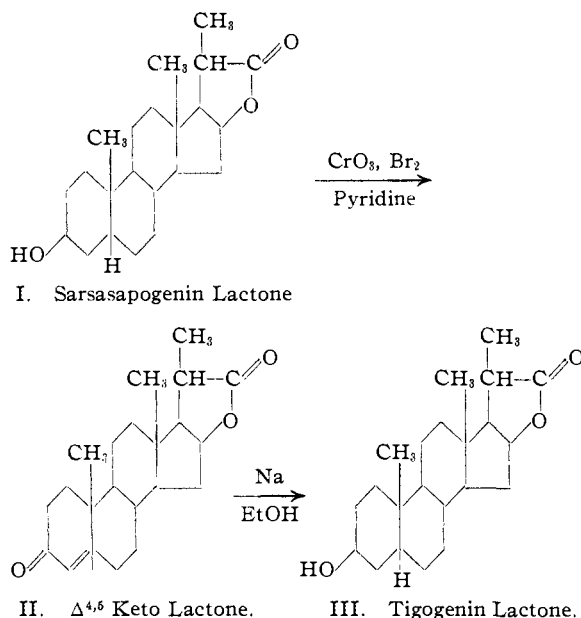


between positions at C-2 and C-3 for the hydroxyl group of tigogenin and sarsasapogenin. Likewise, it has not been shown that the hydroxyl group occupies the same position in both compounds. We have converted the hydroxy sarsasapogenin lactone into its *allo* form and find that this is identical with the hydroxy tigogenin lactone. This transformation indicates that the sarsasapogenin lactone differs from the tigogenin lactone only in regard to the configuration at C₅ and that the hydroxyl groups are located at C-3.



The keto lactone of sarsasapogenin first described by Farmer and Kon¹ was caused to react with one mole of bromine to give a bromo keto lactone, m. p. 194–195°. *Anal.* Calcd. for C₂₂H₃₁O₃Br: C, 62.4; H, 7.4. Found: C, 62.3; H, 7.5. Treatment of this compound with boiling pyridine gave an unsaturated keto lactone, m. p. 213–214°. *Anal.* Calcd. for C₂₂H₃₀O₃: C, 77.15; H, 8.8. Found: C, 77.1; H, 8.9. This was reduced with sodium and ethanol and the epimeric forms separated with digitonin. Decomposition of the insoluble digitonide yielded a hydroxy lactone, m. p. 234–235°. *Anal.* Calcd. for C₂₂H₃₄O₃: C, 76.25; H, 9.9. Found: C, 76.3; H, 10.0. This formed an acetate, m. p. 217–218°, which gave no depression with an authentic sample of tigogenin lactone acetate, m. p. 217–218°, first described by Tschesche and Hagedorn.² *Anal.* Calcd. for C₂₄H₃₆O₄: C, 74.15; H, 9.4. Found: C, 73.9; H, 9.3. Mild oxidation of the lactone with chromic anhydride gave a keto lactone, m. p. 252–254°. *Anal.* Calcd. for C₂₂H₃₂O₃: C, 76.7; H, 9.4. Found: C, 76.7; H, 9.6.

We wish to thank Parke, Davis and Company for their

(1) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).

(2) Tschesche and Hagedorn, *Ber.*, 68, 1412 (1935).

generous help and assistance in the various phases of this work.

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On the Combination of Electrophoretic and Chromatographic Adsorption Methods

BY HAROLD H. STRAIN

Resolution of mixtures of water-soluble compounds on Tswett adsorption columns¹ is facilitated by application of electrical potential to the ends of the columns. This combination of chromatographic and electrophoretic methods extends and enhances the utility of both methods for the isolation of pure substances from mixtures.

Colored substances are separated upon adsorption columns by electrophoresis in the following manner. An electrode of coiled wire covered loosely with cotton is placed in the constricted end of a glass tube and the tube is filled with the adsorbent, usually cotton, Hyflo Super Cel (heat-treated siliceous earth) or mixtures of this with talc. Before use, the adsorption column is attached to a suction flask and filled with water. A little of the mixture of charged or ionized colored substances to be separated is then drawn into the upper portion of the column where it forms a rather homogeneous colored band. The suction is released; a second electrode is placed at the top of the column and covered with water; and potential is applied to the two electrodes. This causes the pigments to migrate toward the electrodes of opposite charge and gives rise to a series of colored bands or zones each of which contains a single pigment.

