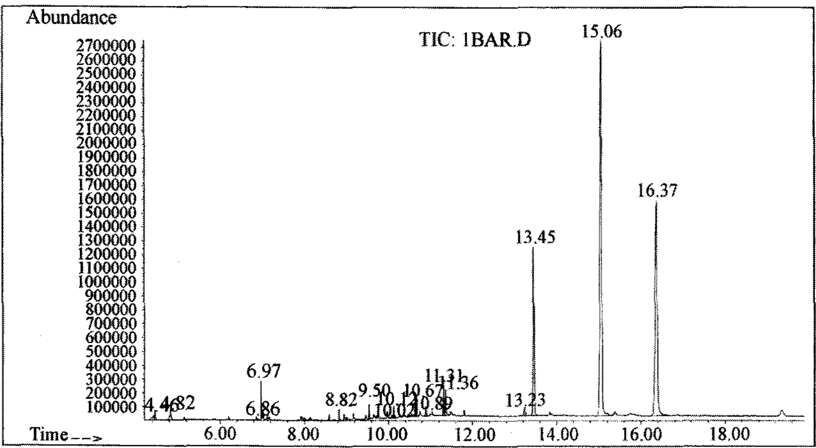

Fig. 3. GC chromatogram of standard berberine dye.

Table 1. Product assignment of the GC peaks of amur cork tree extract before degradation treatment. Peaks yielding above 68% reliability by NIST database

No.	Retention time (min)	Product assignment		Major ion (m/z)	Relative abundance		Reliability (%)
		Name (molecular weight)	Structure		Library	Sample	
1	15.05	Dihydroberberine (MW: 337)		336 337 338	54.3 100 20.3	81.3 100 21.0	97.9
2	7.53	Quinic acid (MW: 192)		60 112 156	100 45.0 5.6	100 54.5 5.0	94.5
3	10.24	Canthine-6-one (MW: 220)		164 192 220	13.0 65.0 100	11.9 59.5 100	89.3
4	13.21	Tetrahydroberberine N-oxide (MW: 355)		164 338 339	80.3 90.3 100	88.8 64.4 100	73.0

extract, they cannot be used as the signature products of amur cork tree since they are commonly detected products of many other plant species such as sappanwood (Ahn, 2007).

Consistent with the previous papers (Ahn, 2009a, 2009b), berberine was not detected from the amur cork tree extract nor in the standard berberine dye. Instead, dihydroberberine (15.05 min) was detected as the signature of berberine dye following Turner et al. (2008a), Turner et al. (2008b), and Song et al. (2002). Dihydroberberine is one of the derivatives of berberine used as medication, and it is known to metabolize to berberine immediately after it enters the body (Turner et al., 2008a). Turner et al. (2008b) illustrated the experimental process of synthesizing dihydroberberine from berberine. Song et al. (2002) suggested a method of producing dihydroberberine by a one-electron reduction process of C=N bond of berberine. While a more detailed discussion of dihydroberberine transformation from berberine is presented in Ahn (2009a), in the present investigation dihydroberberine was detected at 15.05 min with a high reliability ion fragmentation match of 97.9% by NIST database, indicating that the product detected is indeed dihydroberberine (Table 1).

The peak of 7.53 min was assigned as quinic acid or in other name, -(1R,3R,4R,5R)-(-)-quinic acid. Considering the high reliability of 94.5% it is highly probable that the product is indeed quinic acid. Quinic acid was not detected in the previous study on the degradation of standard berberine dye (Ahn, 2009b). Quinic acid is a white crystal non-nitrogenous powder found in many different plants such as tobacco leaves, carrot leaves, apples, peaches, etc. as alkaloid (Buchler GmbH, 2008; Wikipedia, 2010). Gentry et al. (1998) found out that berberine and also quinic acid were isolated from the root sample of Hydrastis canadensis. Considering the low peak intensity of quinic acid in the present result, it is suggested that the amur cork tree under investigation comprised of quinic acid as well in trace amount. And since quinic acid is an independent compound found in natural

plants, it is not surprising that it was not found in the standard berberine dye of the previous study.

