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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF TANNIN HYDROLYSATES FROM THE INK OF ANCIENT MANUSCRIPTS (XIth TO XVIth CENTURY)

P. ARPINO* and J.-P. MOREAU

Laboratoire de Chimie Analytique Physique, École Polytechnique, Route de Saclay, 91128 Palaiseau (France)

and

C. ORUEZABAL and F. FLIEDER

Centre de Recherches sur la Conservation des Documents Graphiques, Muséum National d'Histoire Naturelle, 36, rue Geoffroy Saint-Hilaire, 75005 Paris (France)

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SUMMARY

A procedure has been devised for determining the constituents of ink from ancient hand-written parchments. Some of the tannic acids and sugars resulting from acid hydrolysis of ink could be identified at the nanogram level (thus necessitating the destruction of only a small amount of ink from the manuscript) by gas chromatography–mass spectrometry of their trimethylsilyl derivatives. The presence of "ferrogallic" inks on some manuscripts was assumed from the detection of gallic acid and glucose by selective ion monitoring and capillary-column gas chromatography. The method has been applied to inks from eleven European manuscripts dating from the XIth to the XVIth century, and to various possible ink precursors (extracts from gall nuts, pods of Tara (*Caesalpinia spinosa*) and gum arabic).

INTRODUCTION

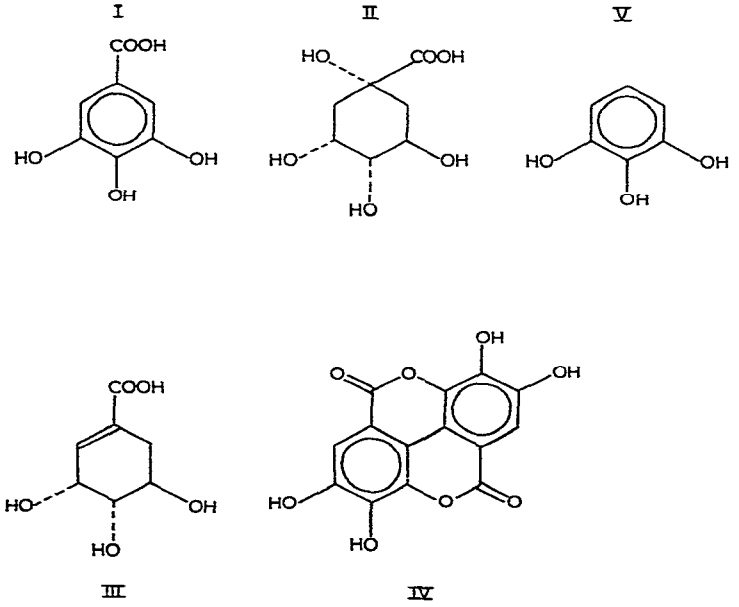
The chemical identification of ink constituents plays a major role in the preservation and restoration of ancient manuscripts[1]. Some recipes for the various ink preparations used many centuries ago are still known, so that relevant information can be obtained by comparing the ink remnants on a manuscript with present-day ink constituents: indeed, changes in chemical composition may reflect geographical variations among local starting materials or different aging conditions. Modern analytical technology and artificial aging have brought new tools to museums and libraries faced with conservation of ancient written documents. Of course, only non-destructive or ultra-sensitive methods requiring minute pieces of manuscript can be considered, and such a method is described here; preliminary investigations by infrared spectroscopy

* To whom correspondence should be addressed.

and gas chromatography (GC) have been reported[1]. To overcome the limitations of GC (infrared analysis provides limited amounts of information), we investigated the combination of GC with mass spectrometry (MS); use of the combined technique (GC–MS) and the preliminary results are reported here.

