



Figure 8 Typical TLC retention order on cellulose of anthocyanidins with respect to their B-ring substituents. See Table 4 for explanation of abbreviations. Top row: Aqueous solvents as mobile phase. This retention order is also observed for anthocyanin 3-*O*-monoglycosides. Bottom row: Alcoholic solvent as mobile phase.

nol–conc. hydrochloric acid–water (190:1:10). The chromatogram (Fig. 7) reveals complete separation of the six common anthocyanidins.

The observed R_f values are related to structural differences in the B-ring. After development in the first direction, the anthocyanidins are located in three groups according to the number of hydroxyl groups on their B-rings (Fig. 8, top). The solvent used in the second direction resolves the pigments largely according to the number of oxygen-containing substituents on the B-rings (Fig. 8, bottom).

4. Separation of Anthocyanins on Cellulose

Separation of typical anthocyanins is carried out on cellulose layers in three solvent systems that all contain concentrated hydrochloric acid, formic acid, and water in different proportions: system 1, 19:19:62; system 2, 7:51:42; and system 3, 25:24:51. The mobile phase is run 18 cm (about 120 min). The R_f values of the individual compounds are given in Table 4.

The anthocyanidins are completely separated from the anthocyanins in systems 2 and 3. System 2 gives the best division between the anthocyanidins; however, none of the six common anthocyanidins is completely separated (Fig. 6). Because the A-ring substituents of the common anthocyanidins are identical, the observed R_f values seem to be related to the number of hydroxyl groups on the B-rings (Fig. 8, top). The introduction of sugar units gives higher R_f values; however, the same separation pattern with respect to the B-ring substituents is observed.

Densitometric profiles of the separation of anthocyanins isolated from black currant (*Ribes nigrum*) and raspberry (*Rubus idaeus*) are given in Figs. 9 and 10, respectively.

V. CAROTENOIDS

A. General

1. Structure

The carotenoids are yellow to red tetraterpenoids in which eight isoprene units are arranged in a symmetrical linear pattern and after biosynthetic dehydrogenation provide a polyene chromophore that absorbs light in the visible part of the spectrum. Skeletal variation is largely confined to cyclization of the endgroup, whereas oxygenation yields alcohols, ketones, *etc.* (see Fig. 11). The carotenoid hydrocarbons are sometimes distinguished from the oxygenated carotenoids by using the class names carotene for the former and xanthophyll for the latter.

2. Distribution

Carotenoids are essential components of all photosynthetic tissue, in which they are largely located in the chloroplasts. They are also responsible, either alone or together with other pigments, for