The peak found at 10.24 min was assigned by the NIST library database (Agilent Technologies, 2000) as canthine-6-one or in other name, 6H-Indolo[3,2,1-de][1,5]naphthyridin-6-one, with reliability yield of 89.3% (Table 1). The strong reliability level suggests that the product assignment is correct. And such GC-MS result of the present investigation is consistent with the findings of Ikuta et al. (1998a, 1998b) who successfully isolated canthine-6-one from the callus tissue of the stme of P. amurense (amur cork tree) along with the main constituents of berberine, palmatine, jatrorrhizine, etc. Canthine-6-one also was not detected in previous study on the degradation of standard berberine dye (Ahn, 2009b). Another product in <Table 1> is the peak at 13.21 min which was assigned as tetrahydroberberine N-oxide based on the ion fragmentation pattern by the NIST library with the reliability yield of 73.0%. According to Vennerstrom et al. (1990), tetrahydroberberine N-oxide is another derivative of berberine which can be synthesized directly from berberine. Tetrahydroberberine also was not detected in the previous study on the degradation of standard berberine dye (Ahn, 2009b)

Based on the above findings, it can be summarized that dihydroberberine is the signature product of berberine when amur cork tree or standard berberine dye are examined by GC-MS. Besides dihydroberberine, canthine-6-one and tetrahydroberberine N-oxide are also the fingerprint products of amur cork tree dye. Quinic acid is a non-nitrogenous coumpound present in the amur cork tree and other plant species.

Other peaks of interest in the GC-MS of amur cork tree extract are peaks found at 13.43 min and 16.35 min. The two peaks were assigned as 8-methoxy-11-[3-methylbutyl]-11H-indolo[3,2-c]-quinoline, 5-oxide-(13.43 min) and 2-pteridinamine, 6,7-dimethyl-N-[(trimethylsilyl)oxy]- (16.35 min) by the NIST library and the product assignment was consistent with that of the previous investigation on the degradation of standard berberine dye (Ahn, 2009b). However, since the present study is limiting the product assignment of the NIST library with reliability yield of 68% or more, the above assignment of the products at 13.43 min and 16.35 min cannot be accepted. Their reliability levels of the NIST spectral match are 22.25% and 29.4% each. Nevertheless, the two unknown products need full attention since they have a high potential as the possible signature products of berberine and amur cork tree dye considering the consistency in the occurrence and the high intensities in both amur cork tree extract and standard berberine dye. The two products are labeled unknown product 7 (13.43 min) and unknown product 8 (16.36 min).

As degradation progressed, the peak intensities of the above assigned products changed, and after PER degradation new peaks immerged at different retention times (Fig. 4). <Table 2> illustrates the two new products detected after the PER degradation treat-

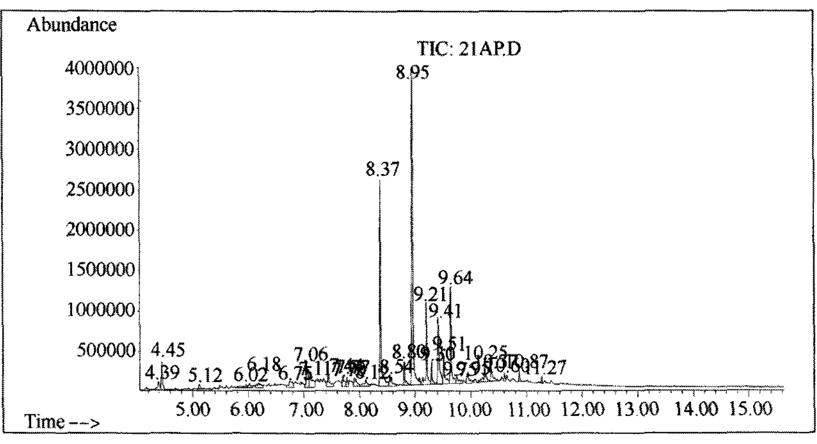

Fig. 4. GC chromatogram of 21-day PER sample.