"Ferro-gallic" ink is ink obtained by precipitation of natural gallotannins, mainly of plant origin, on iron sulphate. A gum, frequently gum arabic, is usually added as a binding agent. It differs from other types of ink, such as carbon ink, which is a suspension of carbon particles in oil (see the appropriate literature[2,3] for more details). Evidence of tannin residues from the ink on a manuscript is an important parameter and confirms the use of ferro-gallic ink: an analytical procedure was required for this purpose.

Many of the chemical constituents of tannins of plant origin used for preparing inks are known[1–4]. Tannins can be partly degraded by acid hydrolysis yielding the tannic acids [gallic acid (I), digallic acid, trigallic acid, quinic acid (II), shikimic acid (III) and ellagic acid (IV)], various other high-molecular-weight phenolic acids, carbohydrates (pentoses and hexoses) and phenols [*e.g.*, pyrogallol (V)]. Attempts to further degrade acid-resistant tannins from ink have not been considered in this study. Thus, acid hydrolysis of an allegedly ferro-gallic ink may yield a complex mixture of mutarotated monosaccharides and tannic acids. Despite the additional complexity introduced by trimethylsilylation of the sugars[5–8], trimethylsilyl (TMS) derivatives of both tannic acids and carbohydrates can be rapidly and quantitatively prepared and analysed by GC–MS. Similar analytical procedures for detection of theaflavins[9], gallotannins from beer[10] and shikimic and quinic acids[11] based on GC of their TMS derivatives have been reported; such methods do not usually pose difficult problems. However, in our work, as the amount of material recovered from hydrolysis was limited, separation of tannic acids from sugars by column or thin-layer chromatography was avoided.



EXPERIMENTAL

Preparation of derivatives

Tannins from known ink precursors (extracts from Chinese and Turkish gall, and pods of Tara), and binders (gum arabic) were hydrolysed and derivatized as described below, using about 0.3 mg of starting material. Inks from various European manuscripts were carefully detached from the parchment by using a scalpel and examined under a binocular microscope: about 1 mg of ink was taken from various letters on several pages. Parchment is made of collagen and differs from such cellulosic supports as paper, and from such tanned materials as leather, in that no interference is likely to arise from contaminating sugars or tannic acids. In a blank experiment on 1 mg of unused parchment, no sugars or tannic acids were found.

Hydrolysis is carried out in a small stoppered vial by heating 2 ml of a suspension of the test substance in 3% aqueous hydrochloric acid at 105° for 4 h; the resulting solution is evaporated to dryness under vacuum, and TMS derivatives are prepared.

Our earlier studies[1], and those of other workers[9,10], showed that tannic acid constituents were best silylated by N,O-bis(trimethylsilyl)acetamide. However, this reagent yields from a pure monosaccharide a complex mixture of anomer derivatives[7]. In this work, therefore, silylation was carried out with hexamethyldisilazane–trimethylchlorosilane–pyridine (2:1:10), which is effective for both free sugars[6] and tannic acids[9,11]. The reaction was allowed to continue for 10 min at ambient temperature, then 1 μl was withdrawn with a syringe and injected into a DuPont Model 21–492B GC–MS apparatus.

Reference carbohydrates (D-glucose, D-galactose, L-arabinose and L-rhamnose), tannic acids (gallic, quinic and ellagic acids) and pyrogallol were silylated as described above and analysed by GC–MS. Identification of the substances from ink hydrolysates was confirmed by co-injection, with the standards, into a Girdel Model 3000 gas chromatograph.

Gas chromatography

High-performance capillary columns and long analysis times are needed for complete resolution of a mixture of the TMS derivatives of natural sugars[8,12]. Our problem was made more complex because the TMS derivatives of the tannic acids interfered with some of the sugar derivatives. When available, a capillary column is recommended, but valuable information can be obtained from ink hydrolysates with use of classical packed columns[1]. The injection technique of Grob and Grob[13] was useful for the transfer of sample to column with minimum loss. Metallic or glass open-tubular columns coated with OV-101 or OV-17 have been used for GC and GC–MS.

