# Characterization of Procyanidins of *Vitis vinifera* Variety Tinta del País Grape Seeds

Teresa Escribano-Bailón, Yolanda Gutiérrez-Fernández, Julián C. Rivas-Gonzalo, and Celestino Santos-Buelga*

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain

HPLC has been applied for the analysis of procyanidins in seeds from grapes of *Vitris vinifera* variety Tinta del País. Use of a diode array detector permitted the location on the chromatogram of peaks corresponding to procyanidins and procyanidin gallates and also a possible procyanidin esterified with *p*-coumaric acid. A seed extract was fractionated by gel chromatography on Sephadex LH-20 and then by preparative HPLC to isolate compounds and permit their identification. Structures were characterized by TLC, complete and partial acid hydrolysis, enzymatic hydrolysis, and thiolysis in the presence of phenylmethanethiol with later desulfuration of the benzyl thioethers obtained. The heptamers could be the compounds with the highest degree of polymerization present in the extract, although only dimer, trimer, and tetramer procyanidins were isolated.

## INTRODUCTION

Recently, the procyanidins have received considerable attention owing to their pharmacological effects, in particular their effects on arteriosclerosis (Masquelier, 1987), and their radical scavenger ability (Ariga et al., 1990; Ricardo da Silva et al., 1991a; Uchida et al., 1986). The proanthocyanidins esterified with gallic acid, whose antiinflammatory and antiallergic properties seem to be superior to those of nonacylated tannins, are of special interest (Boukharta et al., 1988).

The most important sources of these compounds in the diet, at least in the Mediterranean region, are grapes and wine. Together with other phenolics, they contribute to the sensory properties in wines, beers, and cider, in fruits and juices, and in plant foods in general (Cheynier et al., 1990; Lea, 1984; Metche, 1988). They also have an important role in the maturation and aging of red wine and in the processes of oxidative browning of grapes and juices (Cheynier et al., 1988; Cheynier and Ricardo da Silva, 1991; Lea, 1980; Oszmianski et al., 1985; Singleton, 1989). In grapes and wines the major proanthocyanidins are the procyanidins, formed by condensation of units of (+)-catechin and (−)-epicatechin. The richest part of the grape is the seed, from where procyanidins are extracted during the wine-making process (Bourzeix et al., 1986).

Different authors have studied the procyanidin composition of grape seeds (Boukharta et al., 1988; Bourzeix et al., 1986; Jaworski and Lee, 1987; Lea et al., 1979; Lee and Jaworski, 1987, 1990; Oszmianski and Lee, 1990; Oszmianski and Sapis, 1989; Oszmianski et al., 1988; Revilla et al., 1991; Ricardo da Silva et al., 1990, 1991b; Rigaud et al., 1991). The highest compounds cited are hexamers (Lea et al., 1980), but only the structures of some dimer and trimer procyanidins and their acylated derivatives have been elucidated. All of the acylated procyanidins found in grape seeds are esters of gallic acid, esterification normally occurring on the oxygen at position 3 of the heterocycle (Boukharta et al., 1988). Some authors have also found derivatives of gallocatechin in grapes (Czochanska et al., 1979; Lea et al., 1979; Lee and Jaworski, 1990).

The aim of the present work was to characterize procyanidins present in seeds of grapes of the *Vitis vinifera* variety Tinta del País, a grape employed for the elaboration of wines with certified brand of origin belonging to the Ribera de Duero region in Spain.

## MATERIALS AND METHODS

**Samples.** Grapes from *V. vinifera* variety Tinta del País were harvested at Pedrosa de Duero, Spain, a town located in the region known as Ribera de Duero, and collected at their optimum point of maturity in the last week of September 1990.

**Preparation of Extracts.** Approximately 100 g of seeds was frozen at −40 °C and, in that state, ground and homogenized in methanol (three times 100 mL) containing 0.5 mg/mL of ascorbic acid to avoid oxidation; water was added, and the mixture was concentrated under vacuum until all of the methanol had been removed. The extract obtained was washed three times with petroleum ether to eliminate liposoluble substances.

**Gel Chromatography Fractionation.** Fractionation of the aqueous extract of grape seeds was performed in a column (50 × 2.5 cm) filled with Sephadex LH-20 (Sigma) suspended in 96% ethanol. The gel was previously conditioned by successive washing with 70% acetone, water, and 96% ethanol, in which it was allowed to swell for 14–16 h. Elution was carried out with 96% ethanol, as indicated by Oszmianski and Sapis (1989), maintaining a flow rate of 2.5 mL/min with the aid of a peristaltic pump. The absorbance of the eluate was measured at 280 nm. The fractions collected were added to a small volume of water and concentrated under vacuum to eliminate the alcoholic solvent, and the aqueous extracts were analyzed by HPLC.

