CHROM. 3762

CELLULOSE THIN LAYERS FOR ANTHOCYANIN ANALYSIS, WITH SPECIAL REFERENCE TO THE ANTHOCYANINS OF BLACK RASPBERRIES

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SUMMARY

The anthocyanins of the American black raspberry (*Rubus occidentalis*) were reinvestigated, since quite different glycosidic characteristics had been attributed to them by different authors. New evidence was presented by means of two-dimensional thin-layer chromatography to show that the four black raspberry anthocyanins are: cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sambubioside, and cyanidin 3-xylosylrutinoside.

Boysenberries were also reinvestigated and found to contain the following four anthocyanins: cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside and cyanidin 3-glucosylrutinoside.

INTRODUCTION

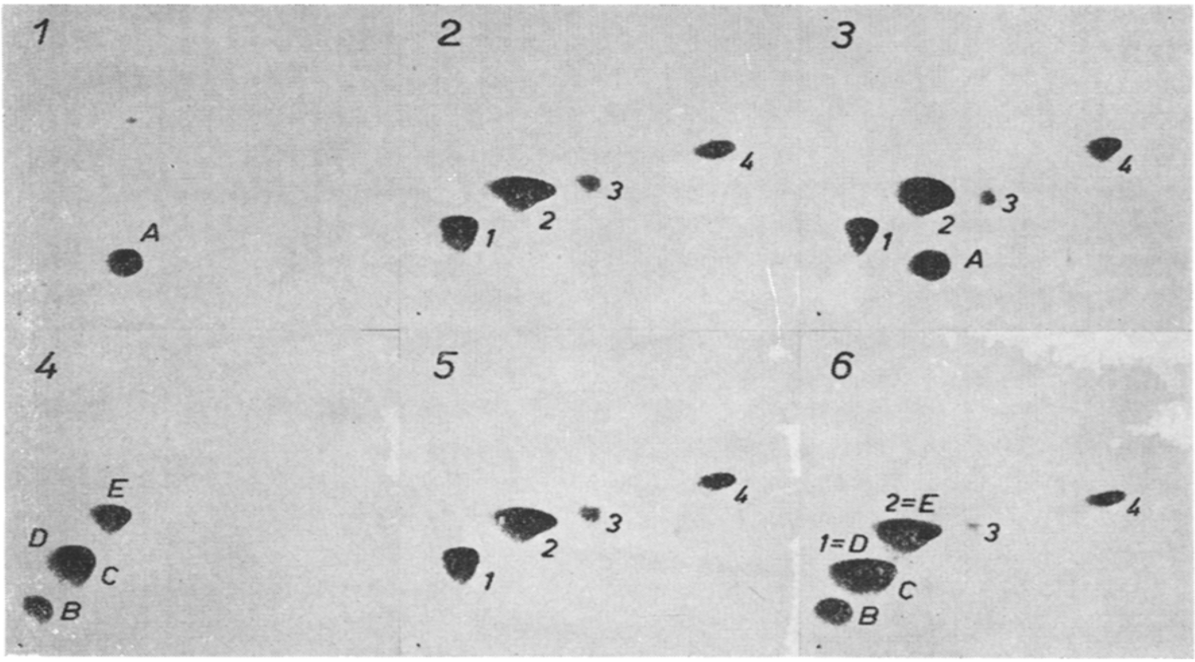
The American black raspberry, belonging to the species *Rubus occidentalis*, was shown by HARBORNE AND HALL[1] to contain four cyanidin glycosides, all with the sugars attached to the 3-position, namely glucoside, rutinoside, sambubioside and xylosylrutinoside. The same number and nature of the sugar elements had been found also by the present writer[2,3].

In contrast to these findings, extensive studies led DARAVINGAS AND CAIN[4] to describe the four pigments as 3-rhamnoglucoside, 3,5-diglucoside, 3-diglucoside and 3-rhamnoglucoside-5-glucoside. In a new publication[5] these authors retain their interpretation, which was felt to be a reason for reinvestigating the matter.