Selective ion monitoring provides another tool for separation and identification when GC separation is incomplete: mass spectra of reference mixtures of the TMS derivatives of gallic, ellagic and quinic acids and pyrogallol showed abundant ions that did not interfere with those from the derivatives of the carbohydrates encountered. Thus, when such a facility is available, lesser resolution and a shorter GC time of analysis are acceptable. A standard packed column [2 m × 2 mm I.D.; SE-30, 1.5% on Gas-Chrom Q (100–120 mesh)] can be used for the detection of gallic

acid, even though its peak overlaps that of β-galactopyranose (see Fig. 2). A good compromise between inertness, efficiency and speed of analysis is offered by glass micro-packed columns of I.D. 0.5 mm filled with Chromosorb W (100–120 mesh); use of such a column for the rapid screening of complex mixtures, together with GC–MS, should be emphasized. The columns were prepared in lengths ranging from 5 to 10 m and were dynamically coated with OV-101 or OV-17 as previously described[14].

Mass spectrometry

Packed or micro-packed columns were connected to the mass spectrometer via a single-stage jet-type separator; capillary columns were directly connected to the spectrometer source. Simple glass-to-metal connections were made as reported elsewhere[15]. Mass spectra were taken at a scan speed of 1 sec/decade in a cyclic mode with a period of 4 sec. Data acquisition and processing were performed with the help of a DuPont Model 094B-2 data system.

RESULTS

Chromatograms reconstructed by summing the ion intensities of each scan are shown in Fig. 1 for the reference materials and in Fig. 2 for one of the eleven inks studied. This chromatogram, obtained from a XVIth century ink, is typical of those from the other inks, except that some of them contained less gallic acid (or even an undetectable amount), but the pattern for arabinose, galactose and glucose was very similar.

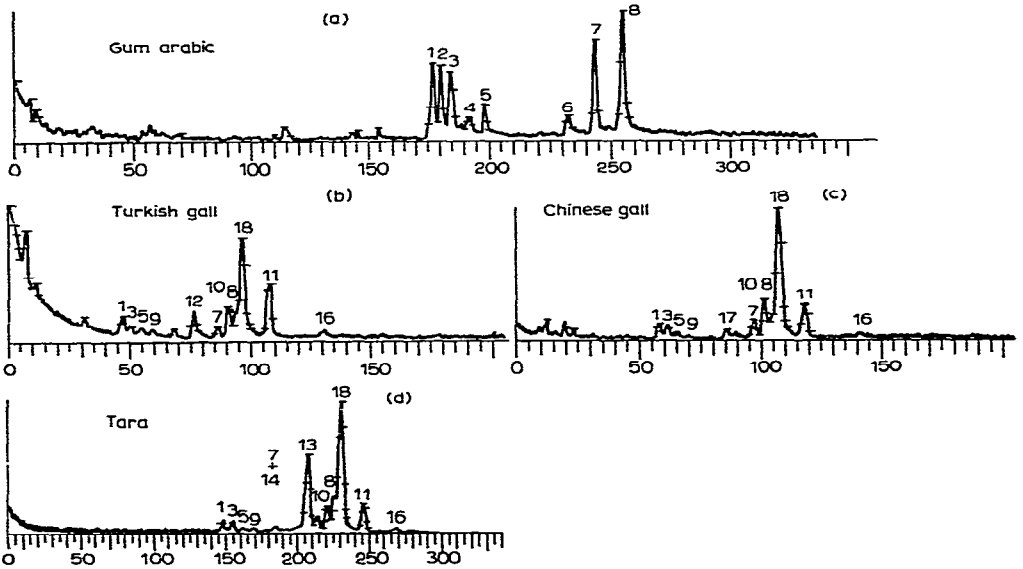


Fig. 1. Computer-reconstructed GC plot from GC–MS of reference material. (a), OV-101 capillary column; 20 m × 0.25 mm I.D.; temp. 130 to 250° at 2°/min. (b), (c) and (d), SE-30 packed column, 2 m × 2 mm I.D.; temperature, 150 to 250° at 6°/min. The compounds responsible for the peaks numbered 1 to 18 are listed in Table I.