**Analytical HPLC.** A Waters Model 600E pump equipped with a U6K manual injector was used. The column (25 × 0.46 cm) was a 4-μm Ultracarb C18 ODS 20, thermostated at 32 °C and protected by a RP-18 guard column (15 × 3.2 mm) from Brownlee Laboratories Inc. The solvents were, A, 4.5% formic acid, and, B, acetonitrile/4.5% formic acid (10:90 v/v). For elution, a convex gradient (curve 4) from 0 to 50% B over 30 min was established followed by a linear gradient from 50 to 100% B over 50 min, with a flow rate of 1.5 mL/min. Detection was made at 280 nm with an HP 1040M Series II diode array detector coupled to a Hewlett-Packard ChemStation 79994 data treatment station. Before injection into the chromatographic system, all extracts were filtered through a 0.45-μm membrane filter (Millipore).

**Preparative HPLC.** Preparative fractionation for the isolation of compounds was performed with the same chromatograph and detection system employed for analytical HPLC. The column (25 × 1 cm) was a 5-μm Ultracarb C18 ODS 20 thermostated at 42 °C. The elution conditions were as follows: flow rate, 4 mL/min; solvent A, methanol; solvent B, 2.5% acetic acid; elution was begun with 2% A isocratic over 50 min followed by

0021-8561/92/1440-1794$03.00/0 

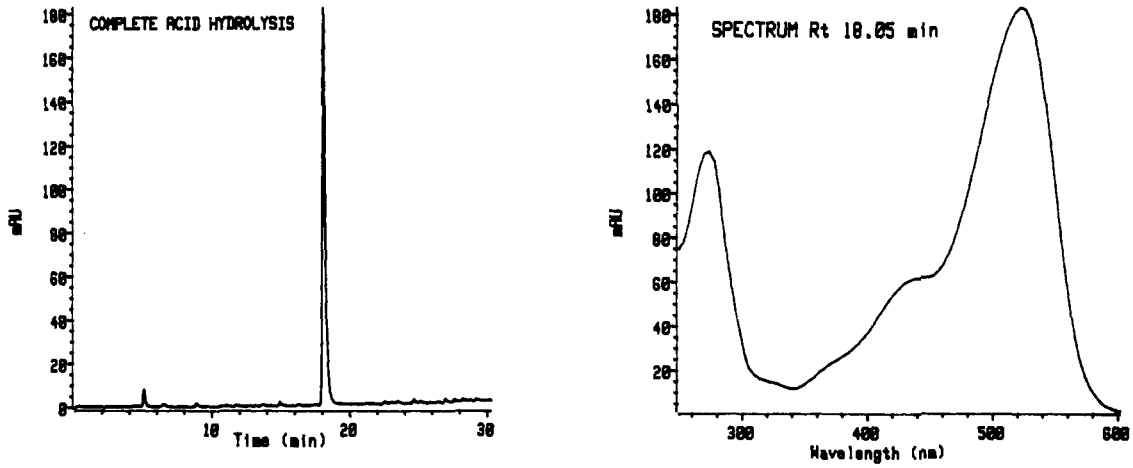


**Figure 1.** (A, Left) HPLC chromatogram recorded at 520 nm after complete acid hydrolysis of grape seed extract. (B, Right) Spectrum corresponding to the peak present in the previous chromatogram obtained with the diode array detector and corresponding to cyanidin.

