Journal of Chromatography, 259 (1983) 291 300
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,580

SEPARATION OF SOME ANTHOCYANIDINS, ANTHOCYANINS, PROANTHOCYANIDINS AND RELATED SUBSTANCES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

KAREL VANDE CASTEELE
Laboratory of Plant Biochemistry, Rijksuniversiteit Gent, K.L. Ledeganckstraat 35, 9000 Ghent (Belgium)
HANS GEIGER
Institut für Chemie, Universität Hohenheim, Garbenstrasse 30, D-7000 Stuttgart 70 (G.F.R.)
ROGER DE LOOSE
Rijksstation voor Sierplantenteelt, Caritasstraat 21, 9230 Melle (Belgium)
and
CHRISTIAAN F. VAN SUMERE*
Laboratory of Plant Biochemistry, Rijksuniversiteit Gent, K.L. Ledeganckstraat 35, 9000 Ghent (Belgium)
(Received November 29th, 1982)

SUMMARY

The separation of some anthocyanidins, anthocyanins, proanthocyanidins and related substances by means of reversed-phase high-performance liquid chromatography is described. The system consists of a LiChrosorb RP-18 (10 μm) Knauer column and a combination of isocratic and linear gradient elution (solvent A: water–formic acid (95:5 v/v); solvent B: methanol; 35°C). These results and those of previous studies on related compounds are employed to present a "review chromatogram" demonstrating the separatory power of the RP–HPLC technique. The possible use of this system for the analysis of complex mixtures of natural products is discussed.

INTRODUCTION

Anthocyanins which occur in flower and fruit tissues and in the superficial cells of organs such as leaves and stems[1,2] constitute one of the most important groups of plant pigments. In 70 species of 33 families of angiosperms they were found in membrane-bounded anthocyanoplasts located within the main cell vacuole[2].

Apart from contributing cyanic colours to flowers and fruits, the pigments also play an important rôle during pollination and are of great economic and genetic importance[3–6]. The aglycones (anthocyanidins) are chemically rather unstable. They occur in flower tissues mainly as 3-glucosides, 3,5-diglucosides or 3,7-diglycosides[5,6]. Other glycosidation patterns which may have consequences in terms of flower colour may also occur[5], while even certain sugar moieties may be further substituted by acyl residues [*e.g.*, malvidin-3-(6-O-*p*-coumaroylglycoside)-5-glucoside]. Sugar attach-

0021-9673/83/0000-0000/$03.00

ment is thought to be of importance for pigment stability[4], although it has recently been shown by Preston and Timberlake[7] that malvidin-3-glucosides and malvidin-3,5-diglucoside (and probably also other anthocyanins) can occur in four structural forms between pH 0 and 6, *viz.*, the flavylium cation, the quinoidal base, the carbinol base and the chalcone.

While sugars are certainly an important structural feature of the anthocyanins and promote solubility and possibly also a certain stability for insoluble anthocyanidins, much of the variation is at present mainly of taxonomic interest as there is evidence that most of the plant families are characterized by a particular glycosidic pattern in their anthocyanins[5].

Proanthocyanidins[8–12] or condensed tannins, which arise from flavan-3-ols and exist in a range of forms (monomers, dimers, trimers, oligomers and polymers[8]), are mostly confined to plants with a woody growth habit[13]. They are found in numerous gymnosperms and angiosperms in amounts as high as 40% of the fresh weight of the tissues extracted[11]. Unfortunately, with increasing polymerization, the colourless proanthocyanidins, which form anthocyanidins when heated with acid, become more difficult to dissolve in aqueous and alcoholic media[8,14]; however, according to Czochanska *et al.*[15], polymeric flavan-3-ols with molecular weights up to 7000 (corresponding to 20 flavan-3-ol units) may be solubilized.

Due to their ability to tan protein[16,17], oligomeric proanthocyanidins may play a variety of important biological and economic rôles. On the one hand they may produce unwanted precipitates or hazes in beers and wines[17,18], and on the other they contribute to the taste and flavour of foods[9,16,17]. In addition, their rôle in the ageing of wine is well documented[14]. They also inhibit enzymes[17], influence the ways in which soil organic matter is produced[16,17] and are very probably important in the defence mechanism of plants against predators and micro-organisms[17,19]. Some procyanidins possess antihypertensive and cardiotonic activity[20].

