Introducing hydroxyl functions onto the B-ring produces the same increase in retention as was observed for flavonoid aglycones chromatographed on silica systems. However, the effects of hydroxyl substituents at positions 5 and 3 are somewhat different. No significant change in retention is observed when a hydroxyl function is introduced in the 5-position. This may indicate that the contribution of intramolecular hydrogen bonding is less effective in the case of polyamide than in the case of silica layers. Polyamide is known to form extensive hydrogen bonds between the amide carbonyl function and the hydroxyl substituents of phenolic compounds. This effect can be observed for flavonols, which are more retained than the corresponding flavones. Instead of taking part in internal hydrogen bonding, the 3-hydroxyl group interacts with the polyamide layer. Methylation of any hydroxyl group prevents the formation of hydrogen bonding between the flavonoid and the stationary phase, and a marked decrease in retention is observed.

c. Octadecyl-Bonded Silica (RP-18) Layers. Samples (3 μL) are applied on RP-18 support and developed over 8.5 cm (35 min) with methanol–formic acid–water (58:10:16) as the mobile phase. The pigments are visualized with NP/PEG-4000 reagent, and the plate is inspected under longwave UV. The colors observed are comparable to those seen with the preceding systems. Measured R_f values are given in Table 2 under system 4.

The retention of flavones and flavonols is partly determined by their polarity, as evidenced by the decreased retention with increasing hydroxylation on the pigments (Table 2). In contrast to this trend, a 5-hydroxyl group takes part in intramolecular hydrogen bonding to the carbonyl group, and increased retention is observed; the R_f values of 7-hydroxy- and 5,7-dihydroxyflavone are 0.41 and 0.33, respectively, in system 4 (Table 2). Whereas addition of a new methoxyl function on the pigment has little effect on its mobility on RP-18 layers, the replacement of a hydroxyl with a methoxyl function gives markedly increased retention (Table 2).

4. Separation of Flavonoid Glycosides

Samples (3 μL) of flavones and flavonols are applied on a silica plate and developed over 8.5 cm (35 min) with ethyl acetate–formic acid–acetic acid–water (100:11:11:27) as the mobile phase. After development, the plate is dried and inspected under longwave UV before and after it has been sprayed with the NP/PEG 4000 reagent. Typical R_f values and colors for some flavonoids are given in Table 3.

The flavonoids that are separated on silica (Table 3) give well-defined bands with R_f values ranging from 0.20 to 0.72. The monoglycosides are less retained than the diglycosides. The retention with respect to the type of monosaccharide substituent increases as follows: arabinofuranoside > rhamnoside > arabinopyranoside > glucoside > galactoside.

The separation of flavonoid glucosides in *Betula* spp. with fluorescence quenching as the detection mode is shown in Fig. 4.

Methanolic extracts of *Coreopsis* spp., *Dahlia* spp., and *Helichrysum bracteatum* containing chalcones and aurones were tested on silica gel plates with ethyl acetate–formic acid–water (60:12:16) as the mobile phase. The plates were developed over 8.5 cm (about 40 min), dried, and sprayed with NP/PEG 4000. The clear yellow zones turned to violet and red, and the red-to-orange fluorescent colors seen under longwave UV light confirmed the presence of chalcone and aurone pigments. Ammonia vapor intensifies the colors after spraying.

IV. ANTHOCYANINS

A. General

1. Structure

Anthocyanins are water-soluble glycosides of anthocyanidins and are part of the phenolic group known collectively as flavonoids (see Sec. III). The anthocyanidins (aglycones) are polyhydroxy and polymethoxy derivatives of the 2-phenylbenzopyrylium cation (Fig. 5). Eighteen different anthocyanidins (aglycones) have been reported, but only six of them (Table 4) are widespread. With the exception of the rare deoxyanthocyanins, the 3-hydroxyl is always replaced by a sugar; however, glycosylation at the 7-, 3′-, 5′-, and, especially, the 5-hydroxyl groups is encountered