E. Practical Experiments

1. Isolation

Small quantities of plant material are directly extracted with host methanol, whereas Soxhlet extraction with the same solvent is used for larger quantities. Successive extractions with several solvents of increasing polarity are often performed when a complete analysis is required.

2. Hydrolysis of Glycosides

The glycosidic extract is evaporated to dryness and heated under reflux for 25 min with 7.5% hydrochloric acid. After cooling, the aglycones are extracted with portions of diethyl ether. Concentrated extracts are used directly for TLC.

3. Separation of Glycosides

Extracts from rhubarb root (*Rheum palmatum*), alder buckthorn bark (*Rhamnus frangula*), and aloe (*Aloe capensis*) are used as pigment sources. The extracts (3 μL) are applied to silica gel as streaks (2 cm), and the plate is developed to a distance of 8.5 cm (35 min) with ethyl acetate–methanol–water (100:13.5:10) as the mobile phase. An illustrative example of separated glycosides from *R. frangula* is given in Fig. 23. Yellow bands are observed in daylight, and the pigments produce orange-yellow colors under longwave UV. Spraying the plates with 5% ethanolic KOH gives red to purple bands in the visible and dull red bands in the longwave UV, except for the C-glycosides from aloe, which give an intense yellow-orange fluorescence.

R_f values for several anthraquinone glycosides are given in Table 11. The pigments are generally separated according to the number and nature of the sugar units. The monoglycosides are well separated from the diglycosides. Within each of these groups of glycosides, variation of the sugar moiety is reflected in further separation, although changes in the aglycone do not appear to result in similar separation. Rhein-8-*O*-glucoside does not follow the general pattern and is strongly sorbed due to the acid group. Rhein itself is located at R_f 0.28, and the system is thus useful for preparative separation of rhein from a hydrolyzed extract where the major compounds move with the solvent front.

4. Separation of Sennosides

Extracts (3 μL) from leaves and fruits of senna (*Cassia angustifolia*) are applied as streaks (2 cm) on silica gel sheets and developed over 8.5 cm (100 min) with 2-propanol–ethyl acetate–water (36:36:28) as the mobile phase. The sennosides occur as pale yellow zones in daylight and give characteristic dull red zones in longwave UV. After spraying with KOH reagent, the colors of the sennoside pigments are intensified in daylight and a characteristic yellow-green fluorescence appears in UV light. Alternatively, the bianthrones can be detected indirectly by oxidation to the corresponding anthraquinones. The plate is first sprayed with concentrated nitric acid, then heated on a thermoplate for 10 min at 120°C to complete the reaction. Further spraying with 5% KOH gives the common colors of the free anthraquinones.

The separated pigments are indicated in Fig. 24. Bianthrone glycosides dominate in the extracts. Sennoside B and sennoside A appear at R_f 0.18 and 0.30, respectively. Sennoside D is located directly below rhein-8-glycoside at R_f 0.44, and small amounts of sennoside C are detected at R_f 0.52 in the leaf extract. An additional pigment occurs above the sennoside A band and reacts like the sennosides with the common spray reagents.

5. Separation of Aglycones

Hydrolyzed root extracts from rhubarb and madder (*Rubia tinctorum*) are applied as bands (2 cm) on silica gel layers and developed in three different solvent systems. All systems separated at least five yellow pigments fro rhubarb and several pink-to-purple pigments from madder. Calculated R_f values are given in Table 12.

System 1 required development over 18 cm (100 min) with light petroleum (40–60°C)–ethyl acetate–formic acid (75:25:1) as the mobile phase. A representative separation of pigments from rhubarb is given in Fig. 25. It should be noted that this separation was achieved on a plate prepared