Although the above list seems more than convincing evidence for the importance of the anthocyanidins, anthocyanins and proanthocyanidins, only high-performance liquid chromatographic (HPLC) separation of some of the anthocyanins has been studied in any detail[7,21–31], less effort being devoted to separation of the flavan-3-ols, proanthocyanidin dimers and oligomers[11,18,32–34]. In three previous papers[35–37] the reversed-phase (RP–HPLC) separation of a relative large series of phenolics was described. It seemed of interest to extend this reference system to anthocyanidins, anthocyanins, flavan-3-ols, procyanidin dimers (procyanidin B-1 to B-5) and one procyanidin trimer (C-2). Indeed, the extended reference system has proven to be of great value when plant extracts have to be analyzed. A review chromatogram showing the separation of 41 phenolics demonstrates the separatory power of our RP–HPLC system.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 1084B liquid chromatograph equipped with a variable-wavelength Pye-Unicam LC3 UV detector and a Knauer prepacked analytical column (250 × 4.6 mm) of LiChrosorb RP-18 (10 μm) was used throughout this work.

Elution

Two solvents were used: A, formic acid–water (5:95, v/v); B, methanol. The elution profile was: 0–2 min, 7% B in A (isocratic); 2–8 min, 7–15% B in A (linear gradient); 8–25 min, 15–75% B in A (linear gradient); 25–27 min, 75–80% B in A (linear gradient); 27–29 min 80% B in A (isocratic). The temperature of the oven thermostat was set at 35°C. The flow-rate was 2.5 ml/min and the column pressure 80–100 bar.

Detection

The UV detector was set at 280 nm (optical bandwidth 8 nm). Usually, anthocyanins are separated as their coloured flavylium cations and detected at *ca.* 525 nm. However, the compounds also absorb in the UV region at 280 nm[7].

Samples

Samples of 0.0025–0.025% solutions in aqueous methanol were applied[31] to the column by means of a 20-μl loop valve.

Pelargonidin and delphinidin chloride as well as (±)catechin and (−)epicatechin were purchased from Fluka (Buchs, Switzerland). Petunidin was kindly donated by D. Strack (Universität Köln, Köln, G.F.R.). All other anthocyanins were kindly supplied by W. Steck (PRL, Saskatoon, Canada) and G. Hrazdina (Cornell University, Geneva, NY, U.S.A.). The proanthocyanidin dimers were donated by E. Haslam (University of Sheffield, Sheffield, Great Britain). Except for the pure A-2 compound (light brown), the other dimers (brown solids) contained impurities, but all consisted of one major component. The procyanidin trimer C-2 was kindly supplied by J. Jerumanis (Department of Brewing, Université Catholique de Louvain la Neuve, Belgium).

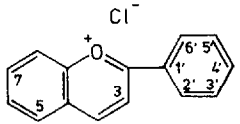
RESULTS AND DISCUSSION

Table I shows the retention times, t_R, of nineteen anthocyanidins and anthocyanins and Fig. 1 shows the separation of a series of nine anthocyanidins and anthocyanins. All separated compounds were eluted as narrow (high plate number) and symmetrical peaks. From Table I it is seen that certain compounds form "critical groups or pairs". This is especially the case for some glycosides, *e.g.*, peonidin-3,5-diglucoside, cyanidin-3-galactoside and malvidin-3,5-diglucoside; cyanidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-glucoside and pelargonidin-3-glucoside. In addition, anthocyanidins such as peonidin and malvidin also form critical pairs.

However, all these compounds do not always occur in a single organism, although it may sometimes be necessary to separate critical groups or pairs by another HPLC system or any other suitable method. In this regard reference can be made to Strack and co-workers[29,31] who suggest that an acetonitrile-containing system is superior to other solvents in resolving methoxylated phenolic compounds, *e.g.*, peonidin and malvidin. Unfortunately, systems containing acetonitrile are much more expensive than our general system, which was elaborated for the separation of different classes of phenolics[35–37]. The separation of certain critical pairs can probably be improved further by using a slower flow-rate (*e.g.* 1 ml/min)[31], although this would increase both bandwidth and the duration of the analysis. The latter may even increase ten-fold.