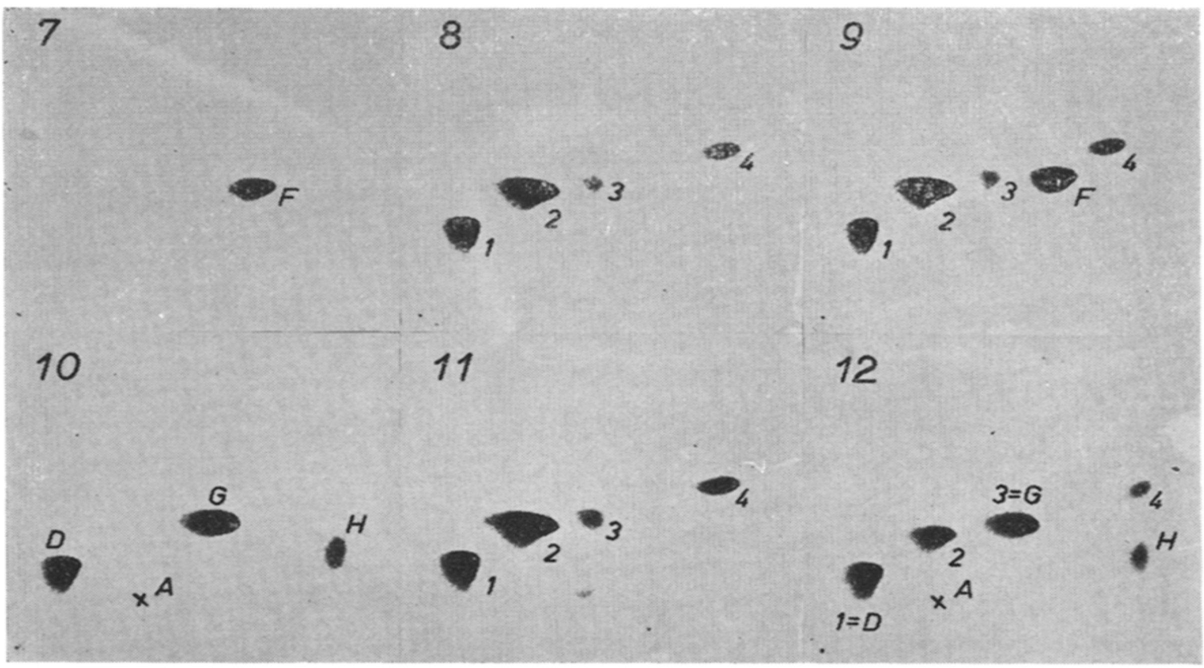
The anthocyanins of boysenberries were also checked, as the cyanidin 3-rhamnoglucoside 5-glucoside was reported from this type of berry by LUH *et al.*[6]; this being also at variance with the results of HARBORNE AND HALL[1] and with unpublished results of the present author.

EXPERIMENTAL

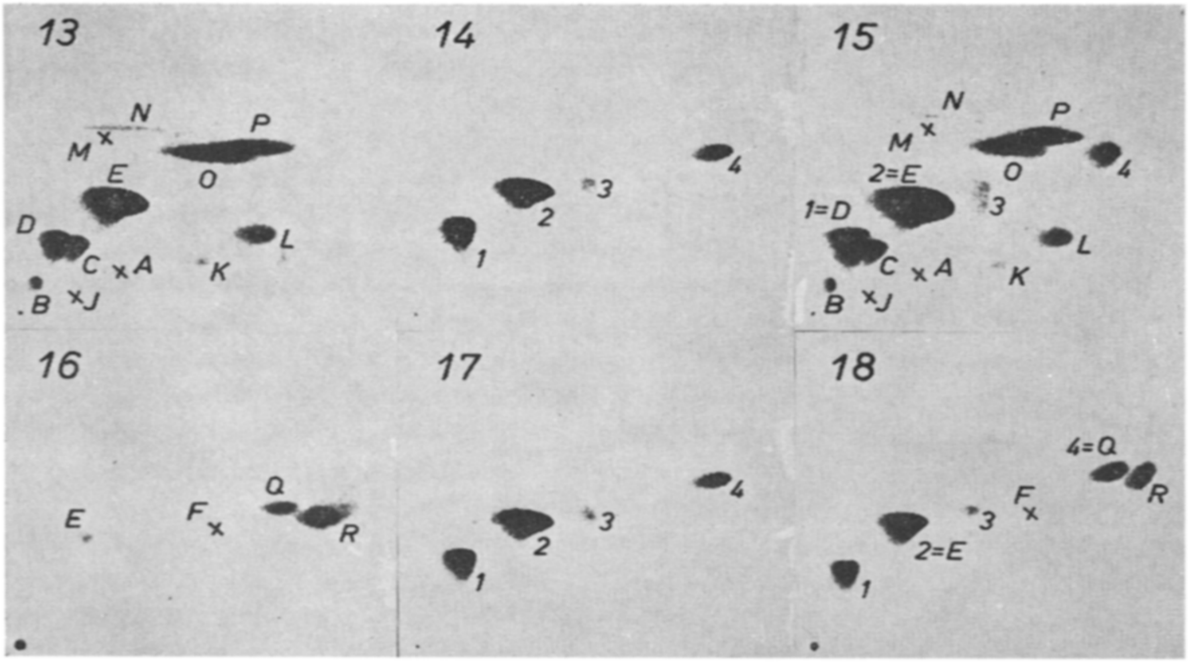
A special system of thin-layer chromatography was used[3,7]. Glass plates 6 in. × 6 in. were coated with a suspension of Merck microcrystalline cellulose, slit width 0.35



