2. Separation

Extracts (3 μL portions) were applied as bands (2 cm) on plastic-backed silica gel (0.2 mm) (Macherey-Nagel) and developed over 8.5 cm with petroleum ether (40–60°C)–acetone–acetic acid (75:25:1.5) as the mobile phase. Separated extracts showed several clearly located yellow and orange-yellow bands (Fig. 22). Spraying with 10% methanolic KOH intensified the pigments from *L. alba*, and the naphthaquinones in *J. regia* and *D. rotundifolia* changed to blue-violet. The main compounds were identified as lawsone (R_f 0.26), juglone (R_f 0.48), and 7-methyljuglone (R_f 0.50).

The location of the hydroxy substituent in compounds such as juglone and 7-methyljuglone (Fig. 18) has a great influence on the retention of these compounds. Intramolecular hydrogen bonding between the keto group and the hydroxyl substituent reduces the polarity and results in relatively high R_f values. In contrast, lawsone (Fig. 18) is strongly sorbed to the TLC layer.

D. TLC of Anthraquinones

Common anthraquinone glycosides are best separated on silica layers with ethyl acetate–methanol–water (100:13.5:10) as the mobile phase (58). More polar compounds, such as the sennoside pigments, are well separated on a silica gel system reported by Khafagy et al. (59).

Suitable solvent systems for the separation of aglycones on silica layers include petroleum ether–ethyl acetate–formic acid (75:25:1) (58) and petroleum ether–ethyl formate–formic acid (75:25:5). A mobile phase reported by Nyiredy et al. (60) offers no advantages compared with the former systems. Finally, a method developed by Ebel and Kaal (61) involves direct hydrolysis of the glycosides on the TLC plate; the solvent systems suggested may be advantageously replaced by the solvents described above.

A two-dimensional TLC separation on silica of a complex mixture of anthraquinone aglycones and glycosides from the fungus *Dermocybe sanguinea* was recently reported (61a). The eluent systems used were *n*-pentanol–pyridine–methanol (6:4:3) and toluene–ethyl acetate–ethanol–formic acid (10:8:1:2).

The prediction of retention data for quinones on the basis of their structure and the physical chemistry of the chromatographic system used also received attention, and results are reported to be consistent with the forecast behavior (61b,61c).

Figure 22 Separation of naphthaquinone pigments from (A) *Lawsonia alba*, (B) *Juglans regia*, and (C) *Drosera rotundifolia*. Solvent system: light petroleum (40–60°C)–acetone–acetic acid (75:25:1.5). Staionary phase: POLYGRAM silica gel N-HR/UV_{254} (0.2 mm, Macherey-Nagel). Developing distance: 8.5 cm. Detection: visible light with KOH reagent. Band identities: (1) lawsone, (2) juglone, (3) 7-methyljuglone.