TABLE I

RETENTION TIMES OF ANTHOCYANIDINS AND ANTHOCYANINS



Substance	*Structure*			t_R *(min)*
	OH	OCH_3	*Glycosyloxy*	
Delphinidin-3,5-diglucoside	7,3′,4′,5′	—	3-β-D-Glucopyranosyloxy, 5-β-D-glucopyranosyloxy	9.98
Cyanidin-3,5-diglucoside	7,3′,4′	—	3-β-D-Glucopyranosyloxy, 5-β-D-glucopyranosyloxy	11.13
Petunidin-3,5-diglucoside	7,4′,5′	3′	3-β-D-Glucopyranosyloxy, 5-β-D-glucopyranosyloxy	11.78
Peonidin-3,5-diglucoside	7,4′	3′	3-β-D-Glucopyranosyloxy, 5-β-D-glucopyranosyloxy	12.66
Cyanidin-3-galactoside	5,7,3′,4′	—	3-β-D-Galactopyranosyloxy	12.77
Malvidin-3,5-diglucoside	7,4′	3′,5′	3-β-D-Glucopyranosyloxy, 5-β-D-glucopyranosyloxy	12.88
Cyanidin-3-arabinoside	5,7,3′,4′	—	3-β-D-Arabinopyranosyloxy	13.30
Cyanidin-3-rutinoside	5,7,3′,4′	—	3-β-D-(6-O-Rhamnosyl)glucosyloxy	13.73
Delphinidin-3-glucoside	5,7,3′,4′,5′	—	3-β-D-Glucopyranosyloxy	13.82
Cyanidin-3-glucoside	5,7,3′,4′	—	3-β-D-Glucopyranosyloxy	13.86
Pelargonidin-3-glucoside	5,7,4′	—	3-β-D-Glucopyranosyloxy	13.99
Malvidin-3-glucoside	5,7,4′	3′,5′	3-β-D-Glucopyranosyloxy	14.97
Delphinidin chloride	3,5,7,3′,4′,5′	—	—	15.13
Cyanidin chloride	3,5,7,3′,4′	—	—	16.55
Petunidin chloride	3,5,7,4′,5′	3′	—	16.66
Malvidin-3-(6-O-*p*-coumaroyl-glucoside)-5-glucoside	7,4′	3′,5′	3-(6-O-*p*-Coumaroylglucopyranosyloxy), 5-β-D-glucopyranosyloxy	17.61
Pelargonidin chloride	3,5,7,4′	—	—	17.93
Peonidin chloride	3,5,7,4′	3′	—	18.54
Malvidin chloride	3,5,7,4′	3′,5′	—	18.68

