out on DEAE cellulose (67). Two developments with 2-propanol–water–acetic acid (75:20:5) were required, but this system failed to separate pigments from beetroot.

A TLC system using cellulose plates (0.5 mm) was reported for the preparative separation of betalains from beetroot (65). A high sample load (200 mg of pigment) required a prerun in the polar solvent 2-propanol–ethanol–water–acetic acid (6:7:6:1). After development over 10 cm followed by extensive drying of the plate, betanin was finally separated from the betaxanthins using two successive developments (15 cm) with the same solvent components but in different proportions (10:4:4:1).

C. Practical Experiments

1. Extraction

Beetroot (*Beta vulgaris*) is a good source when testing the chromatographic properties of the betalains by TLC. Fresh beetroot (50 g) is first homogenized with 100 mL of methanol–water (1:1). The suspension is allowed to stand for 2 h at 4°C. The extract is recovered by filtration, and the process is repeated, now with water (50 mL) as solvent. The combined filtrates are concentrated below 30°C under reduced pressure.

2. Separation

The concentrated extract is applied as a streak (5 cm) on a cellulose plate (0.1 mm) (Merck) and developed twice (2 × 15 cm) with 2-propanol–ethanol–water–acetic acid (6:7:6:1) as the mobile phase. The plate is dried in a nitrogen stream, and five sharply yellow bands (R_f 0.66, 056, 0.48, 0.43, and 0.36) are observed. The bands with R_f values at 0.48 and 0.43 are tentatively identified as vulgaxanthin I and vulgaxanthin II, respectively. The violet betanin band appears at R_f 0.27; however, some trailing may be observed. For semipreparative isolation of betalains in beetroot, homemade cellulose plates (0.3 mm) (Macherey Nagel 300) give similar results.

If a shorter developing distance (2 × 8.5 cm) is selected, the relatively long separation time (6 h) for the experiment given above may be reduced to about 2 h. Even though the resolution will suffer, three betaxanthine zones are observed in addition to the violet betanin band.

3. Differentiation Between Betalains and Anthocyanins

The pigment extract is administered as a small band on a cellulose plate (0.1 mm) (Merck) and developed in 1-butanol–acetic acid–water (6:1:2) for 2 h. Betalains move slowly in this solvent, whereas anthocyanins have much higher R_f values and are often separated into individual bands.

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