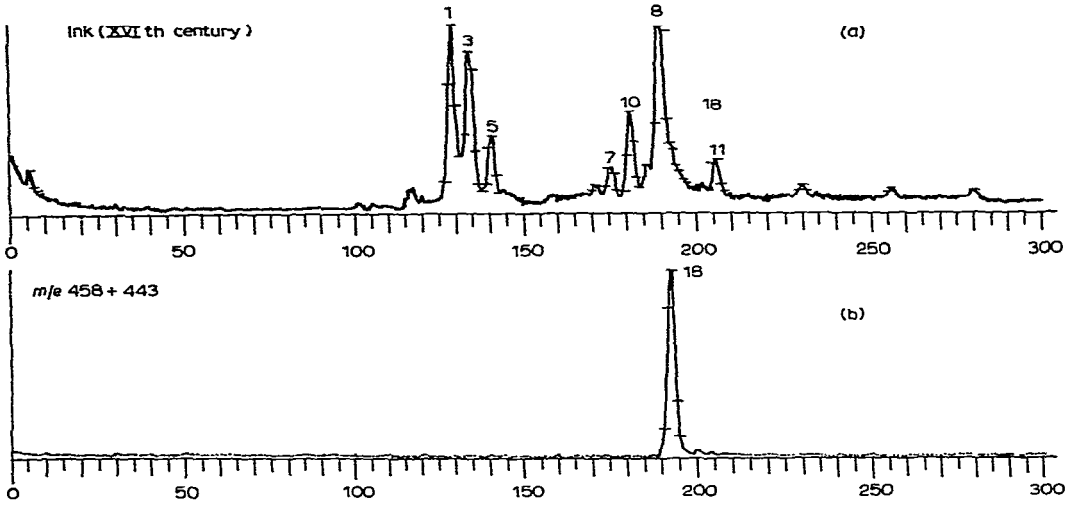


Fig. 2. Computer reconstructed plots of (a) the GC–MS analysis of a XVIth century ink hydrolysate [GC conditions and peak numbering as in Fig. 1 (b)], and (b) mass chromatogram at *m/e* 458 plus 443 from the same GC–MS analysis; this selectively displays the presence of the TMS derivative of gallic acid in the mixture.

Carbohydrates

Acid hydrolysis and formation of TMS derivatives causes mutarotation of sugars, and both furanoside and, less abundantly, pyranoside forms of α- and β-anomers were regularly encountered. Assumptions on ring size were made from mass-spectrometric evidence[16,17], the criteria being the abundance of *m/e* 204 over 217 for pyranosides, and *m/e* 217 largely predominant over 204, with a significant peak at *m/e* 332, for furanosides. The mass spectra of α- and β-anomers do not differ, and identification was confirmed by co-injection with the standards and comparison of retention values with those found in the literature[7].

Simple monosaccharides are the major constituents of fossil inks and gum arabic; they were relatively less abundant than gallic acid in the gall and Tara extracts

TABLE I

COMPOUNDS RESPONSIBLE FOR PEAKS ON CHROMATOGRAMS

Peak No.	*Compound*	*Peak No.*	*Compound*
1	β-Arabinopyranose	10	α-Glucopyranose
2	α-Rhamnopyranose	11	β-Glucopyranose
3	α-Arabinopyranose	12	Unknown (M^{+} = 434)
4	β-Rhamnopyranose	13	Unknown (M^{+} = 554)
5	β-Arabinofuranose	14	Quinic acid
6	Unknown (M^{+} = 490)	16	Hexopyranose (unknown)
7	α-Galactopyranose	17	Shikimic acid
8	β-Galactopyranose	18	Gallic acid
9	Pentopyranose (unknown)		

examined. A mass chromatogram at *m/e* 204 and 217 selectively displayed the sugar pattern in a mixture.