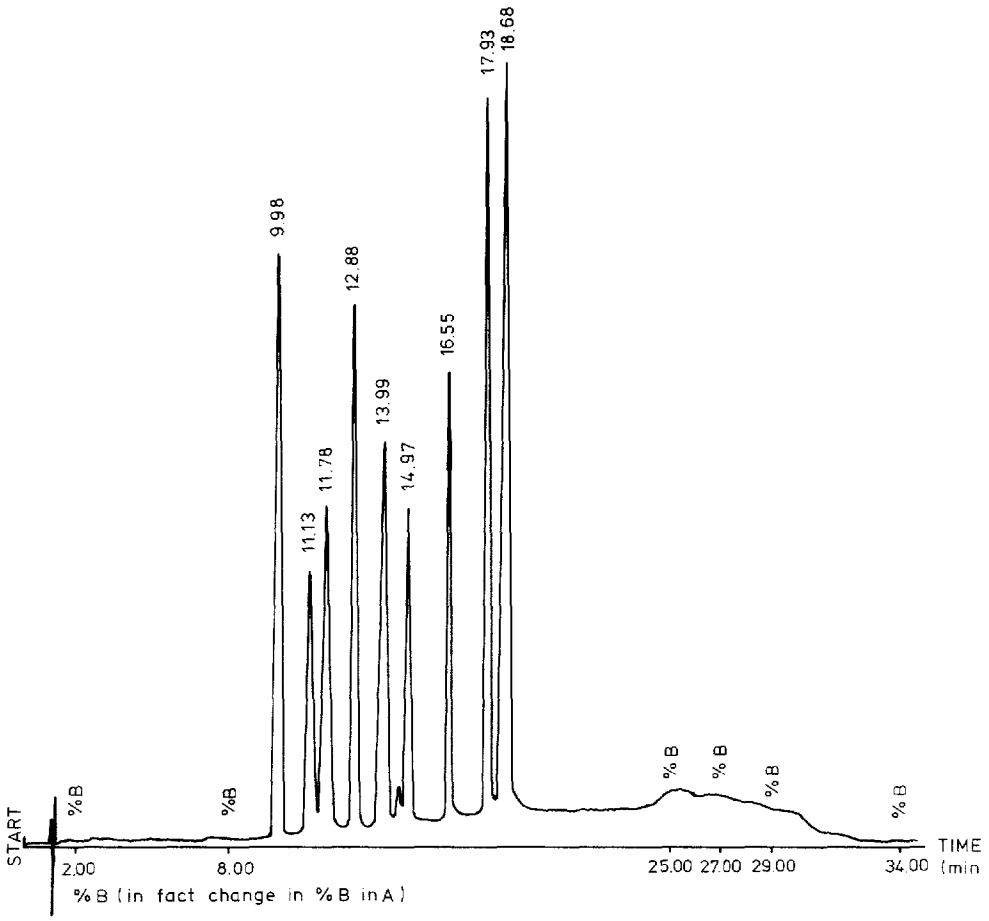


Fig. 1. The separation of anthocyanidins and anthocyanins on a Knauer prepacked column (250 × 4.6 mm) of LiChrosorb RP-18 (10 μm). For the eluting system see Experimental. See Table I for identity of peaks.

Returning to our general system (see Elution), in agreement with Strack *et al.*[31] we also found that the key factors for the separation of the anthocyanidins and anthocyanins are:

the overall polarity of the molecule;
the substitution of the B ring;
and for the glycosides, the nature of the attached sugar

From Table I it is seen that diglucosides are generally more rapidly eluted than monoglycosides which in turn are more rapidly eluted than the aglycones. The importance of the type of substitution of the B ring is illustrated by the fact that delphinidin (15.13 min) is more rapidly eluted than cyanidin (16.55 min) which precedes pelargonidin (17.93 min), and the methoxylated compounds peonidin (18.54 min) and malvidin (18.68 min).

Again in agreement with Strack *et al.*[31], we also found (see Table I) that ga-

lactosides exhibit a shorter retention time than arabinosides or glucosides; cyanidin-3-galactoside (12.77 min) is eluted faster than cyanidin-3-arabinoside (13.30 min) and cyanidin-3-glucoside (13.86 min). However, although malvidin-3-(6-O-*p*-coumaroylglucoside)-5-glucoside (17.61 min) is eluted more slowly than malvidin-3,5-diglucoside (12.88 min) and malvidin-3-glucoside (14.97 min), the acylated compound is eluted before the aglycone malvidin (18.68 min). This is not in agreement with Strack's finding for pelargonidin pigments acylated with *p*-coumaric acid. Our method has also proven to be applicable to the rapid identification of anthocyanins by examination of the kinetics of appearance and disappearance of the intermediate glycosides formed under strictly controlled hydrolysis of the original glycoside[31,38].

The application of our RP-HPLC method (see also refs. 35–37) to the characterization and flower pigment genetics of 60 different cultivars of *Rhododendron simsii* Planch. (*Azalea indica* L.), by fingerprinting the flavonoid pattern (including the anthocyanins) of petals, yielded good resolution and very characteristic prints[38] (see also refs. 30, 31). In regard to the proanthocyanins, which as a whole were less strongly retained than the anthocyanins and anthocyanidins (Table II), it can be stated that the trimer (C-2) (Fig. 2) is eluted before the procyanidin dimers (B-1, B-2, B-3, B-4)(Fig. 3) and that the latter compounds are generally eluted more rapidly than the monomers (Fig. 5), although there is some overlapping between the two groups of substances, *e.g.*, (+)catechin (6.04 min) has a smaller t_R than procyanidin B-2 (8.77 min). The recorded sequence, trimers, dimers, monomers, may be due to a molecular sieve effect and this could well be an advantage. Indeed, if the molecular weights of the proanthocyanidin oligomers were high enough (± 5 times the mo-