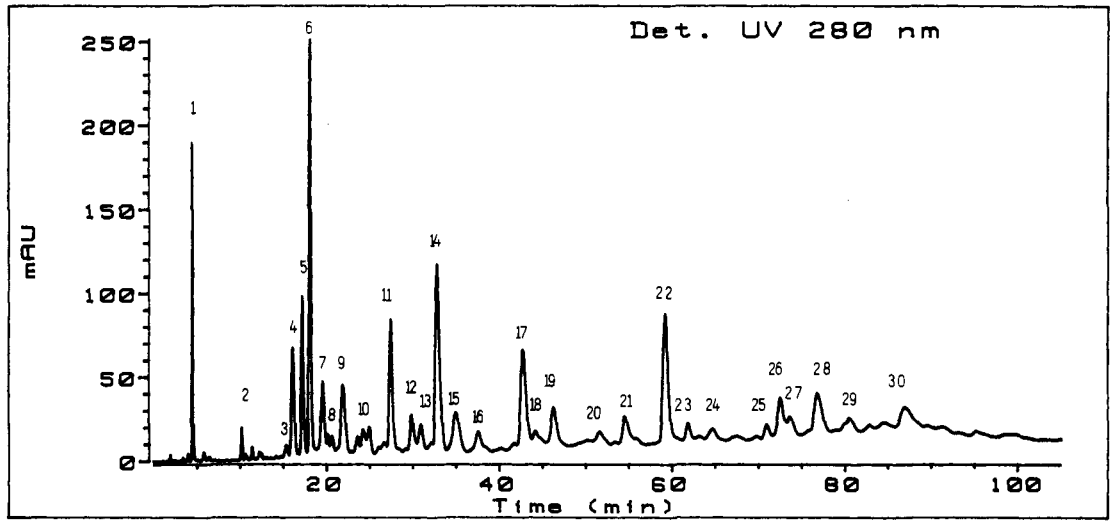


**Figure 2.** Analytic HPLC chromatogram recorded at 280 nm of grape seed extract. The identification of the peaks is shown in Table I.

a linear gradient from 2 to 10% A over 60 min and from 10 to 25% A over 60 min. Detection was performed at 280 nm, and the peaks were collected manually at the time of their elution. Each fraction was evaporated under vacuum, and the aqueous extracts were kept at −40 °C until the compounds were identified.

**TLC.** To classify the procyanidins according to their degree of polymerization, TLC of the compounds isolated was carried out using an adaptation of the method reported by Lea et al. (1979), using precoated silica gel plates (Scharlau), 0.25 mm thick, using an ascending elution with toluene/acetone/acetic acid (3:7.5:1). A solution of 1% vanillin (Merck) in 70% hydrochloric acid was employed for detection.

**Identification of Procyanidins.** The following methods were used for the identification of compounds.

*Complete Acid Hydrolysis.* This was performed by treating with 1-butanol + HCl (2 + 1) over 10 min at 100 °C. The hydrolysate was analyzed by HPLC under the conditions reported by Hebrero et al. (1988), carrying out the identification by comparison with anthocyanidin standards obtained at our laboratory.

*Partial Acid Hydrolysis.* This was performed with 0.1 N HCl according to the method described by Thompson et al. (1972), but with aqueous medium instead of ethanol. The hydrolysate was later analyzed by HPLC.

*Partial Acid Degradation with Phenylmethanethiol and Later Desulfuration.* A degradation of procyanidins by thiolysis was carried out in the presence of sulfur dioxide and phenylmethanethiol (Sigma); the benzyl thioethers obtained were later desulfurized with hydrogen using Raney nickel according the method Rigaud et al. (1991). The extracts were filtered through a Millex HV4 (Millipore) and analyzed by HPLC.

*Enzymatic Hydrolysis.* Aqueous extracts of the procyanidins were subjected to incubation with tannase T 981005 (Kikkoman) under the conditions reported by Ricardo da Silva et al. (1991b), and the hydrolysates were analyzed by HPLC.

## RESULTS AND DISCUSSION

The extraction solvent was chosen after a series of tests were carried out with ethanol, methanol, and acetone, both alone and mixed with water. The extraction with methanol proved most efficient, since, after the seeds were extracted with it, nothing remained in the residue which could be extracted with any of the other solvents tested, which did not occur when ethanol and acetone media were used first.

Figure 1 shows the HPLC chromatogram obtained after complete acid hydrolysis of the extract of grape seeds. The peak corresponds to cyanidin, which was identified by comparison of its retention time and spectrum with those of a standard of this anthocyanidin. This showed that the proanthocyanidins present were of the procyanidin type, that is, (+)-catechin and (−)-epicatechin derivatives.

For the analytical HPLC, first the elution conditions reported by other authors were assayed; with these, satisfactory results were not obtained. A common problem found with all of them was that a considerable drift from the baseline was produced; additionally, the resolution of the peaks was insufficient. Accordingly, different columns, eluents, and gradients were assayed until, finally, the

