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PYROLYSIS GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE IDENTIFICATION OF ANTHOCYANINS

GAETANO LANZARINI, LUCIANO MORSELLI and PIER GIORGIO PIFFERI
Istituto di Tecnologie Chimiche Speciali, Facoltà di Chimica Industriale, Università di Bologna, Bologna (Italy)
and
ANGELO GIOVANNI GIUMANINI
Centro di Gascromatografia–Spettrometria di Massa, Università di Bologna, Bologna (Italy)
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SUMMARY

A new method is described for the rapid qualitative identification of anthocyanins which is based on recognition of differential features of the individual pyrograms and identification by gas–liquid chromatography coupled with mass spectrometric analysis.

INTRODUCTION

Anthocyanins are naturally occurring pigments which possess the 2-phenyl-5,6-benzopyrylium ion (also called flavylium ion) and are derivatized as glucosides. The difficulties encountered in the identification of these compounds have been well documented[1–7], the main problems being their complex structure and high reactivity. Attempts to synthesize their trimethylsilyl derivatives led to incomplete results[8].

Some compounds which are similar to anthocyanins, when subjected to pyrolysis, give characteristic gas chromatographic diagrams (pyrograms) that can be used for qualitative and quantitative determinations[9]. The effectiveness of pyrolysis combined with gas chromatography has been demonstrated for the identification of high-molecular-weight substances[10–18] or highly complex structures[19–25]. We previously reported the results obtained by this technique for some anthocyanidins[26] and anthocyanins[27]. The present paper describes a method based on pyrolysis gas chromatography (GC)–mass spectrometry (MS) for the rapid and accurate identification of the most widely distributed anthocyanins.

EXPERIMENTAL

Cyanidin-, delphinidin-, peonidin- and malvidin-3,5-diglucosides were obtained from natural sources; pelargonidin-3,5-diglucoside was purchased from Fluka,

Buchs, Switzerland and Baker, Deventer, The Netherlands. Each pigment was carefully purified by paper chromatography just before use[28–31].

A Varian-Aerograph Model A-425 pyrolyzer equipped with a platinum filament was coupled with a Perkin-Elmer Model 900 gas chromatograph and a Perkin-Elmer Model 270 mass spectrometer. No strong absorptions of the eluates were encountered in the use of the Perkin-Elmer Model 270 mass spectrometer, as assessed by independent gas–liquid chromatographic (GLC) traces of the pyrolyzates using a flame-ionization detector. The GLC separation of the pyrolysis products was accomplished by use of a steel column (3 m × 5 mm I.D.) packed with Carbowax 20M (10%) on Chromosorb W (60–80 mesh). The injection port was kept at 240°. The oven temperature was programmed as follows: 15 min at the initial temperature (30°), then at 6.5°/min up to 200° and finally isothermic at 200° for 20 min. Nitrogen was the carrier gas for the GLC analyses; helium was used for the GLC–MS determinations. Flow-rates were adjusted to *ca.* 25 ml/min. Under these conditions the analysis time was *ca.* 60 min.

Mass spectra were recorded at 75 eV and at a filament output of *ca.* 100 μA; the scan time was 30 sec/decade Repetitive scanning of ten a.m.u. was employed especially for unsymmetrical and broad GLC peaks. The pyrolysis time and temperature were optimized in order to obtain meaningful results (peak number and intensity); the platinum filament of the pyrolyzer was charged with *ca.* 2–4 mg of wet pigment through which was passed a current of *ca.* 8 A for 20 sec.

RESULTS AND DISCUSSION

The complete pyrograms of the anthocyanins examined are shown in Figs. 1–6. A summary of the main GLC peaks obtained on pyrolysis of all of the anthocyanins, together with their GLC characteristics and relative abundances in the individual pyrograms, is given in Table I. The chemical identification of the individual peaks was based solely on the data gathered from the electron-bombardment spectra; in fact, for 19 of the major peaks, the identification was achieved with various degrees of certainty by comparison with known spectra.