TABLE II

RETENTION TIMES VALUES OF PROANTHOCYANIDINS AND RELATED SUBSTANCES

Substances		*Absolute configuration*	t_R *(min)*
Monomers			
(+)Catechin	5,7,3′,4′	2*R* : 3*S*	6.04
(+)Afzelechin	5,7,4′	2*R* : 3*S*	8.77
(−)Epicatechin	5,7,3′,4′	2*R* : 3*R*	10.60
Epicatechin-3′-methylether (3′-methoxyepicatechin)	5,7′,4′	2*R* : 3*R*	11.54
Dimers		*Absolute configuration of interflavonyl link*	
Procyanidin B-3	(+)catechin-(+)catechin	4 (*S*)	4.10
Procyanidin B-1	(−)epicatechin-(+)catechin	4 (*R*)	4.68
Procyanidin B-4	(+)catechin-(−)epicatechin	4 (*S*)	5.86
Procyanidin B-2	(−)epicatechin-(−)epicatechin	4 (*R*)	7.89
Procyanidin A-2		(scc Fig. 4)	13.17
Procyanidin B-5	(−)epicatechin (−)epicatechin (isomeric with B-2)	*	13.72
Trimer			
Procyanidin C-2	(+)catechin-(+)catechin-(+)catechin	4 (*S*), 4″(*S*)	3.71

* The isomerism may be structural (position of linkage to "lower" flavan-3-ol unit) or stereochemical (at position 4 of "upper" flavan-3-ol)[39].

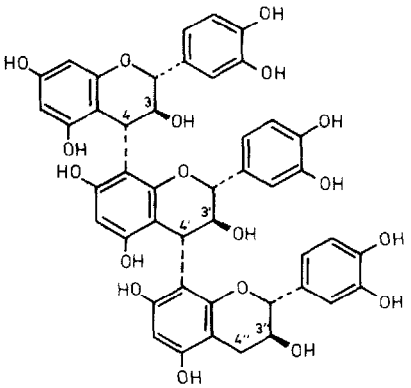
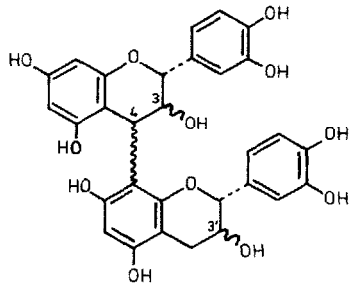


Fig. 2. Structure of the procyanidin C-2 trimer.



Procyanidins :

	3	3'	4
B-1	R	S	R
B-2	R	R	R
B-3	S	S	S
B-4	S	R	S

Fig. 3. Structure of β-type procyanidins.

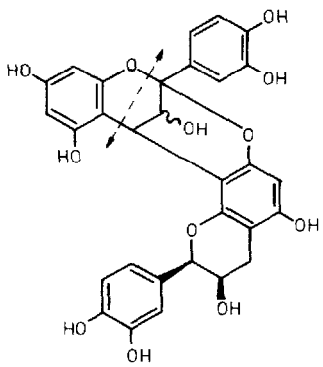
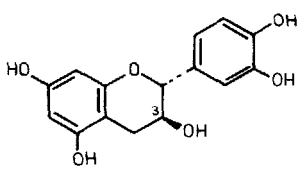
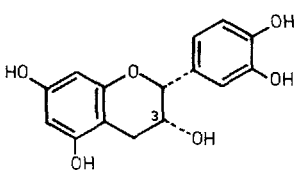


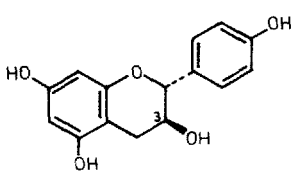
Fig. 4. Structure of the procyanidin A-2 dimer.