Figs. 1–6. Photo-copies of anthocyanins on two-dimensional thin-layer chromatograms. Letters refer to Table I. Fig. 1. Extract of cornflower petals. Fig. 2. Extract of black raspberries, cv. Bristol. Fig. 3. Cornflower and black raspberries co-chromatographed. Anthocyanin A does not occur in black raspberries. Fig. 4. Extract of black-currants. Fig. 5. Extract of black raspberries, cv. Bristol. Fig. 6. Black-currants and black raspberries co-chromatographed. Anthocyanins D and E match with black raspberry pigments 1 and 2, respectively.



Figs. 7–12. Photo-copies of anthocyanins on two-dimensional thin-layer chromatograms. Letters refer to Table I. Fig. 7. Extract of hibiscus petals. Fig. 8. Extract of black raspberries, cv. Bristol. Fig. 9. Hibiscus and black raspberries co-chromatographed. Anthocyanin F does not occur in black raspberries. Fig. 10. Extract of elderberries. Fig. 11. Extract of black raspberries, cv. Bristol. Fig. 12. Elderberries and black raspberries co-chromatographed. Anthocyanin G matches with black raspberry pigment 3. The x signs show the position of spots visible in the original chromatograms but too weak to show up in the prints.



Figs. 13–18. Photo-copies of anthocyanins on two-dimensional thin-layer chromatograms. Letters refer to Table I. Fig. 13. Extract of petals of Swiss pansy. Fig. 14. Extract of black raspberry, cv. Bristol. Fig. 15. Swiss pansy and black raspberries co-chromatographed. Anthocyanin L does not occur in black raspberries. Fig. 16. Extract of red-currants belonging to the species *Ribes petraeum*. Fig. 17. Extract of black raspberries, cv. Bristol. Fig. 18. *Ribes petraeum* and black raspberries co-chromatographed. Anthocyanin matches with black raspberry pigment 4. The x signs show the position of spots visible in the original chromatograms but too weak to show up in the prints.

mm. After application of the extracts, the plates were stacked on top of each other[7], so that up to 15 plates could be processed at the same time.

Two different solvent systems, were used:

(A) 1st direction, 5 parts *n*-BuOH + 2 parts conc. HCl + 1 part water; 2nd direction, 8 parts water + 4 parts conc. HCl + 1 part conc. formic acid.

(B) 1st direction, 6 parts *n*-BuOH + 1 part conc. acetic acid + 2 parts water; 2nd direction, 10 parts water + 2 parts conc. HCl + 3 parts conc. propionic acid.

Corresponding results, though with somewhat different R_F values, were obtained with the two solvent systems (*cf.* Figs. 19 and 20). The chromatograms in Figs. 1–18 were all made with solvent system A.

Before application on the plates, all pigments were purified with the aid of Dowex cation exchanger, 50 W-X 4 (ref. 3). In several cases, acid or alkaline hydrolysis was made in order to check the aglyconic or glycosidic pattern of the anthocyanins used for comparison.