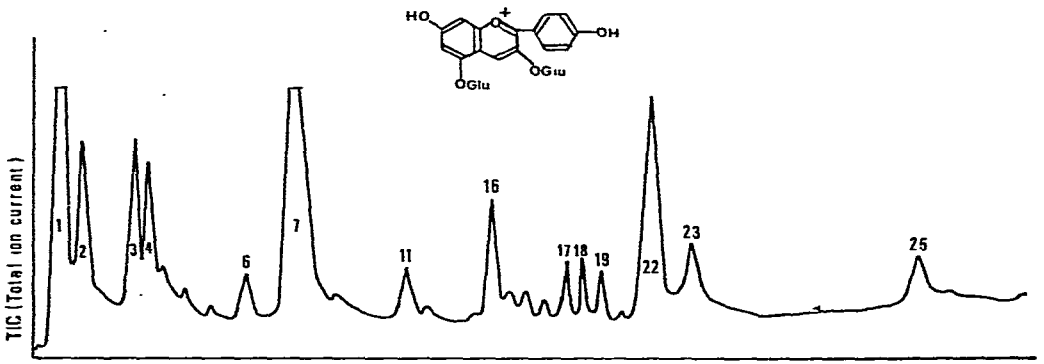

Fig. 1. Pyrogram of pelargonin. Column (3 m × 5 mm I.D.): steel packed with Carbowax 20M (10%) on Chromosorb W (60–80 mesh). Temperature: 15 min at 30°, then at 6.5°/min up to 200° and 20 min at 200°; injector, 240°. Carrier gas: helium at *ca.* 25 ml/min. Analysis time: *ca.* 60 min; sample size: *ca.* 2–4 mg. Peaks as in Table I.

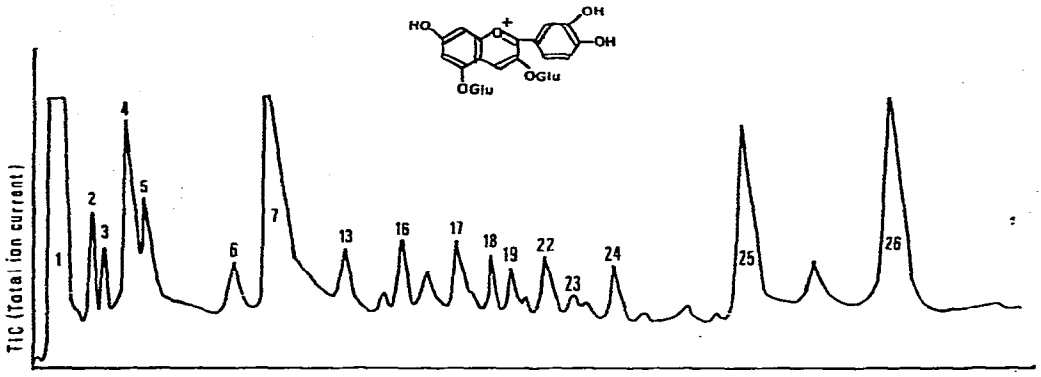

Fig. 2. Pyrogram of cyanin. Conditions as in Fig. 1.

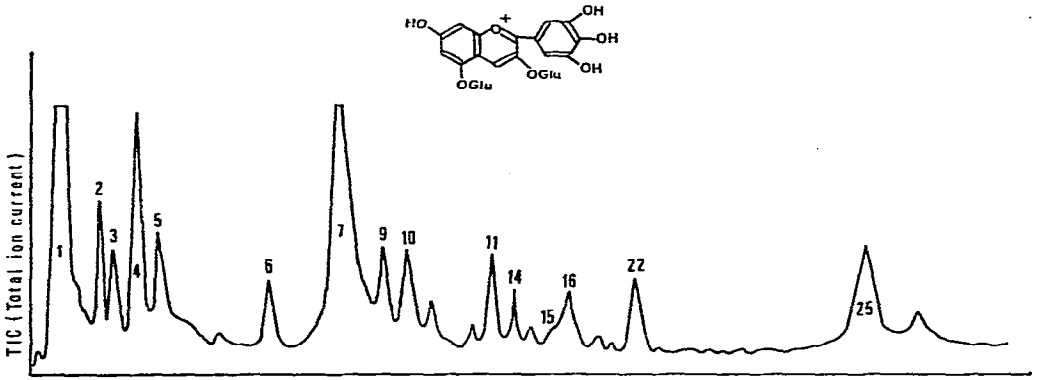

Fig. 3. Pyrogram of delphinin. Conditions as in Fig. 1.