3-(S)-(+)-catechin



3-(R)-(-)-epicatechin



3-(S)-(+)-afzelechin

Fig. 5. Structures of 3-(S)-(+)-catechin, 3-(R)-(−)-epicatechin and 3-(S)-(+)-afzelechin.

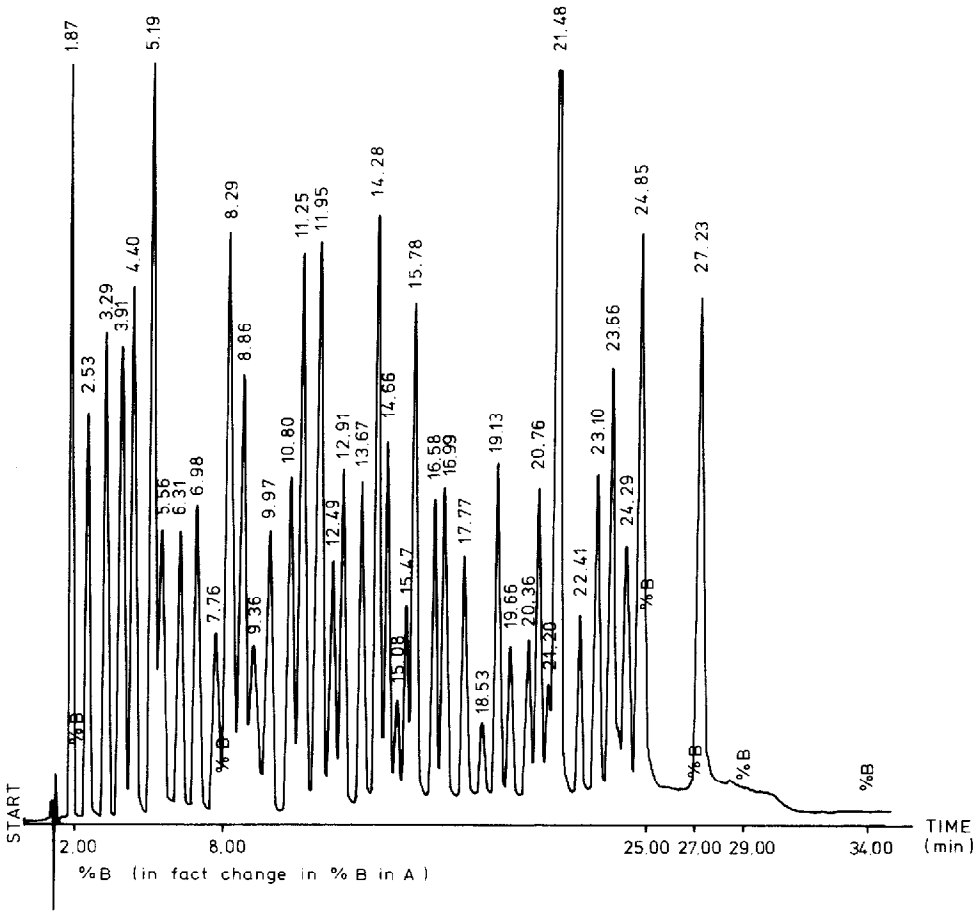


Fig. 6. The separation of a mixture of 41 phenolics and related compounds on a Knauer prepacked column (250 × 4.6 mm) of LiChrosorb RP-18 (10 μm). For the eluting system see Experimental.