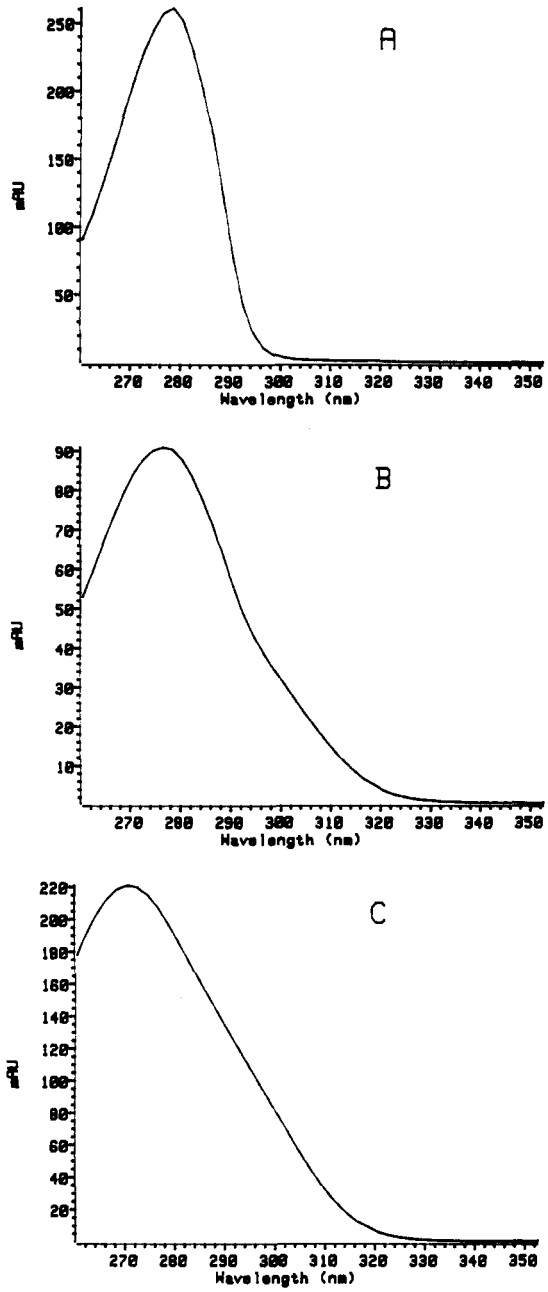


Figure 3. Spectra corresponding to (+)-catechin (A), epicatechin 3-*O*-gallate (B), and gallic acid (C) obtained with the diode array detector at the time of chromatographic elution.

conditions described under Materials and Methods were obtained. With these, it was possible to reduce the drift from the baseline to a considerable extent and also obtain a good separation of the peaks. Figure 2 shows a chromatogram of the complete extract of grape seeds obtained by using the conditions set up. Comparison with commercial standards purchased from Extrasynthèse (Genay, France) permitted the location on the chromatograms of the peaks corresponding to gallic acid (peak 1), (+)-catechin (peak 6), (−)-epicatechin (peak 14), and epicatechin 3-*O*-gallate (peak 22).

An attempt to identify the procyanidins was made from the spectra corresponding to each peak of the chromatogram obtained with the diode array detector. As a previous step, spectra of (+)-catechin, (−)-epicatechin, epicatechin 3-*O*-gallate, and gallic acid were obtained from different mixtures of acetonitrile and formic acid, which reproduced the gradient conditions present during the chromatographic elution. It was observed that the composition of

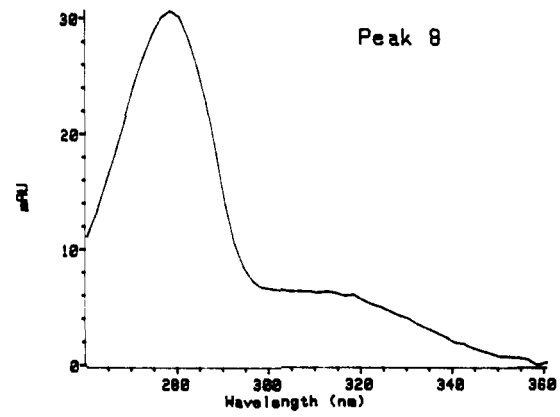


Figure 4. Spectrum of peak 8 obtained with the diode array detector at the time of chromatographic elution.

the solvent did not affect the absorption maximum or the shape of the spectrum. The spectra (see Figure 3) of both (+)-catechin and (−)-epicatechin were identical, with a maximum at 279 nm, whereas epicatechin 3-*O*-gallate showed a wavelength of maximum absorption at 276 nm together with a broader spectral band. To localize the peaks corresponding to procyanidins on the chromatogram, it was considered that the spectrum of the nonacylated procyanidins should be identical to that of the (+)-catechin and that of the procyanidins esterified with gallic acid should be similar to that of epicatechin 3-*O*-gallate. According to this, peaks 3–5, 7, 9–11, 16, 18–20, and 24 would correspond to nonacylated procyanidins and peaks 12, 13, 15, 17, 21–23, and 25–30 would correspond to procyanidin gallates.

The spectrum of peak 8 (Figure 4) showed a maximum at 279 nm and a pronounced shoulder around 310 nm. These features suggest that we may be dealing with a procyanidin esterified with an acid other than gallic acid, possibly *p*-coumaric acid, which shows a maximum at 310 nm. It was not possible to confirm this aspect since we were unable to isolate a sufficient amount of the compound for its identification because it was present in very low amounts in the extracts. It will be interesting to establish the type of esterification of this peak, since the presence of procyanidins acylated with acids different from gallic has never been cited in the grape.