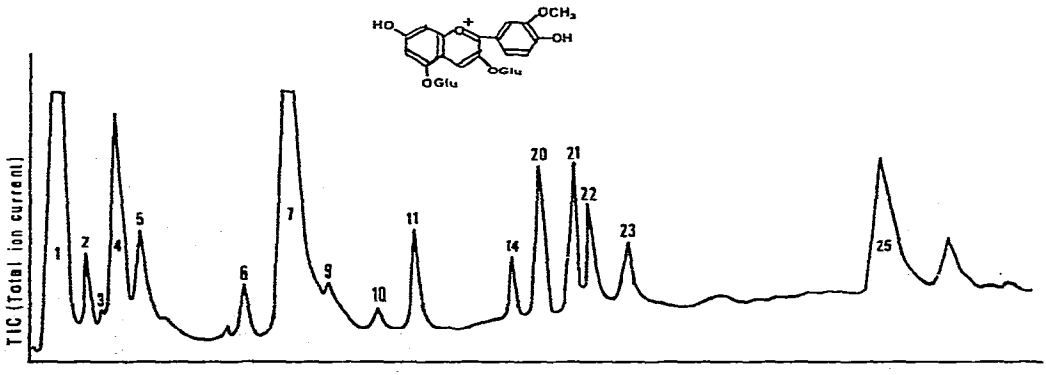

Fig. 4. Pyrogram of peonin. Conditions as in Fig. 1.

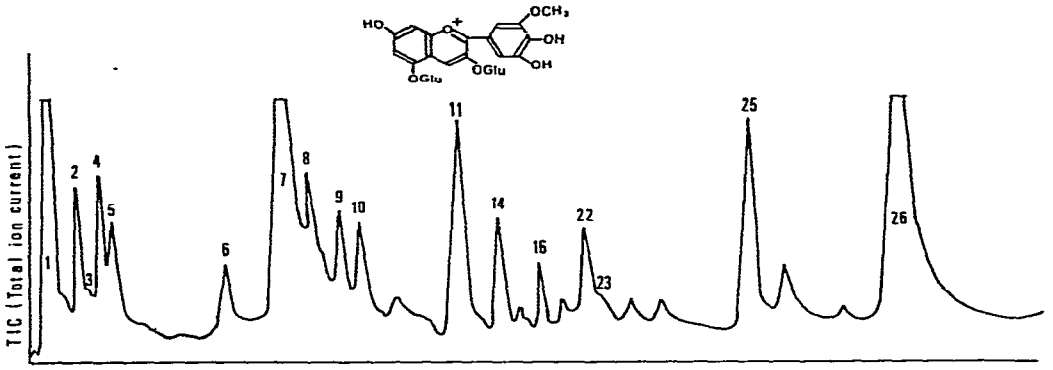

Fig. 5. Pyrogram of petunin. Conditions as in Fig. 1.

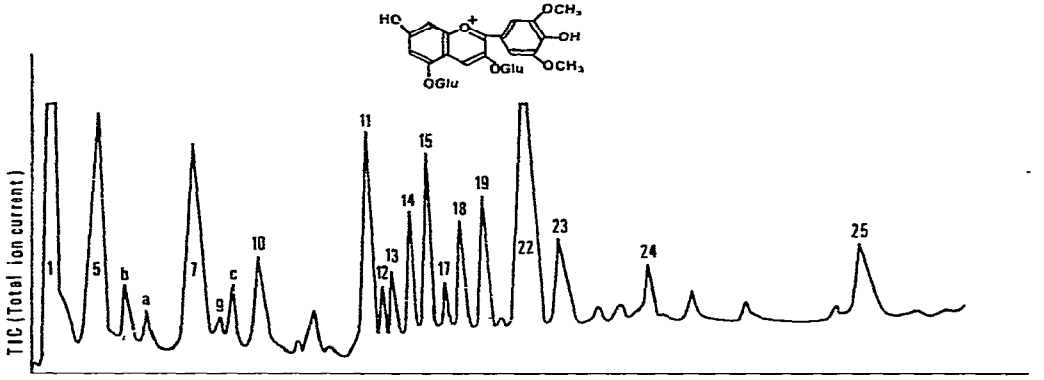

Fig. 6. Pyrogram of malvin. Conditions as in Fig. 1.

The pyrolysis time was an important variable. Short pyrolysis times led to simpler, but much less meaningful, pyrograms. Under the chosen conditions, a characteristic "fingerprint" could be assigned to each anthocyanin on the basis of the presence and relative abundance of the individual peaks. The reproducibility of the pyrogram profiles was good, but no attempt was made to calculate peak-area ratios; the relative intensities of the peaks were established by visual inspection. While any detailed discussion of the mechanism of pyrolysis is outside the scope of this work, however, a few remarks about the prominent pyrolysis features of the individual anthocyanins can be made.

