Vorschrift für LDH; an Stelle von 1 ml Natriumlaktat in der Lösung I wird 0.580 mg Apfelsäure, gelöst in 5 ml dest. Wasser und neutralisiert mit 0.1 *N* Natronlauge, zugefügt (Fig. 4).

Die Anwendung der genannten Farbreaktionen ergab bei Pflanzenextrakten (Fig. 4), die durch disc-Elektrophorese getrennt worden waren, scharfe und eindeutige Banden (LINSKENS[7]).

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Separation of alcohol mixtures by thin-layer chromatography

Thin-layer chromatography (TLC) was applied by LAWSON AND GETZ[1] to the purification of selachyl alcohol and later by SUBBARAO *et al.*[2] to mixtures of alcohols, especially hydroxy-acids, employing petroleum ether–diethyl ether solvent systems. In the present study, ternary, quaternary and quinary artificial mixtures of alcohols were submitted to TLC resolution and the sequences of separation compared with the boiling points of the individual components.

Glass plates measuring 20 × 20 × 0.4 cm were cleaned with chromic acid and washed successively with tap and distilled water. The slurry of silica gel G was applied uniformily at a thickness of 0.25 mm. The coated plates were air-dried at 25° for 16 h, heated in an oven for 30 min at 110°, cooled and stored over silica gel. The alcohols of high purity originated from Union Carbide and Carbon Co., Stephan Chemical Co., Eastman Kodak Co., Armour and Co. and Air Reduction Chemical Co. The last source supplied 2-methyl-3-butyn-2-ol and 3-methyl-1-pentyn-3-ol, boiling at 103–104° and 120–121°, respectively.

A series of alcohol mixtures was prepared containing equivalent concentrations of each component and the total volume of the solutions was kept constant. The samples in amount of 5 μl were applied with a microsyringe 2.0 cm from the lower edge of the plate and dried by air blower. Ascending development was affected at

TABLE I

SEPARATION OF ALCOHOL MIXTURES BY TLC

Mixture	*Solvent system*	*Sequence of resolution*[a]				
		A	*B*	*C*	*D*	*E*
I	95% Ethanol[b]	1-Tetradecanol (0.93)	1-Octadecanol (0.84)	1-Heptadecanol[c] (0.84)		
II	1-Butanol saturated with water	2-Methyl-3-butyn-2-ol (0.88)	3-Methyl-1-pentyn-3-ol (0.76)	1-Hexadecanol (0.24)		
III	1-Butanol saturated with water	4,9,12-Trimethyl-7-pentadecanol (0.81)	Oleyl alcohol (0.70)	Ricinolyl alcohol (0.64)	1-Hexadecanol (0.25)	
IV	1-Butanol saturated with water	2-Ethylhexanol (0.89)	1-Decanol (0.74)	1-Tetradecanol (0.67)	1-Hexadecanol (0.25)	1-Octadecanol[d] (0.00)
V	1-Butanol saturated with water	2-Methyl-3-butyn-2-ol (0.87)	3-Methyl-1-pentyn-3-ol (0.75)	1-Dodecanol (0.64)	1-Heptadecanol (0.57)	
VI	1-Butanol saturated with water	Tetrahydrofurfuryl alcohol (0.93)	1-Dodecanol (0.64)	1-Octadecanol (0.00)		
VII	1-Butanol + 2% NH_4OH[b]	3-Methyl-1-pentyn-3-ol (0.90)	D-Borneol (0.78)	1-Hexadecanol (0.09)		
VIII	Water[b,e]	Tetrahydrofurfuryl alcohol (0.53)	Phenylethyl alcohol (0.34)			

[a] Position of the spot or zone in relation to the point of application, the furthermost one being designated as A. Average R_F values appear in parentheses and the standard deviation was $\pm$ 0.02 in each case.

[b] No satisfactory separation occurred with 1-butanol saturated with water as solvent.

[c] Sequence as indicated or virtually of the same R_F as 1-octadecanol; trailing was very prominent with the latter.

[d] This component did not migrate.

[e] Although resolution was rather poor, the above sequence is indicated.

24° in a glass chamber equilibrated at least 8 h previously with the solvent. The plates were then dried and the spots located by spraying with concentrated sulfuric acid containing 0.5 % potassium dichromate. The spots were identified by use of reference standards. The respective findings are presented in Table I. Generally sharp separations resulted, except for mixtures I and VIII. Of great interest is the observation that the sequence of separation of components appeared to parallel the respective boiling points of the alcohols.

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Electrophoresis and detection of proteins on thin layers of alumina

The usefulness of electrophoretic techniques for separation of proteins is well-established. In recent years chromatography and electrophoresis on thin layers have been used to advantage in separations of a great variety of organic and inorganic compounds. Thin-layer techniques are frequently favored over chromatography or electrophoresis on, for example, paper because they are far faster and permit a wide variation in composition of supporting medium. Thin-layer chromatography has been used to some extent on proteins, but the only solid supports that have been used are hydroxyl-apatite[1] and various forms of Sephadex[2–7] (Pharmacia, Uppsala, Sweden), a gel filtration medium composed of cross-linked dextran. We are not aware of any published reports of electrophoresis of proteins on thin layers.

A major difficulty in the application of thin-layer techniques to proteins has been the locating of the proteins on the finished plates[6]. Conventional methods for staining proteins on paper strips[8] include steps involving washings, and are therefore inapplicable to thin-layer plates. MORRIS[6] has succeeded in circumventing this problem by overlaying the developed plates with filter paper, but a simpler method is clearly desirable. One method is outlined in this report.

Experimental

Glass plates (20 × 20 cm) were coated with alumina (aluminum oxide G, Stahl) thin layers by the usual methods. These were equilibrated with 0.1 *M* phosphate buffer (pH 7.7), and spotted with 2–10 µg of trypsin, α-chymotrypsin, or bovine