Table 2. Product assignment of the GC peaks of amur cork tree extract after PER degradation treatment

No.	Retention time (min)	Product assignment		Major ion (m/z)	Relative abundance		Reliability (%)
		Name (molecular weight)	Structure		Library	Sample	
5	8.37	Isobenzofuran-1,3-dione,4,5-dimethoxy- (MW: 208)		78 163 208	90.0 33.2 100	59.3 50.1 100	96.0
6	9.41	1,3-Dioxolo[4,5-g] isoquinolin-5(6H)-one,7,8-dihydro- (MW: 191)		134 162 191	100 72.1 80.9	94.7 73.8 100	97.4

ment of amur cork tree extract. The two peaks were detected at 8.37 min and 9.41 min and the appearance of the two peaks was consistent with that of the PER degradation of standard berberine dye reported earlier (Ahn, 2009b). Consistent with the previous result on standard berberine (Ahn 2009b), the peaks were assigned as isobenzofuran-1,3-dione,4,5-dimethoxy- (8.37 min) and 1,3-dioxolo[4,5-g]isoquinolin-5(6H)-one,7,8-dihydro (9.41 min) by the NIST library with reliability of spectral match 96.0% and 97.4% respectively on the ion fragmentation pattern (Table 2). However, these two products were detected only after PER degradation treatment. And this is probably due to the fact that PER is the most accelerated mode of degradation treatment among the four degradation parameters applied in this investigation.

2. Degradation Behavior of Each Products

The change of relative abundance of the chromatogram peak of each product identified by GC-MS analysis is illustrated in <Fig. 5>–<Fig. 8>; <Fig. 5> depicting RT degradation, <Fig. 6> depicting LT degradation, <Fig. 7> depicting OV degradation, and <Fig. 8> depicting PER degradation.

During the degradation time up to 24 days (control, 1, 2, 4, 7, 10, 14, 17, 21, 24 day), there has been a notable decrease in the relative abundance of dihydroberberine (product 1 in the figures). The most notable change was observed in the PER samples in

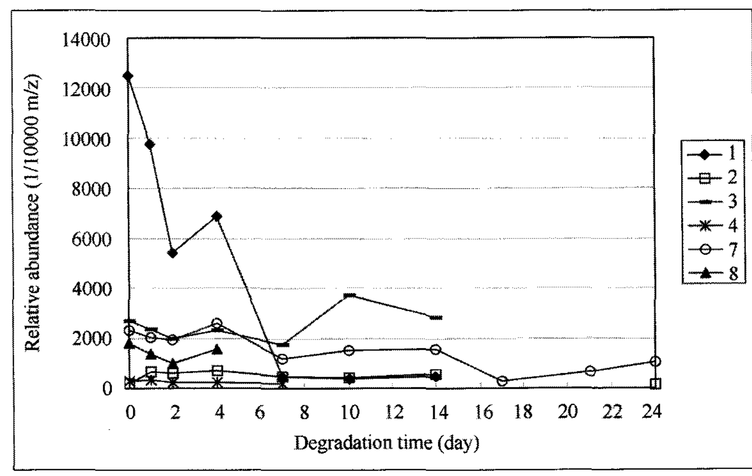

Fig. 5. Change of relative amount of products in room temperature. 1: dihydroberberine, 2: quinic acid, 3: canthine-6-one, 4: tetrahydroberberine, 7: unknown (13.43 min), 8: unknown (16.35 min).

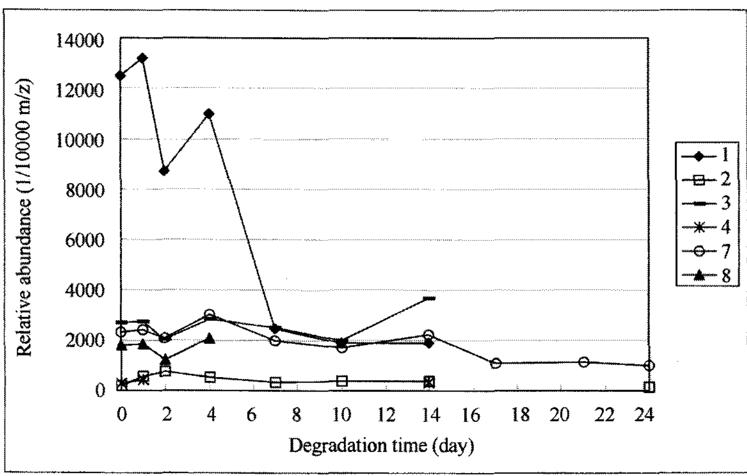

Fig. 6. Change of relative amount of products in 4°C refrigeration. 1: dihydroberberine, 2: quinic acid, 3: canthine-6-one, 4: tetrahydroberberine, 7: unknown (13.43 min), 8: unknown (16.35 min).