Substance	t_R *(min)*	*Substance*	t_R *(min)*
Gallic acid	1.87	3,4-Dimethoxyphenylacetic acid	14.28
N-Protocatechuoylglycine	2.53	Isoferulic acid	14.66
Protocatechuic acid	3.29	Luteolin-5-glucoside	15.08
2,3,4-Trihydroxybenzoic acid	3.91	4-Methylumbelliferon	15.47
Pyrocatechol	4.40	Coumarin	15.78
Protocatechualdehyde	5.19	Cyanidin chloride	16.58
p-Hydroxybenzoic acid	5.56	Astragalin	16.99
Esculin	6.31	3,4,5-Trimethoxycinnamic acid	17.77
Dihydrocaffeic acid	6.98	Quercetin	18.53
m-Hydroxybenzoic acid	7.76	*p*-Methoxycinnamic acid	19.13
Caffeic acid	8.29	Feruloyl-L-phenylalanine	19.66
Afzelechin	8.86	Kaempferol	20.36
m-Hydroxybenzaldehyde	9.31	Apigenin	20.76
Delphinidin-3,5-diglucoside	9.97	*p*-Methylcinnamic acid	21.20
Syringic acid	10.80	N-Feruloyl-L-phenylalanyl-L-phenylalanine	21.48
Cyanidin-3,5-diglucoside	11.25	4,6-Dimethoxyherniarin	22.41
p-Coumaric acid	11.95	Tricetin-3′,4′,5′-trimethyl ether	23.10
Umbelliferon	12.49	Chrysin	23.66
3-O-Feruloyl-D-quinic acid	12.91	Piperin	24.29
Ferulic acid	13.67	β-Phenylcinnamaldehyde	24.85
		Tectochrysin	27.23

lecular weights of the monomers under investigation) they would appear in the fore run and as such would not interfere with the analysis of the lower-molecular-weight substances.

Next to the size of the compounds, the overall polarity and substitution of the B-ring, the stereochemical position of the free OH group at carbon-3 or carbon-3′ is also of great importance. 3-(S) monomers are eluted before 3-(R) substances, *e.g.*, 3-S-(+)catechin(6.04min),3-S-(+)afzelechin(8.77min)and3-R-(−)epicatechin(10.60 min) (Table II; Fig. 5). The same holds true for carbon-3′ in the proanthocyanidin dimers, but in this case the stereochemistry at carbon-3 and -4 must also be taken into account. The position carbon-3′ in the dimers (Fig. 3) is more important than carbon-3 and the latter is most probably of greater importance than carbon-4, *e.g.*, B-3 (4.10 min) is eluted before B-1 (4.68 min) and B-4 (5.86 min) and B-2 (Table II). Thus it seems possible that this type of RP–HPLC could be very helpful in a preliminary determination of the stereochemistry of flavonoids and especially proanthocyanidin dimers.

Finally Fig. 6 shows the separation of different classes of phenolics and related compounds. Forty-one different substances, including phenols, N-protocatechuoylglycine[40], phenolic aldehydes, phenolic acids and derivatives, flavonoids (including some of the present compounds) and coumarins, could be separated within 28 min.

ACKNOWLEDGEMENTS

We are greatly indebted to all those who generously furnished us with samples. H.G. thanks the Fonds der Chemischen Industrie for financial support and C.F.V.S. is grateful for a grant from the Belgian Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw. The technical assistance of Mrs. G. Persoon-Saey, Mrs. H. Goetgeluck-Vermeulen and Mr. W. Hutsebaut is gratefully acknowledged.

REFERENCES

1 C. Nozzolillo, *Can. J. Bot.*, 50 (1972) 29.
2 R. C. Pecket and C. J. Small, *Phytochemistry*, 19 (1980) 2571.
3 C. F. Timberlake and P. Bridle, in J. B. Harborne, T. Mabry and H. Mabry (Editors), *The Flavonoids*, Chapman and Hall, London, 1975, p. 215.
4 J. B. Harborne, *Introduction to Ecological Biochemistry*, Academic Press, London, 1977, p. 32.
5 J. B. Harborne, in T. Swain, J. B. Harborne and C. F. Van Sumere (Editors), *Biochemistry of Plant Phenolics*, Plenum, New York, 1979, p. 466.
6 K. Halbrock, in P. K. Stumpf and E. E. Conn (Editors in Chief), *The Biochemistry of Plants*, Vol. 7, E. E. Conn (Editor), *Secondary Plant Products*, Academic Press, London, 1981, p. 450.
7 N. Preston and C. Timberlake, *J. Chromatogr.*, 214 (1981) 222.
8 T. Swain, in T. Swain, J. B. Harborne and C. F. Van Sumere (Editors), *Biochemistry of Plant Phenolics*, Plenum, New York, 1977, p. 626.
9 E. Haslam, *Phytochemistry*, 16 (1977) 1625.
10 L. Y. Foo and L. J. Porter, *Phytochemistry*, 19 (1980) 1747.
11 H. A. Stafford and H. L. Lester, *Plant Physiol.*, 66 (1980) 1085.
12 E. Haslam, in P. K. Stumpf and E. E. Conn (Editors in Chief), *The Biochemistry of Plants*, Vol. 7, 1981, p. 545.
13 E. C. Bate-Smith and T. Swain, *Chem. Ind. (London)*, (1954) 433.
14 E. Haslam, *Phytochemistry*, 19 (1980) 2577.
15 Z. Czochanska, L. Y. Foo, R. H. Newman, L. J. Porter, W. A. Thomas and W. T. Jones, *J. Chem. Soc., Chem. Commun.*, (1979) 375.