Compounds separated upon adsorption columns by electrophoresis may be isolated in two ways. The bands of colored substances may be removed separately from the column and the pigments themselves eluted from the adsorbent with additional water, ethanol, alkalis or acids, or the pigments may be washed through the columns and collected separately in successive portions of the percolate.

In striking contrast to the effect obtained in the usual electrophoretic experiments where the bands of colored substances migrate with little or no widening, the materials on Tswett columns spread out rapidly until rather homogeneous colored zones are obtained. The pigments in

(1) M. Tswett, *Ber. deut. botan. Ges.*, 24, 384 (1906).

these regions appear to migrate along the surface of the adsorbent. This latter phenomenon was not observed in columns filled with weakly adsorptive, impervious materials such as sand and small glass beads.

The width of the bands of adsorbed substances formed in columns by electrophoresis depends upon the quantity of pigment present and upon the adsorptive capacity of the adsorbent. In experiments at room temperature with large columns, the bands of migrating pigments always exhibit well-defined boundaries throughout the mass of the adsorbent. The adsorbent thus serves to prevent mixing of the colored substances as well as to aid in their separation.

A pronounced electro-osmotic effect has been observed in all the experiments carried out thus far. Since the water usually moves toward the cathode, this electrode is placed at the top of the column so that the head of water may be adjusted to balance the electro-osmotic pressure. Passage of the water through the adsorbent also may be regulated, as customary in the Tswett method, by application of suction to the base or of pressure to the top of the column.

A great number of colored, water-soluble, organic substances such as nitro, hydroxy, amino, azo, carboxy and sulfonic compounds can be isolated from mixtures by electrophoresis upon Tswett columns. Combination of colorless organic compounds with colored, water-soluble radicals before adsorption would doubtless facilitate the separation of mixtures of colorless substances by the electrophoretic and chromatographic methods.²

Thus far, compounds separable by electrophoresis but inseparable by chromatographic adsorption have not been found. However, with columns of the same size and with equal quantities of pigments, electrophoresis often produces greater separation of the pigments than percolation alone.

The mixtures separated on Tswett columns by electrophoresis (the first compound named in each group remaining nearest the cathode) were: aminoazobenzene and indigo carmine, 3-nitro-4-aminoanisole and indigo carmine, methyl orange and methyl red, methyl orange and 2,6-dichlorophenol indophenol, methyl orange and methyl red and indigo carmine, picric acid and methyl orange. For the separation of these compounds, potentials ranging from 175 to 200 volts and

(2) H. H. Strain, *THIS JOURNAL*, **57**, 758 (1935).

currents from 0.5 to 2 m. a. were used with columns ranging in size from 2 by 13 cm. to 3 by 23 cm. The quantity of each pigment adsorbed varied from 2 to 15 mg.

From weakly acid solutions, two forms of the indicators were separated on Tswett columns both by chromatographic adsorption and by electrophoresis. The forms present in acid solutions were much more strongly adsorbed than those present in weakly alkaline solutions. Indicators adsorbed from acid solutions were gradually converted into the isomers observed in alkaline solutions upon prolonged washing of the columns with water or upon extended passage of the electric current. These methods provide another means for investigation of the tautomerism of indicators.

Resolution of mixtures by adsorption upon Tswett columns depends upon the properties of the adsorbent, of the solvent and of the compounds in the mixture. Since the properties of the adsorbent vary with the method employed in its preparation and since the properties of the solvent are altered by small quantities of impurities, considerable experience is often required to adapt this effective adsorption technique to the isolation of new compounds from mixtures.

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The Temperature of Maximum Density of D₂O and of its Mixtures with H₂O

BY ELIJAH SWIFT, JR.

The temperature of maximum density of deuterium oxide has been reported as 11.4¹ and 11.6^o,² the latter being the generally accepted value. This measurement was made on a sample of only 0.12 ml. volume in a small dilatometer, and the temperature taken as the temperature of maximum density was apparently that at which the meniscus was at its minimum position in the capillary. A temperature obtained in this manner is not the true temperature of maximum density, but what might be termed *the apparent temperature of maximum density in glass*, the difference between the two amounting to about 0.5^o in the case of Pyrex.³ It was hoped that a rede-

(1) T. Takeuchi and T. Inai, *Japan J. Phys.*, **11**, 67 (1936).
(2) G. N. Lewis and R. T. Macdonald, *THIS JOURNAL*, **55**, 3057 (1933).
(3) "Pyrex" brand chemical glass.