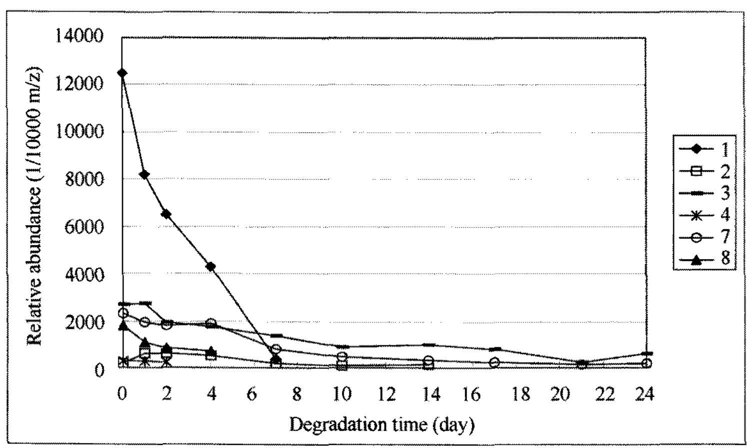

Fig. 7. Change of relative amount of products in 110°C oven. 1: dihydroberberine, 2: quinic acid, 3: canthine-6-one, 4: tetrahydroberberine, 7: unknown (13.43 min), 8: unknown (16.35 min).

which dihydroberberine dramatically decreased after only one day and was not detected at all after 2 day degradation treatment (Fig. 8). Although slightly slower than the PER treatment, the OV degradation treatment also illustrated a fast decrease of dihydroberberine with the product disappearing after 7 day degradation treatment (Fig. 7). Even in RT and LT degradation environment dihydroberberine was not free of degrading. Dihydroberberine was not detected after 14 days, and there was a visible decrease in the relative abundance after 7 days (Fig. 5)-(Fig. 6). However, comparing the four degradation systems LT system seems to provide the most preservable environment for the amur cork extract and this phenomenon is in agreement with the similar degradation study on other natural dyes (Ahn & Obendorf, 2004, 2007). It is notable that the relative abundance of dihydroberberine showed a slight increase after 1 day refrigeration and that the decreasing rate was much lower than putting it in room temperature.

Compared to dihydroberberine, the relative abundance of quinic acid (product 2 in the figures), canthine-6-one (product 3), tetrahydroberberine (product 4), and the two unknown products at 13.43 min (prod-

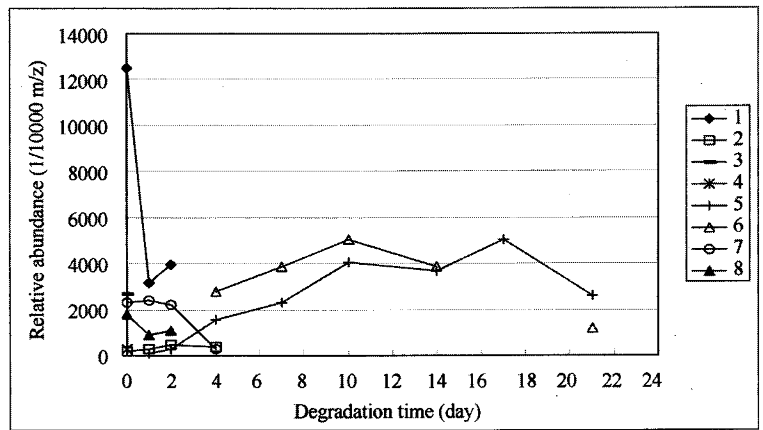

Fig. 8. Change of relative amount of products in $H_2O_2/UV/O_2$ degradation. 1: dihydroberberine, 2: quinic acid, 3: canthine-6-one, 4: tetrahydroberberine, 5: isobenzofuran-1,3-dione,4,5-dimethoxy-, 6: 1,3-dioxolo[4,5-g]isoquinolin-5(6H)-one,7,8-dihydro, 7: unknown (13.43 min), 8: unknown (16.35 min).