16 R. L. M. Synge, *Qual. Plant. Plant Foods Hum. Nutr.*, 24 (1975) 337.
17 C. F. Van Sumere, J. Albrecht, A. Dedonder, H. De Pooter and I. Pé, in J. B. Harborne and C. F. Van Sumere (Editors), *The Chemistry and Biochemistry of Plant Proteins*, Academic Press, London, 1975, p. 211.
18 J. Jerumanis, *European Brewery Convention Proc. 17th Congress Berlin West, 1979*, DWS, Dordrecht, The Netherlands, p. 309
19 T. Swain, in G. A. Rosenthal and D. H. Jaryen (Editors), *Herbivores their Interaction with Secondary Plant Metabolites, Tannins and Lignins*, Academic Press, New York, 1979.
20 H. Wagner, in T. Swain, J. B. Harborne and C. F. Van Sumere (Editors), *Biochemistry of Plant Phenolics, Phenolic compounds of Pharmaceutical Interest*, Plenum, New York, 1977, p. 603.
21 C. H. Manley and P. Shubiak, *Can. Inst. Food Sci. Technol. J.*, 8(1) (1975) 35.
22 J. Adamovics and F. R. Stermitz, *J. Chromatogr.*, 129 (1976) 464.
23 M. Wilkinson, J. G. Sweeney and G. A. Iacobucci, *J. Chromatogr.*, 132 (1977) 349.
24 P. Symonds and R. Catagrel, *Bull. Liaison Groupe Polyphenols*, 8 (1978) 379.
25 M. Williams, G. Hrazdina, M. M. Wilkinson, J. G. Sweeny and G. A. Iacobucci, *J. Chromatogr.*, 155 (1978) 389.
26 L. Wulf and Ch. Nagel, *Amer. J. Enol. Vitic.*, 29 (1978) 42.
27 S. Asen, *J. Amer. Hort. Sci.*, 104 (1979) 223.
28 A. L. Camire and F. M. Clydesdale, *J. Food Sci.*, 44 (1979) 926.
29 N. Akavia and D. Strack, *Z. Naturforsch., C*, 35(1) (1980) 16.
30 R. De Loose, *Meded. Fac. Landbouwwet., Rijksuniv. Gent*, 45(1) (1980) 69.
31 D. Strack, N. Akavia and H. Reznik, *Z. Naturforsch.*, 35e (1980) 533.
32 A. G. H. Lea, *J. Chromatogr.*, 194 (1980) 62.
33 I. McMurrough, *J. Chromatogr.*, 218 (1981) 683.
34 P. Mulkay, R. Touillaux and J. Jerumanis, *J. Chromatogr.*, 208 (1981) 419.
35 C. F. Van Sumere, K. Vande Casteele, R. Hanselaer, M. Martens, H. Geiger and L. Van Rompaey, *J. Chromatogr.*, 234 (1982) 141.
36 K. Vande Casteele, H. Geiger and C. F. Van Sumere, *J. Chromatogr.*, 240 (1982) 81.
37 K. Vande Casteele, H. Geiger and C. F. Van Sumere, *J. Chromatogr.* , in press.
38 C. F. Van Sumere, R. De Loose and K. Vande Casteele, in preparation.
39 R. S. Thompson, D. J. E. Haslam and R. J. N. Tanner, *J. Chem. Soc., Perkin Trans. I*, (1972) 1387.
40 R. Hanselaer, L. Dhaenens, M. Martens, K. Vande Casteele and C. F. Van Sumere, in preparation.