For the isolation of compounds, an initial fractionation of the grape seed extract was made on Sephadex LH-20. Three fractions were collected and analyzed by analytical HPLC (Figure 5). Fraction I contained almost exclusively gallic acid, (+)-catechin, and (−)-epicatechin. Fraction II contained important amounts of peaks 4, 5, 9, 11, and 22 (corresponding to epicatechin 3-O-gallate), together with lesser amounts of the substances corresponding to peaks 7, 14 [(−)-epicatechin], 17–19, and 23. Fraction III proved to be more complex, showing some peaks already present in fraction II together with most of those not eluted in the previous fractions.

Following this, fractions II and III were fractionated by preparative HPLC to obtain pure compounds. Sufficient amounts for the identification of peaks 4, 5, 9, and 11 were obtained from fraction II, together with a small amount of the substance corresponding to peak 3. Peak 19 was obtained from both fractions II and III. From fraction III peaks 7, 13, 15, 17, 26, and 28 were isolated. Preparative HPLC split peak 17 into two different ones, designated 17a and 17b.

TLC of the compounds isolated was performed to obtain preliminary information concerning their degree of polymerization (Figure 6). Seven different levels of polym-

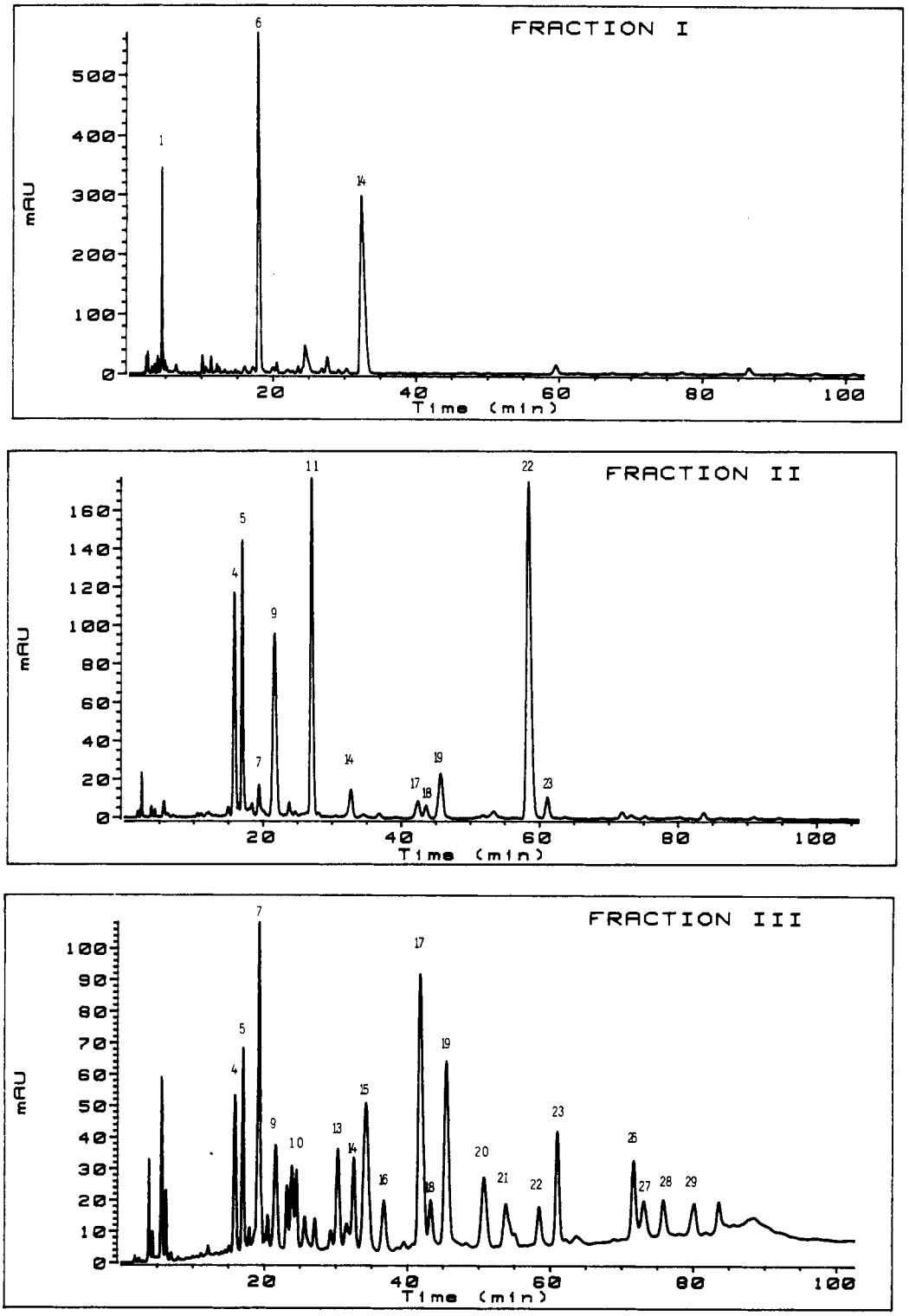


**Figure 5.** Analytic HPLC of the three fractions obtained by fractionation of the grape seed extract in Sephadex LH-20. The numbering of peaks is the same as in Figure 2.

