falling of the iron through the mantle generates a large amount of heat, and if there is a sharp layer some distance below the crust of the earth, perhaps at the Rapetti discontinuity, high temperature silicate phase is generated at depth. This leads to convection in the outer mantle of the earth and the formation of mountains, as has been postulated by Griggs. The forces are adequate to account for the formation of mountains and roughly the time of convection estimated is approximately that required by geological evidence, being somewhat too rapid, using a viscosity of 10^{22} poise for the outer earth.

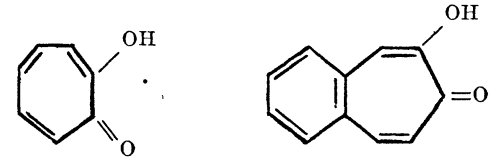
The chemical differentiation in the crust can be explained as separation of olivene from other constituents by sinking in a molten magma at a temperature in the neighborhood of 1500–1600° A. This temperature also is appropriate for explaining the constituents of the atmospheres of Mars, Venus, and the earth on the basis of chemical reactions taking place at this temperature and below. The difference in density of the moon and the earth may be explained if low density material condensed first and higher density material (iron) later. This hypothesis leads to an initial structure of the earth with a core of moon-like material surrounded by a layer of silicate and iron phase. If this central core remained at the center of the earth for some time, then on rising to the surface it should have produced the Pacific Basin, with its floor of basaltic rock. It is interesting to speculate upon what time in the earth's geological history this may have occurred.

Studies on the Structure of Colchicine[6]

D. S. Tarbell, ***Department of Chemistry, University of Rochester***

(Introduced by W. A. Noyes, Jr.)

The alkaloid colchicine is of biological interest because of its antimitotic action, but its chemical structure is not completely elucidated. Evidence will be presented to support the suggestion of Dewar that colchicine contains the "tropolone" structure (I) in ring C. This evidence



I II

is based on periodic acid oxidation of hexahydrocolchiceine, and on the study of infrared absorption spectra of colchicine derivatives and other compounds known to contain the tropolone ring. The synthesis of simpler compounds containing the tropolone ring has been studied, and a compound believed to be benztropolone (II) has been obtained by condensation of hydroxyacetone with phthalaldehyde; a by-product in this condensation has been proved to be phthiocol (2-hydroxy-3-methyl-1,4-naphthoquinone). The chemical behavior of II will be discussed in relation to that of the colchicine series.

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TECHNICAL PAPERS

General Method for Paper Chromatographic Analysis of Reducing and Nonreducing Carbohydrates and Derivatives

E. Pacsu, T. P. Mora, and P. W. Kent

Frick Chemical Laboratory, Princeton University, Princeton, New Jersey

The paper chromatographic procedure for the investigation of reducing sugars (*3, 4*) or their methylated derivatives (*1, 2*) depends for its success on the ease with which the substances under test will reduce certain inorganic ions at elevated temperatures. It has been found that not all ketosugars or disaccharides will bring about reduction. Efforts have been made to find a more general method of developing chromatograms in order to detect these nonreducing substances. It was proposed by Partridge (*4*) that acidic naphthoresorcinol could be used to detect such carbohydrates. In the course of investigation in this laboratory of complex mixtures of these substances, we have found the use of acidic reagents and of excessive drying undesirable. Experiment showed that excellent results can be obtained by developing the chromatograms with aqueous potassium permanganate (1%) containing sodium carbonate (2%). The chromatograms were removed from the chamber and dried at room temperature, or rapidly at 100° C. By spraying with the permanganate reagent, the bands appeared, with varying rapidity, as striking yellow zones on purple background, even at room temperature. In a short time, as the chromatograms dried, the spots took up their final color as gray regions on a brown background. In view of this change of color, it was expedient to mark the position of each band as soon as it was developed.

Using the partitioning solvents as specified by Partridge (*3, 4*)—viz., butanol (40%), ethanol (10%), water (50%)—it was possible to separate sugar alcohols, glycosides, and reducing and nonreducing sugars. Attempts to separate acetylated or partly methylated sugars using this modified procedure were not successful, since these substances did not readily reduce permanganate.

In an attempt to obtain a better separation of trisaccharides and the higher sugars, the usual solvent mixture was reversed, i.e., the aqueous layer was used as the eluting solvent in place of the usual butanol layer. Some separation was indeed obtained, but this was ac-

companied by a considerable spreading of the bands. However, some substances, e.g., streptomycin, which were immobile in the Partridge procedure, migrated rapidly and could be readily developed by the permanganate reagent. Further investigation of this approach would be profitable.

