7. Two-Dimensional TLC of Aglycones

The methanolic extract is applied in a corner on a 10 × 10 cm commercial glass plate and developed over 9 cm in the system described for the separation of glycosidic compounds. After development, the plate is thoroughly dried and placed in a desiccator containing a beaker with 25% HCl. The air outlet must be open, and a tube is used to lead acid fumes into a beaker containing 10% aqueous sodium hydroxide. The apparatus is placed in an oven, and the hydrolysis is completed after heating for 35 min at 90°C. The plate is carefully removed and dried in a nitrogen stream. Second-direction development is done with petroleum ether (40–60°C)–ethyl acetate–formic acid (75:25:1) as the mobile phase.

The chromatographic pattern from two-dimensional TLC analysis of an extract of alder buckthorn bark is shown in Fig. 27. Hydrolyzed extracts of rhubarb and alder buckthorn are used as references in the second, aglycone-separating, direction. The observed pattern gives valuable data about the glycosides with respect to the aglycone moiety. The pattern obtained for alder buckthorn indicates that chrysophanol, physcione, and emodin occur in free form. Emodin derived from an emodin glycoside is located in the middle. The remaining emodin spots are due to the glycofrangulins. Diglycosides of chrysophanol and physcione give three spots at the upper right.

IX. BETALAINS

A. General

1. Structure

There are two main groups of betalains, the red-violet betacyanins and the yellow betaxanthins. The betacyanins, which are made by condensation of betalamic acid with cyclo-DOPA (dihydroxyphenylalanine), occur mainly as *O*-glycosides with a sugar attached to one of the hydroxyl groups of the dihydroindole unit (Fig. 28). Glucose and glucuronic acid are the monosaccharides most commonly present. The sugar residues may be acylated, usually with cinnamic acids. The betaxanthins result from the condensation of betalamic acid with amines or amino acids (Fig. 28). They have hitherto not been reported to be glycosylated.

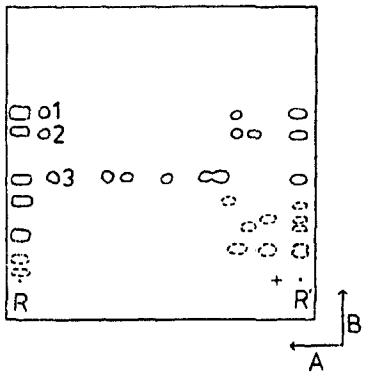

Figure 27 Two-dimensional separation of anthraquinone pigments of *Rhamnus frangula*. First direction (glycoside separation): solvent system A, ethyl acetate–methanol–water (100:13.5:10); developing distance 9 cm. Second direction (aglycone separation): solvent system B, light petroleum (40–60°C)–ethyl acetate–formic acid (75:25:1); developing distance 8.5 cm. Stationary phase: silica gel 60 F_{254} (0.25 mm, Merck). Detection: KOH reagent, visible light/UV_{365}. Hydrolyzed extracts used as references in the second direction: R, *Rheum palmatum*; R', *Rhamnus frangula*. Spot identities: (1) chrysophanol, (2) physcione, (3) emodin.