columns can also be used for removing proteins, lipids, and salts. For such precleaning, samples can be accepted in virtually any form, provided they are stable and not complexed. It is desirable, however, to bring the pH to 5–8 before purification.

D. Sugars Isolated from Polysaccharides and Conjugated Carbohydrates

A wide variety of naturally occurring compounds contain sugars. Monosaccharides are linked, by glycosidic bonds, to another sugar component or to a moiety of another origin, called an aglycone. Individual sugar components of conjugated sugars can be analyzed after appropriate enzymatic or chemical hydrolysis. Acid hydrolysis is most frequently used. It is suitable for cleavage of polysaccharides and almost all types of conjugated compounds such as glycolipids, glycoproteins, and various glycosides. Various acid hydrolytic conditions may be chosen for different types of compounds depending on factors such as the monosaccharide composition of the individual compound, the ring form of the component sugars, and the configuration of the glycosidic bonds. It is known that furanoside linkages are much more labile than pyranoside linkages, that α-glycosidic bonds are usually more labile than β-glycosidic bonds, and that pentaglycans, in the pyranoside form, are more readily hydrolyzed than pyranoside hexoglycans. The presence of uronic acid groups and amino sugars also increases the resistance to acid hydrolysis. Hydrolysis is usually done with hydrochloric acid, sulfuric acid, or trifluoroacetic acid at concentrations ranging from 0.5 M to up to several moles per liter, and at elevated temperatures. Detailed procedures are described in the literature (25–30).

Thin-layer chromatographic analysis of the hydrolysates requires removal of the mineral acid used for hydrolysis. Ion-exchange resins are widely used, but some classical procedures are also useful. After hydrolysis with 1 M sulfuric acid, the acid may be removed as barium sulfate by adding barium carbonate solution. Small amounts of 1 M hydrochloric acid can be removed in vacuo, and larger amounts of this acid can be conveniently removed from an aqueous hydrolysate by repeated washing with a 10% solution of di-*n*-octylmethylamine in chloroform. The hydrolysates of plant glycosides usually contain interfering compounds such as phenolics. These can be removed by extracting the hydrolysates with diethyl ether or ethyl acetate (26).

TLC is occasionally used to assay the activity of carbohydrate-degrading enzymes. For example, Whitehead et al. (31a) used TLC to assay the activity of an agarase isolated from a marine bacterium.

E. Glycolipid Analysis

Of all the glycoconjugates mentioned above, only glycolipids have chemical properties suitable for TLC analysis as intact molecules without hydrolysis of the sugar moiety. In fact, the amphipathic nature of glycolipids is well suited to TLC, which has many applications in lipid analysis. Lipid fractions may be applied directly to TLC plates, or glycolipids may be purified or enriched prior to analysis (31b,31c,31d). Synthesis of neoglycolipids using oligosaccharides isolated from complex carbohydrates is useful in some structural studies involving TLC coupled to mass spectrometry (see Sec. IV.E).

III. SEPARATION

Carbohydrates are weakly acidic (pK_a 12–14), strongly hydrophilic compounds (31e). Their separation by thin-layer chromatography depends on partition and adsorption phenomena, and occasionally, resolution incorporates anion-exchange mechanisms. The high polarity of sugars requires very polar solvents and sorbents with low activity. The essential component of typical solvent systems is water. Because water decreases the layer activity, nonmodified inorganic sorbents such as alumina and silica as well as modified sorbents and organic materials can be used for separation of carbohydrates. Their mobility on polar layers depends primarily on the molecular weight of the carbohydrates and the number of hydroxyl groups. Consequently, the diastereoisomers are often poorly resolved. The water content of the mobile phase as well as polar solvents