erization were observed in the grape seed extract, which could indicate the presence of heptamers as more polymerized compounds. Among the compounds isolated, those corresponding to peaks 4, 5, 9, 11, 13, 15, 17a, and 17b were classified as dimers and peaks 7, 19, 26, and 28 as trimers; peak 3 was classified as a tetramer.

To characterize the compounds, different types of hydrolysis were performed. Partial acid hydrolysis was used to identify the lower subunits. In this type of hydrolysis, part of the interflavan bond is broken, releasing free lower subunits; thus, for example, from a trimer procyanidin the nonhydrolyzed trimer and the lower dimer and monomer are obtained. The lower subunits are also obtained in free state in the thiolysis with phenylmethanethiol. In this degradation, the 4-flavanyl carbocations formed in the breakage of the interflavan union, corresponding to the higher subunits of procyanidin, are captured by the nucleophile (phenylmethanethiol) to give 4-flavanylbenzyl thioethers. Later desulfuration of the thioderivatives allows one to obtain the upper subunits in the free state which can thus be identified. Enzymatic hydrolysis was carried out to identify the acylated procyanidins and the type of acid involved. A description of

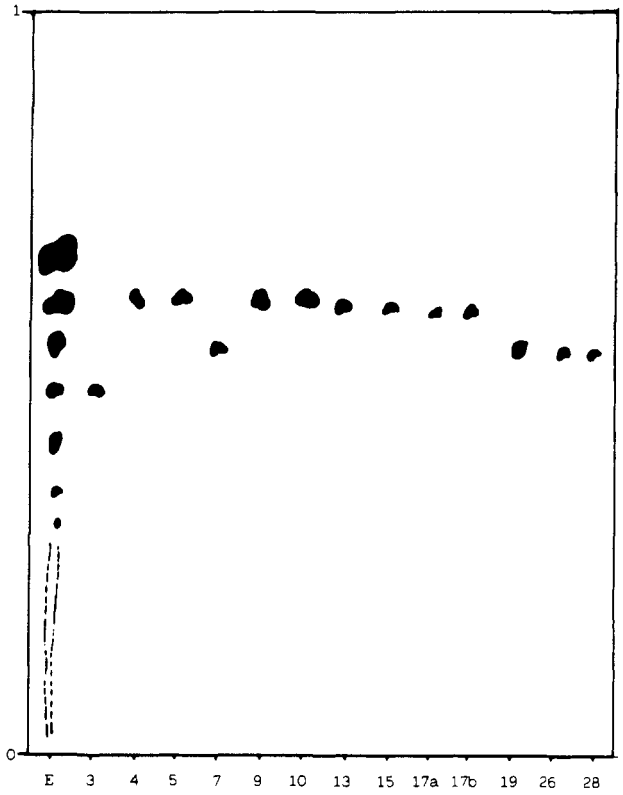


**Figure 6.** TLC of the complete extract of grape seeds (spot E) and of the procyanidins isolated. The numbering of spots is the same as for the peaks in Figure 2.

the results obtained in the assays carried out is offered in the following paragraphs.

**Peaks 4, 5, 9, and 11.** Partial acid hydrolysis and thiolytic degradation released (+)-catechin from peaks 4 and 5 and (−)-epicatechin from peaks 9 and 11. Desulfuration of the thio derivatives obtained in thiolysis carried out in the presence of Raney Ni formed (+)-catechin from peaks 4 and 9 and (−)-epicatechin for peaks 5 and 11. These results showed that we were dealing with the following dimers: peak 4, B3 [catechin-(4α→8)-catechin]; peak 5, B1 [epicatechin-(4β→8)-catechin]; peak 9, B4 [catechin-(4α→8)-epicatechin]; peak 11, B2 [epicatechin-(4β→8)-epicatechin].

**Peaks 13, 15, 17a, and 17b.** Enzymatic hydrolysis released the dimers B2 (peaks 13 and 17b), B4 (peak 15), and B1 (peak 17a). In partial acid hydrolysis and in thiolysis, peak 13 released (−)-epicatechin, peaks 15 and 17b (−)-epicatechin 3-*O*-gallate, and peaks 17a (+)-catechin. Hydrogenation of thio derivatives formed (−)-epicatechin 3-*O*-gallate in peaks 13 and 17a, (+)-catechin in peak 15, and (−)-epicatechin in peak 17b. From these results the following identification was achieved: peak 13, B2-3-*O*-gallate [epicatechin 3-*O*-gallate-(4β→8)-epicatechin]; peak 15, B4-3′-*O*-gallate [catechin-(4α→8)-epicatechin 3-*O*-gallate]; peak 17a, B1-3-*O*-gallate [epicatechin 3-*O*-gallate-(4β→8)-catechin]; peak 17b, B2-3′-*O*-gallate [epicatechin-(4β→8)-epicatechin 3-*O*-gallate].