The prominent peaks of pelargonidin-3,5-diglucoside are due to phenol (peak 22), *o*-cresol (23) and naphthalene (16). Rather flat pyrograms were exhibited both by cyanidin-3,5-diglucoside and by delphinidin-3,5-diglucoside and the GLC profile, rather than any individual peak, may be distinctive: the peaks for chlorobenzene–ethylbenzene (9, not separated), styrene (10) and 2-furaldehyde (11) were evident only for the latter compound. Peonidin-3,5-diglucoside was characterized by the presence of guaiacol (20) and dimethoxybenzene (21), a feature unique for this pigment. *o*-Cresol (23) and 2-furaldehyde were also distinctly present in the pyrogram. The profile for petunidin-3,5-diglucoside was characterized by the abundant peaks of furalde-

TABLE I

TYPICAL PYROGRAM WHICH INCLUDES THE PRINCIPLE PEAKS AND THE PYROLYZATES OBTAINED FROM EACH ANTHOCYANIN

r.t. = Retention time relative to peak 11; e.t. = peak exit temperature; × = product present in large quantity and for which the mass spectrum was recorded; 0 = product present in small quantity for which the mass spectrum was not recorded, the product being identified by comparison with the retention time relative to peak 11; — = product absent in this anthocyanin. Pg = Pelargonin, Cn = cyanin, Df = delphinin, Pn = peonin, Pt = petunin, and Mv = malvin.

Peak No.	*Structure assigned*	*r.t.*	*e.t. (°C)*	*Anthocyanin*					
				Pg	*Cn*	*Df*	*Pn*	*Pt*	*Mv*
1	Mixture of low mol. wt. compounds	0.06	30	×	×	×	×	×	×
2	Acetone	0.15	30	×	×	×	×	×	0
3	2-Methylfuran	0.19	30	×	×	×	0	0	0
4	Methanol	0.22	30	×	×	×	×	×	0
5	Benzene	0.27	30	0	×	×	×	×	×
6	Toluene	0.53	35	×	×	×	×	×	—
7	Water	0.66	60	×	×	×	×	×	×
8	Unknown	0.74	78	—	—	—	—	0	—
9	Chlorobenzene–ethylbenzene	0.79	90	0	—	×	0	×	—
10	Styrene	0.83	98	—	—	×	0	×	×
11	2-Furaldehyde	1.00	133	×	0	×	×	×	×
12	Benzofuran–furylmethylketone	1.02	137	—	—	—	—	—	×
13	*n*-Paraffin	1.04	142	—	×	—	—	—	×
14	Methylfuraldehyde	1.06	147	0	—	×	×	×	×
15	Methylbenzoate	1.08	150	—	—	0	—	—	×
16	Naphthalene	1.12	158	×	×	×	—	×	—
17	*n*-Paraffin	1.17	170	×	×	—	—	—	×
18	*n*-Paraffin	1.20	175	×	×	—	—	—	×
19	*n*-Paraffin	1.22	180	×	×	—	—	—	×
20	Guaiacol	1.27	190	—	—	—	×	—	—
21	Dimethoxybenzene	1.32	200	—	—	—	×	—	—
22	Phenol	1.34	200	×	×	×	×	×	×
23	*o*-Cresol	1.41	200	×	0	—	×	0	×
24	*n*-Paraffin	1.46	200	—	×	—	—	—	×
25	Phthalates	1.77	200	×	×	×	×	×	×
26	Phthalates	2.05	200	—	×	—	—	×	—

hyde (11) and methylfuraldehyde (14). The pyrogram of malvidin-3,5-diglucoside was dominated by the phenol (22) peak, as that of pelargonidin-3,5-diglucoside was dominated by the *o*-cresol peak; the former diglucoside, however, also showed peaks for benzofuran–acetylfuran (12, unresolved) and methyl benzoate.

In connection with previous work[21–23], it appears that some pyrolysis products, like 2-furaldehyde and 5-methyl-2-furaldehyde, derive from the glucoside moiety of the pigment molecules; thus the presence of such groups enables one to distinguish between anthocyanins and anthocyanidins. Heyns *et al.*[32] demonstrated that products such as benzene, toluene and benzofuran originate from the pyrolysis of D-glucose. The presence of chlorinated compounds in the pyrolyzates can be traced back to the fact that chloride was the pigment anion. Phthalates, and perhaps hydrocarbons, observed in some pyrograms are probably due to contaminants.

The present results support the belief[26,27] that pyrolysis gas chromatography–mass spectrometry can provide the basis of a new analytical method for the identification of anthocyanins.

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