RESULTS

The results are summarized in Figs. 1–18, which are reflex photocopies of actual chromatograms, reduced *ca.* 3.5 times in printing. Anthocyanins of known constitution were extracted from various plants and compared, by means of co-chromatography, with the pigments of the black raspberry cultivar Bristol.

In this way, Figs. 1–3 show that cyanidin 3-glucoside-5-glucoside (A in Figs.

TABLE I

CHEMICAL COMPOSITION OF ANTHOCYANINS REFERRED TO IN THE FIGURES

Reference letter	*Anthocyanin*
A	Cyanidin 3-glucoside-5-glucoside
B	Delphinidin 3-glucoside
C	Delphinidin 3-rutinoside
D	Cyanidin 3-glucoside
E	Cyanidin 3-rutinoside
F	Cyanidin 3-sophoroside
G	Cyanidin 3-sambubioside
H	Cyanidin 3-sambubioside-5-glucoside
J	Delphinidin 3-glucoside-5-glucoside
K	Delphinidin 3-rutinoside-5-glucoside
L	Cyanidin 3-rutinoside-5-glucoside
M	Delphinidin 3-coumaroylglucoside (?)
N	Cyanidin 3-coumaroylglucoside (?)
O	Delphinidin 3-coumaroylrutinoside-5-glucoside
P	Cyanidin 3-coumaroylrutinoside-5-glucoside
Q	Cyanidin 3-xylosylrutinoside
R	Cyanidin 3-glucosylrutinoside

1 and 3), as isolated from cornflower (*Centaurea cyanos*), does not occur in Bristol. On the other hand, the comparison between Bristol and black-currants (*Ribes nigrum*) in Figs. 4–6 indicates that the Bristol pigments 1 and 2 are the same as the two black-currant anthocyanins D and E, cyanidin 3-glucoside and cyanidin 3-rutinoside (*i.e.* rhamnoglucoside).

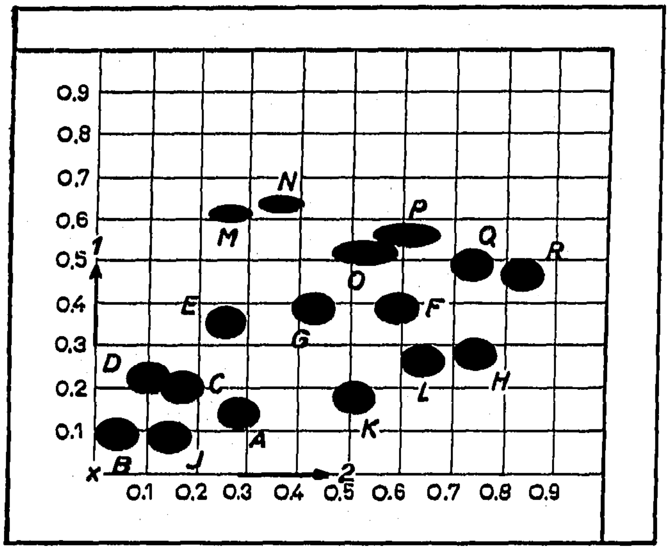
The chemical composition and R_F values of the anthocyanins dealt with in this paper and/or occurring in the figures are summarized in Table I and in Figs. 19–20.

Figs. 7–12 deal with the third Bristol pigment. The comparison with cyanidin 3-sophoroside (*i.e.* diglucoside, F in Figs. 16 and 18) from hibiscus (*Hibiscus rosa-sinensis*) shows that this anthocyanin does not occur in Bristol. The cyanidin 3-sambubioside (*i.e.* xyloglucoside, G in Fig. 10) from elderberry (*Sambucus nigra*) corresponds, however, completely with pigment 3 of the black raspberry.