**Peaks 7 and 19.** Partial hydrolysis and thiolysis of peak 7 released (+)-catechin and the dimer B1, while (−)-epicatechin and dimer B2 were obtained after desulfuration of the thio derivatives; we were therefore dealing with the trimer epicatechin-(4β→8)-epicatechin-(4β→8)-catechin. In the hydrolyses carried out on peak 19, (−)-epicatechin and dimer B2 were released, and these same compounds were obtained following the hydrogenation of the thio derivatives, allowing us to conclude that we were dealing with the trimer C1 [epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin].

**Table I. Compounds Isolated from Grape Seeds: Identification, Distribution by Percentage Areas, and Retention Times under the Analytic HPLC Conditions Employed**

| peak[a] | Rt, min | area, % | compound |
|---|---|---|---|
| 1 | 4.4 | 3.7 | gallic acid |
| 3 | 15.3 | 0.4 | nongalloyled tetramer (unknown) |
| 4 | 16.1 | 3.9 | catechin-(4α→8)-catechin (dimer B3) |
| 5 | 17.2 | 4.5 | epicatechin-(4β→8)-catechin (dimer B1) |
| 6 | 18.0 | 10.8 | (+)-catechin |
| 7 | 19.6 | 3.4 | epicatechin-(4β→8)-epicatechin-(4β→8)-catechin |
| 9 | 21.9 | 4.2 | catechin-(4α→8)-epicatechin (dimer B4) |
| 11 | 27.4 | 5.9 | epicatechin-(4β→8)-epicatechin (dimer B2) |
| 13 | 30.9 | 1.7 | epicatechin 3-*O*-gallate-(4β→8)-epicatechin (B2-3-*O*-gallate) |
| 14 | 32.7 | 10.1 | (−)-epicatechin |
| 15 | 34.9 | 3.5 | catechin-(4α→8)-epicatechin 3-*O*-gallate (B4-3′-*O*-gallate) |
| 17a | 42.7 | 6.9 | epicatechin 3-*O*-gallate-(4β→8)-catechin (B1-3-*O*-gallate) |
| 17b | 42.7 | | epicatechin-(4β→8)-epicatechin 3-*O*-gallate (B2-3′-O-gallate) |
| 19 | 46.2 | 3.2 | epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (trimer C1) |
| 22 | 59.1 | 8.8 | epicatechin 3-*O*-gallate |
| 26 | 72.5 | 2.9 | epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin 3-*O*-gallate |
| 28 | 76.8 | 4.4 | di- or trigalloyled trimer C1 |

[a] Numbering of peaks is the same as in Figure 2.

**Peak 26.** Trimer C1 was released in its enzymatic hydrolysis. Partial acid hydrolysis and thiolysis formed (−)-epicatechin 3-*O*-gallate and B2-3′-*O*-gallate, while (−)-epicatechin was obtained after desulfuration. These results correspond to the trimer epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin 3-*O*-gallate.

**Peak 28.** Enzymatic hydrolysis of this released the trimer C1; however, the compound could not be identified with certainty since no conclusive results were obtained following its thiolysis and later desulfuration with Raney Ni. It could therefore be the di- or trigalloyled trimer C1.

The results obtained in the identifications are summarized in Table I, where the compounds identified are related, together with their distribution by area percentages and the retention times under the chromatographic conditions employed. According to the results obtained, the presence of (−)-epicatechin delays the elution of the procyanidins, especially when it is found in the lower subunit(s). (+)-Catechin has the opposite effect and decreases retention time. This effect is clearly observable when the elution orders of dimers B1–B4 are compared. Acylation with gallic acid leads to a loss of polarity, such that the peaks corresponding to galloyled procyanidins always appear with longer retention times.

In summary, it can be indicated that the application of the entirety of the techniques utilized allows the carrying out of the identification of procyanidins with greater certainty, without the necessity of availability of patterns and advancement in the characterization of these compounds in the seed of *V. vinifera*.

ACKNOWLEDGMENT

We express our gratitude to the Estación Enológica de Castillla y León (Rueda, Spain) for the samples supplied. This work was financed by the Junta de Castilla y León, Reference Project 0907/90. Mz. Escribano-Bailón was a fellowship recipient from the Programa de Formación de

Profesorado y Personal Investigador of the Ministerio de Educación y Ciencia (AP90).

