values on paper as well as those of cyanidin-3-monoglucoside (isolated from *Sedum album*) are given in Table 1.

The acid hydrolysis of the two pigments was performed as described earlier with the exception that the aglycones were extracted with butanol.[3] The two anthocyanins yielded cyanidin identified by absorption and infrared spectra as well as by co-chromatography in forestal with an authentic marker. The sugars were isolated according to the method described earlier.[3] They were identified by chromatography with authentic markers in ethyl acetate-pyridine-water (8:1:1, by vol.) and ethyl acetate-acetic acid-water (3:1:1, by vol). Pigment I gave glucose and pigment II glucose and rhamnose. The position of the attachment of the sugar was determined by spectral measurements.[5,6] The controlled hydrolysis was performed by warming pigment II with 5 % HCl at 100° for 5 min. The resulting solution was then chromatographed on paper in 1 % HCl. Three spots were obtained identified by co-chromatography and spectral measurements as cyanidin, cyanidin-3-monoglucoside (Table 1) and unchanged pigment.

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The Anthocyanins of the Berries of *Majanthemum bifolium*

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The *Liliaceae* is a vast and heterogeneous family and among its many genera, only a few have coloured berries instead of capsules. Earlier these genera (*Asparagus*, *Majanthemum*, *Polygonatum*, *Convallaria*, and *Paris*) formed one family, *Convallariaceae*, but owing to many fundamental differences between them they are now all grouped in the *Liliaceae* family and, according to Krause,[1] in the subfamily *Asparagoideae*.

At least one of the five genera mentioned above differs as regards its pigment content. The three species of *Polygonatum* occurring in Sweden have berries generally described as dark blue. However this impression is due to the occurrence of bloom on the berries and their real colour is dark green. As far as we have found these berries do not contain any extractable pigments. The berries of *Asparagus officinalis* and *Convallaria majalis* are, at least partly, pigmented by carotenoids. The former contain capsanthin [2] and physalein [3] (zeaxanthin dipalmitate) whereas the latter contain α-, β-, and γ-carotene, lutein and lycopene. If they also contain anthocyanins will be investigated later on.

The berries of *Paris quadrifolia* and of *Majanthemum bifolium* owe their colour to the presence of anthocyanins. A preliminary investigation of the berries of *P. quadrifolia* has revealed the presence of at least five different anthocyanins. A fuller report of the anthocyanin content will appear later.

The unripe berries of *M. bifolium* are colourless but as they ripen they turn red, sometimes in a very short time. Since the red colour is due to the presence of anthocyanins the unripe berries ought to contain some precursor the nature of which will be further investigated.

Three anthocyanins have been isolated from the ripe berries. Cyanindin-3-monoglucoside and cyanidin-3-rhamnosylglucoside were identified from spectral data, co-chromatography with authentic specimens, and identification of their hydrolysis

products.[4] The third anthocyanin is not acylated since there was no change in its R_F-values in different solvents after alkaline treatment.[5] According to its spectral data it has a free hydroxyl group in the 5 position.[6] On acid hydrolysis it gave peonidin, glucose, and rhamnose and on controlled hydrolysis peonidin, unchanged pigment, and a new pigment which judging from its R_F-values could be peonidin-3-monoglucoside. From these data the third pigment has been provisionally identified as peonidin-3-rhamnosylglucoside, an anthocyanin probably not yet found in Nature. In 1958 it was reported to occur in cultivated varieties of *Cyclamen* [7] but this was not confirmed on reinvestigation.[8] Also the published R_F-values do not agree with those obtained by us which, however, coincide fairly well with the R_F-values reported by Harborne for peonidin-3-rhamnosylglucoside obtained by hydrolysis of peonidin-5-glucoside-3-rhamnosylglucoside.[8]

Table 1. The R_F-values of the peonidin glycosides.

	BAW	BuHCl	1 % HCl	HAc-HCl
Peonidin derivative	0.40	0.33	0.21	0.48
Peonidin-3-rhamnosylglucoside [8]	0.38		0.21	
Peonidinglucoside obtained on controlled hydrolysis	0.42	0.29	0.09	0.31
Peonidin-3-monoglucoside	0.41	0.30	0.09	0.33

BAW = butanol-acetic acid-water (4:1:5, by vol., top layer),
BuHCl = butanol-2 M HCl (1:1 v/v, top layer),
1 % HCl = conc. HCl-water (3:97 v/v),
HAc-HCl = acetic acid-conc. HCl-water (15:3:82, by vol.).

Experimental. The methods used for TLC, PC, extraction, and purification of anthocyanins have been described earlier.[4] When chromatographed on Whatman No. 3 paper in butanol-acetic acid-water (6:1:2, by vol.) two bands were obtained, one containing the peonidinderivative and the other a mixture of cyanidin glycosides. By paper chromatography in 15 % aq. acetic acid the latter was resolved into two pigments identified as cyanidin-3-monoglucoside and cyanidin-3-rhamnosylglucoside by methods described earlier.[4]

The R_F-values of the third pigment are recorded in Table 1. The absorption maximum in the visible region (530 nm) is somewhat higher than that reported by Harborne for peonidin-3-rhamnosylglucoside (523 nm).[8] No shift of this maximum to the blue region occurred on the addition of ethanolic $AlCl_3$ indicating the absence of two adjacent hydroxyl groups.[6] The ratio $E_{440}/E_{530} = 26$ is characteristic of anthocyanins with a free hydroxyl group at the 5 position.[6] The absence of an acyl group was proved by treating the pigment with 2 M NaOH for 3 h at room temperature. After acidification and extraction with butanol the product was identified as unchanged pigment by co-chromatography with untreated pigment in BAW and 1 % HCl. Peonidin was identified by chromatography in forestal and from spectral data.[5] Glucose and rhamnose were identified by chromatography with authentic markers in two solvents.[4] Peonidin-3-monoglucoside was obtained in a very small amount and could only be characterized by its R_F-values.[5]

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