On Anthocyanins in *Hippuris*

GERD BENDZ and ÅSA HAGLUND

The Institute of Chemistry, University of Uppsala, Uppsala, Sweden

The stems of *Hippuris vulgaris* and also to a lesser degree those of *Hippuris tetraphylla* sometimes exhibit a red colour indicating the presence of anthocyanins. The two species have now been found to contain cyanidin glycosides. From *H. tetraphylla* cyanidin-3-monoglucoside has been isolated and identified by spectral data, by co-chromatography with an authentic specimen and by identification of its hydrolysis products. The more red-coloured *H. vulgaris*, on the other hand, has been found to contain not only cyanidin-3-monoglucoside but also cyanidin-3-monogalactoside. Those two anthocyanins having almost identical R_F-values in all conventional solvent mixtures used were first considered as one pigment which yielded one spot by all chromatographical methods tried. According to its very low R_F-values in 1 % aqueous HCl the pigment ought to be a monoside [1] which was also confirmed by the result obtained upon controlled hydrolysis. Furthermore, judging from its absorption spectrum the hydroxy group at position 5 must be free.[2] However, upon acid hydrolysis the pigment did yield one aglycone, cyanidin, but two sugars, glucose and galactose. All these data indicated that the pigment must be a mixture of two anthocyanins. Their resolution was finally achieved by paper chromatography in 1 % aqueous HCl for 45 h and the two anthocyanins were identified by spectral measurements and by the simultaneous chromatography of authentic markers as well as of a mixture thereof.

Experimental. The methods used for TLC, PC, extraction, and purification of the anthocyanins as well as those used for spectral measurements have been described earlier.[3] The anthocyanin isolated from *H. tetraphylla* (obtained from the University Botanical Garden) was identified as cyanidin-3-monoglucoside by co-chromatography in the usual four solvents with an authentic specimen isolated from *Sedum album*. Upon acid hydrolysis it yielded only cyanidin and glucose identified by chromatography and spectral data.[3] The pigment isolated from *H. vulgaris* (collected in northwestern Dalarna) yielded upon acid hydrolysis cyanidin, glucose, and galactose identified by co-chromatography and spectral data. After controlled hydrolysis and chromatography on paper in 1 % aqueous HCl two spots were obtained, one at the start line identified as cyanidin and the other with $R_F = 0.07$ as unchanged pigment. With PC the following R_F-values were obtained for the pigment, cyanidin-3-monoglucoside (from *Sedum album*) and cyanidin-3-monogalactoside (from *Vaccinium vitis-idaea*):

Butanol-acetic acid-water (4:1:5, by vol., top layer):	0.37	0.37	0.37
Butanol-2 M HCl (1:1, v/v, top layer):	0.25	0.24	0.25
Conc. HCl-water (3:97 v/v):	0.07	0.07	0.07
Acetic acid-conc. HCl-water (15:3:82, by vol.):	0.26	0.26	0.26

After 45 h chromatography on paper in 1 % aqueous HCl the pigment yielded two spots. The faster travelling one was identified as cyanidin-3-monogalactoside and the other one as cyanidin-3-monoglucoside. These two anthocyanins were also chromatographed at the same time and on the same paper alone, mixed together, and mixed with the isolated pigment.

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