TABLE 1

R_f VALUES DETERMINED ON WHATMAN NO. 1 PAPER, USING BUTANOL LAYER AS THE ELUTING SOLVENT (DOWNWARD MIGRATION). TEMPERATURE ~ 25° C.

Name of compound	R_f value
α-Methyl glucoside	0.35
α-Glucose penta-acetate*	0.969
Mannitol	0.08
Sucrose	0.02
Melibiose	0.009
Lactose	0.013
Melezitose	0.01
Glucosamine	0.052
n-Carbobenzoxy-2-amino-α-methyl glucoside*	0.816
Methyl xylofuranoside	0.449
Salicin	0.526
Turanose	0.042
Potassium glucose-1-phosphate	0.003
D-Ribose	0.269

* Test substance was applied to the paper in ethanolic solution and dried before placing in the chamber.

In Table 1 are shown the R_f values of a series of compounds determined by using potassium permanganate.

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Paper Chromatography of Proteins and Enzymes[1]

A. E. Franklin[2] and J. H. Quastel

Montreal General Hospital Research Institute, Montreal, Canada

The original work of Consden, Gordon, and Martin (*1*) on the paper chromatography of substances of biological importance has been extensively developed, and a large literature has accumulated which cannot be adequately summarized here. So far as we are aware, however, there is no report of the separation of protein molecules by paper chromatography.

We wish to report preliminary results of an investigation of the paper chromatography of proteins and enzymes. Our work shows that it is possible to study the movements of proteins on paper, and to determine the conditions under which proteins may be separated by this means. It is also possible by the same means to bring about separation of the components of enzyme mixtures, and to study enzyme activity, manometrically, on paper.

Our work has been greatly facilitated by the use of hemin as a "marker," the presence of a protein-hemin complex on paper being easily shown by the benzidine-hydrogen peroxide reagent. We employ buffer and aqueous salt solutions, instead of nonaqueous solvents, as developing agents.

The technique employed is that of Williams and Kirby (*2*), which involves chromatography by capillary ascent. Whatman No. 1 sheet filter paper has been used throughout this work. When drying is complete, the paper is streaked with freshly prepared benzidine reagent, using a small paint brush. Color development is immediate and intense, and should be photographed almost immediately, as a background color develops gradually and this tends to obscure the color from the protein-hemin complex.

Protein solutions. Ten mg crystalline protein is dissolved in 1 ml of distilled water or saline and to this is added 0.02 ml of 2.0% hemin, which has been dissolved in 3% sodium bicarbonate solution. Aliquots of 0.01–0.02 ml are used for chromatography.

Developing solutions. A search for suitable developing solutions has resulted in the exclusive use of aqueous solutions of salts and buffers. For buffers, 50 ml of a M/5 solution of the salt is adjusted to the desired pH with a *N*/5 solution of HCl or NaOH and the volume is made up to 200 ml. In the case of acetate-HCl buffers, normal solutions are used. Salt solution concentrations are usually in the region of 1–2%.

The use of organic solvents gave unsatisfactory results. *n*-Butanol saturated with water allowed extremely little movement of proteins and favored the formation of an immediate, deeply colored background on application of the benzidine reagent. Aqueous solutions of ethylene glycol, propylene glycol, ethanol, or acetone induced maximum or near maximum movement. All proteins tested moved about the same distance under these conditions.

Benzidine reagent. The preparation is made as follows: Equal volumes of saturated alcoholic benzidine solution and 3% hydrogen peroxide are mixed and made acid with glacial acetic acid. Fresh reagent must be prepared daily. Poor color development results if the hydrogen peroxide stock solution has decreased in strength.

Color development with protein-hemin complexes is generally blue, but may be purple, brown, or green. Some salts, e.g., magnesium sulfate and sodium phosphate, interfere with color development. Citrate, glucose, trisodium phosphate, and potassium hydrogen phthalate at some pH values give rise to a deep purple color.

Background color development varies considerably, depending on the ease with which the benzidine reagent is oxidized. Acid pH values tend to retard background color development, while alkaline pH values give rise to a deeply colored background. Photographs are made of the chromatograms at the optimal development of color. It is necessary to photograph the chromatogram while the paper is still damp.

Hemin. A stock solution of 2% hemin in 3% sodium bicarbonate solution was prepared and 0.02 ml was added to 1 ml of the protein solution. In the proteins studied,

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[2] Holder of a Canada Packers Research Fellowship of McGill University.