uct 7) and 16.35 min (product 8) were very small. Quinic acid which is another form of alkaloid present in the amur cork tree was detected with a similar amount of relative abundance in all four degradation system, present up to 14 days in RT, LT, and OV treatment and up to 4 days in the PER treatment.

Canthine-6-one which is the derivative and the fingerprint of berberine is the product with highest relative abundance besides dihydroberberine. In RT and LT treatments, the relative abundance of canthine-6-one was even higher than that of dihydroberberine when the degradation time reached 7 days. Similarly, in the OV treatment the relative abundance of canthine-6-one was higher than dihydroberberine when the degradation time reached 7 days, and while dihydroberberine disappeared after 7 days canthine-6-one was consistently detected up to the last day of degradation treatment. However, when the amur cork tree extract was degraded under PER condition, canthine-6-one disappeared as the treatment began and it was not detected at all in the degraded samples from 1~24 days. This phenomenon was the same for tetrahydroberberine which was detected only up to 2 days of OV degradation, 4 days of RT degradation, 1 day of LT degradation, and none in the PER system.

On the other hand, the two unknown products (product 7 and 8) showed more consistency in the detection, especially the product 7 detected at 13.43 min. Although its relative abundance is lower than dihydroberberine, its relative abundance was overall higher than the three products discussed above (products 2, 3, and 4) and it was detected consistently throughout the degradation time except for the PERT degradation treatment.

In the PER degradation, the two new products, isobenzofuran-1,3-dione,4,5-dimethoxy- (product 5 in the figure) and 1,3-dioxolo[4,5-g]isoquinolin-5(6H)-one,7,8-dihydro (products 6). emerged. Product 5 especially emerged as soon as the treatment began and product 6 was detected as the degradation time reached 4 days. Anyhow, the relative abundance of the two products were noticeably high until it disappeared after 21 days of treatment. However, the two products were only detected in the PER samples and not in any of the RT, LT, nor OV samples.

V. Conclusions

The above findings illustrate that PER represents the highest intensity of degradation treatment followed by OV treatment among the four degradation parameters selected for this study on the amur cork tree extract. Going back to the earlier discussion on the identification of dye in badly faded archaeological textiles, the present findings suggest that the result of PER degradation and possibly that of the

OV degradation as well can be applied in the GC-MS identification of the dye in the exhumed textiles which had been possibly dyed with the amur cork tree dye, Considering that the exhumed textiles will come into the hands of the analyst after the degradation has progressed extensively, at the time of possible analysis it is highly probable that the main dye component of amur cork tree will have disappeared or left to a minimum amount. Therefore, the products with higher chance of detection will be the ones still remaining at the finishline of the degradation time of this study. In this sense the possible fingerprint products of amur cork tree dye after degradation are isobenzofuran-1,3-dione,4,5-dimethoxy- (product 5), 1,3-dioxolo[4,5-g]isoquinolin-5(6H)-one,7,8-dihydro (product 6), canthine-6-one, and dihydroberberine in the order of higher to lower possibility of detection. Although quinic acid can also be used as a possible match for amur cork tree dye, the presence of quinic acid cannot be the absolute answer to berberine dye since it is a compound present in other plant species as well. Additionally, two other products, unknown product 7 (13.43 min) and unknown product 8 (16.35 min) also can be used as the fingerprint of amur cork tree dye although their identification still needs to be investigated in the future research. To achieve a full understanding of the degradation of amur cork tree dye, along with the verification of the present result future research should focus on the examination of the fabric dyed with amur cork tree dye or standard berberine dye using the same degradation treatments and the instrumental methods as applied in the present investigation.

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