LITERATURE CITED

Ariga, T.; Hamano, M. Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxy radicals. *Agric. Biol. Chem.* **1990**, *54*, 2499.

Boukharta, M.; Girardin, M.; Metche, M. *J. Chromatogr.* **1988**, *455*, 406.

Bourzeix, M.; Weyland, D.; Heredia, N. *Bull. O. I. V.* **1986**, *669–670*, 1171.

Cheynier, V. *Bull. Liaison Groupe Polyphenols* **1990**, *15*, 275.

Cheynier, V.; Osse, C.; Rigaud, J. Oxidation of grape juice phenolic compounds in model solutions. *J. Food Sci.* **1988**, *53*, 1729.

Cheynier, V.; Ricardo da Silva, J. M. Oxidation of grape procyanidins in model solutions containing trans-caffeoyltartaric acid and polyphenol oxidase. *J. Agric. Food Chem.* **1991**, *38*, 1047.

Czochanska, Z.; Foo, L. Y.; Porter, L. J. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **1979**, *18*, 1819.

Hebrero, E.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. High performance liquid chromatography-diode array spectroscopy identification of anthocyanins of *Vitis vinifera* variety Tempranillo. *Am. J. Enol. Vitic.* **1988**, *39*, 227.

Jaworski, A. W.; Lee, C. Y. Fractionation and HPLC determination of grape phenolics. *J. Agric. Food Chem.* **1987**, *35*, 257.

Lea, A. G. H. Reversed-phase gradient high performance liquid chromatography of procyanidins and their oxidation products in ciders and wines, optimised by Snyder's procedures. *J. Chromatogr.* **1980**, *194*, 62.

Lea, A. G. H. Tannin and colour in English cider apples. *Fluess. Obst.* **1984**, *8*, 1.

Lea, A. G. H.; Bridle, P.; Timberlake, C. F.; Singleton, V. L. The procyanidins of white grapes and wines. *Am. J. Enol. Vitic.* **1979**, *30*, 289.

Lee, C. Y.; Jaworski, A. W. Phenolic compounds in white grapes grown in New York. *Am. J. Enol. Vitic.* **1987**, *38*, 277.

Lee, C. Y.; Jaworski, A. W. Identification of some phenolics in white grapes. *Am. J. Enol. Vitic.* **1990**, *41*, 87.

Masquelier, J. *Bull. O. I. V.* **1988**, *689–690*, 554.

Metche, M. *Bull. Liaison Groupe Polyphenols* **1988**, *14*, 119.

Oszmianski, J.; Lee, C. Y. Isolation and HPLC determination of phenolic compounds in red grapes. *Am. J. Enol. Vitic.* **1990**, *41*, 204.

Oszmianski, J.; Sapis, J. C. Fractionation and identification of some low molecular weight grape seed phenolics. *J. Agric. Food Chem.* **1989**, *37*, 1293.

Oszmianski, J.; Sapis, J. C.; Macheix, J. J. Changes in grape seed phenols as affected by enzymic and chemical oxidation in vitro. *J. Food Sci.* **1985**, *50*, 1505.

Oszmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of phenolic compounds in red wine. *Am. J. Enol. Vitic.* **1988**, *39*, 259.

Revilla, E.; Bourzeix, M.; Alonso, E. Analysis of catechins and proanthocyanidins in grape seeds by HPLC with photodiode array detection. *Chromatographia* **1991**, *31*, 465.

Ricardo da Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Heredia, N. Separation and quantitative determination of grape and wine procyanidins by high performance reversed phase liquid chromatography. *J. Sci. Food Agric.* **1990**, *53*, 85.

Ricardo da Silva, J. M.; Darmon, N.; Fernández, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991a**, *39*, 1549.

Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidins dimers and trimers from grape seeds. *Phytochemistry* **1991b**, *30*, 1259.

Rigaud, J.; Perez-Ilzarbe, J.; Ricardo da Silva, J. M.; Cheynier, V. Micro method for the identification of proanthocyanidin using thiolysis monitored by high performance liquid chromatography. *J. Chromatogr.* **1991**, *540*, 401.

Singleton, V. L. Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69.

Thompson, R. S.; Jacques, D.; Haslam, E.; Tanner, R. I. N. Plant proanthocyanidins. Part I: the isolation, structure and distribution in nature of plant procyanidins. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1387.

Uchida, S.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Nonaka, G.; Nishioka, I.; Niwa, M.; Ozaki, M. Condensed tannins scavenge active oxigen free radicals. *Med. Sci. Res.* **1987**, *15*, 831.

Received for review March 17, 1992. Accepted June 26, 1992.