Bristol pigment number 4 finally is dealt with in Figs. 13–18. The idea that pigment 4 would be cyanidin 3-rutinoside-5-glucoside (L in Fig. 13) is not supported by co-chromatography with this anthocyanin from petals of the Swiss pansy (*Viola x wittrockiana*, dark red flowers of unknown cultivar). The red-currants derived from the species *Ribes petraeum* differ from other red-currants by the presence of a branched triglucoside, containing one molecule each of glucose, rhamnose and xylose[1,3]. The comparison in Fig. 18 indicates that this cyanidin 3-xylosylrutinoside (Q in Figs. 16 and 18) corresponds to pigment 4 of Bristol.

In addition to Bristol, the same anthocyanin constitution was found in the black raspberry cultivars John Robertson, Dundee and Black Hawk, as well as in a selected clone without name.

Further evidence that the anthocyanin composition described for these cultivars is typical for *Rubus occidentalis* is given by the study of various purple raspberry cultivars, *i.e.* hybrids between *Ribes idaeus/strigosus* and *R. occidentalis*, in the first place the cultivars Clyde, Marion and Sodus. All these types have six cyanidin 3-



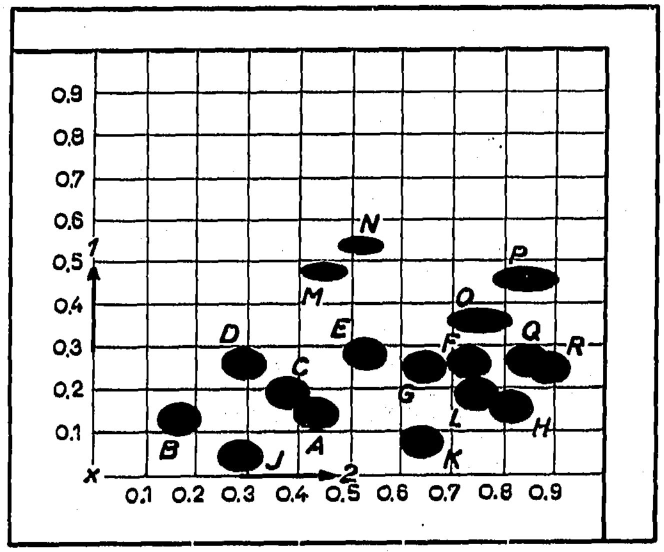


Fig. 19. The composite two-dimensional separation pattern of various anthocyanins on cellulose thin-layer. Solvent system A. Letters refer to Table I.

Fig. 20. The composite two-dimensional separation pattern of various anthocyanins on cellulose thin-layer. Solvent system B. Letters refer to Table I.

glycosides, namely glucoside, rutinoside, sambubioside, sophoroside, xylosylrutinoside and glucosylrutinoside. Some of these compounds have been inherited from the red raspberries (sophoroside and glucosylrutinoside), whereas others are typical of the black raspberries (sambubioside and xylosylrutinoside); glucoside and rutinoside being common to both types of parents. Some raspberries, red, purple and black may contain rather much of the related pelargonidin glycosides.

An analysis of the pigments of the raspberry–blackberry hybrids Boysen and Logan clearly showed that these consist of the four red raspberry anthocyanins: cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside and cyanidin 3-glucosylrutinoside. This is in agreement with the results of HARBORNE AND HALL[1], but does not support the findings of LUH *et al.*[6], who reported cyanidin 3-rutinoside 5-glucoside in boysenberries.

It should be mentioned that the 3,5-anthocyanins of cyanidin, as well as the 5-glucoside and the cyanidin itself, are all red-fluorescent in U.V. light. None of the anthocyanins of black raspberries or boysenberries are fluorescent in U.V. light.

DISCUSSION

This reinvestigation of the pigments of black raspberries and boysenberries thus would show that the earlier results by HARBORNE AND HALL[1] and by the present author[2,3] are still valid. This is not of academic interest only. According to the present writer's experience different types of glycosides behave in rather different ways, at least towards acid hydrolysis. Whether or not this is also true with regard to the anthocyanin destruction in fruit products certainly invites further study. For such technological investigations[5] it seems to be desirable to know, however, what substances one is working with.

Considering the chromatographic procedure, it is obvious that two-dimensional separation is far more elucidative than one-dimensional. Also, thin-layer chromatography does yield considerably more clear-cut results than conventional paper chromatography. Finally, co-chromatography must be considered more reliable than the comparison of R_F-values, as these may vary considerably.

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