Arabinose was found in all samples examined. Rhamnose is eluted separately from arabinose on an OV-101 capillary column, but partly overlaps β-arabinopyranose on a packed column. However, their mass spectra permit unambiguous distinction (*m/e* 243, 259 and 279 for arabinose; and *m/e* 245, 273 and 347 for rhamnose); mass chromatograms at these values, and at the value for $M^+ - CH_3$ (423 for arabinose, 437 for rhamnose) were used in this study. Rhamnose was found in gum arabic and in four of the inks studied.

Both glucose and galactose were present in all samples except gum arabic, which did not contain glucose. The presence of glucose is positive evidence of a ferrogallic ink, as natural gallotannins always yield significant amount of glucose on acid hydrolysis[3,4]. However, as glucose was found in all the inks studied, even when gallic acid could not be detected, its presence alone is inconclusive. Similarly, when arabinose and galactose are present together with rhamnose, a gum (*e.g.*, gum arabic) may be assumed to be a precursor.

Gallotannins

The mass spectra of the TMS derivatives of gallic, quinic and ellagic acids differ significantly from those of sugars; electron-impact MS yields a clear and distinct molecular ion, and the main fragments occur at masses other than those of sugars. Gallic acid partly overlaps β-galactopyranose on a packed OV-101 column, but it can be resolved by using a capillary column or a packed OV-17 column. With our samples, generation of mass chromatograms at *m/e* 458 (M^+) and 443 ($M^+ - CH_3$) offered an unambiguous method of identification, even when GC separation was incomplete. An example is shown in Fig. 2 for the XVIth century ink hydrolysate. Under similar experimental conditions, gallic acid was positively identified in four other samples, the oldest being from the XIth century. The concentration of gallic acid with respect to that of glucose was found consistently to decrease with the passage of time from major abundance in gall nuts to a very small amount in the oldest manuscript on which it was found.

Under the same analytical conditions and with OV-101 columns, quinic acid could not be chromatographically resolved from α-galactopyranose, even with a highly efficient glass capillary column. However, selective ion monitoring at *m/e* 537 ($M^+ - CH_3$) and 462 (M^+ — TMSOH) was effective for the specific detection of this acid. Quinic acid was found in Tara, but never on the manuscripts studied; this could indicate that those inks containing gallic acid were prepared from gall nuts rather than from Tara[1].

Shikimic acid was tentatively identified in Chinese gall, but not in Turkish gall, from its mass spectrum and published retention values[7,11]. Positive identification of this acid could provide a geographical clue as to the origin of an ink; however, none of the inks studied yielded this acid on hydrolysis.

Pyrogallol is a possible degradation product of gallic acid, but we failed to identify it in ink hydrolysates.

Analysis of ellagic acid involved use of different conditions, as a column temperature of 290° was needed for its elution. Although it exhibits a very simple and characteristic mass spectrum, due to its aromatic structure, we failed to identify it in ink hydrolysates.

CONCLUSIONS

From this work, and from previous GC studies[1], we have been able unambiguously to identify gallic acid in ink on ancient manuscripts, thus proving the ferrogallic origin of these inks. The method is rapid, but it still requires an over-large amount of ink (1 mg). To be more useful, the method should be applied to only 100–300 μg of ink: this should be possible by using the full sensitivity of the mass spectrometer and by single-ion monitoring at *m/e* 458. A more elaborate procedure should be considered for a more detailed study of the other tannic acids: limitations in the proposed method are attributable to the sugars liberated, which may prevent detection of tannic acids in low abundance, and a better method would involve selective removal of the sugars from the hydrolysis products; however, such a separation would be difficult to achieve at the microgram level.

The other main fact that we have established is that the concentration of gallic acid relative to that of glucose decreases with the passage of time: artificial aging experiments are now in process involving analysis of the degradation products of gallic acid when exposed to heat, moisture or radiation from a xenon lamp.

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