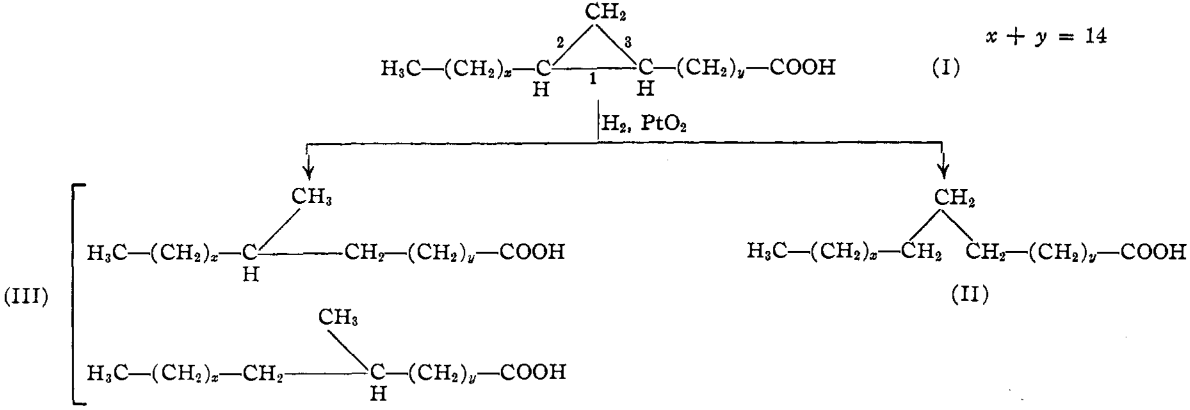
to give two new materials. One of these, a solid fatty acid melting at 68–68.5°, was, by mixed-melting point determinations and X-ray diffraction studies, unequivocally identified as nonadecanoic acid (II). The other melted at 13–14° and gave analytical figures in agreement with a composition of $C_{19}H_{38}O_2$ (*Anal.* Calcd.: C, 76.44; H, 12.83; neut. eq., 298; C-methyl, 2.0. Found: C, 76.29; H, 12.32; neut. eq., 301; C-methyl, 1.3) (tribromoanilide, m. p. 90–94°). The low melting point of this branched-chain, saturated C_{19} acid fraction suggests the presence of a mixture of methyloctadecanoic acids (III).[3]

The behavior of (I), *i. e.*, (1) its stability toward oxidation; (2) its lability toward hydrogenation, and (3) the formation of at least two hydrogenation products, one of which contains a straight carbon chain (nonadecanoic acid), points to the presence of a cyclopropane ring. The infrared absorption maximum at 9.8μ supports this conclusion[4]. As a working hypothesis we propose structure (I) for this fatty acid. Such a formula could well explain the experimental results presented. Thus its hydrogenation could yield the observed hydrogenation products through hydrogenolysis of bonds 1, 2 or 3 in the manner illustrated.



Liquid fraction, m. p. 13–14°

Solid acid m. p. 68–68.5°

Further work on the exact location of the cyclopropane ring as well as on the synthesis of acids of the general structure (I) is presently underway in this laboratory.

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[3] Cason and Winans, *J. Org. Chem.*, **15**, 139 (1950).

[4] Derfer, Pickett and Boord, THIS JOURNAL, **71**, 2482 (1949).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PENNSYLVANIA
KLAUS HOFMANN
ROBERT A. LUCAS

RECEIVED JULY 13, 1950

ELECTROMIGRATION ON FILTER PAPER[1]

Sir:

The recent publication of Cremer and Tiselius[2] of experiments on the electrophoresis of proteins in filter paper prompts us to report similar experiments on the electromigration of inorganic ions. Filter paper electromigration appears to be a rapid method for the determination of the sign of the charge of complex ions and for obtaining estimates of the stability constants of complexes.

The technique consists of moistening a strip of filter paper (E.D. No. 627-030; dimensions, 11 × 3 inches) with the supporting electrolyte, blotting off excess, placing a number of one microliter aliquots of solutions of the ions in question near the center of the paper and measuring the displacement (spot test developing) after applying a known d.c. voltage for a known period of time. The non-polarizable electrodes (silver strips for chloride solutions) are clamped to the paper, whose center portion is cut to *ca.* $^1/_3$ of the total width to increase the voltage drop in the vicinity of the test spots and to provide electrode compartments of fair capacity. For cooling the paper is placed on a 1″ insulated copper block. Temperature is measured with a small thermocouple. Evaporation is reduced by covering the paper with a glass plate to which narrow silver strips are cemented to permit measurement of the voltage drop in the vicinity of the spots. Depending on the conductivity of the solution voltage drops of 3–10 volts/cm. in the test region can be used without rise in temperature of more than 1–2° permitting completion of experiment in as little as 10 to 30 minutes. The extent of water movement primarily due to electro-endosmosis is estimated from the displacement of an "indicator" of zero charge. All data are corrected for adsorption on filter paper by determining the *R* values by standard paper chromatography.

Typical series of results at 26 ± 1° for mercuric ions and, for comparison of iodide ions, in chloride solutions are shown in Fig. 1. The experiments were carried out with hydrochloric acid except for *M* $Cl^- \leq 0.5$ where Cl^--ClO_4^- mixtures of ionic strength 0.5 containing 0.1 *M* H_3O^+ were used. While the mobility (sq. cm. min.$^{-1}$ volt^{-1}) of iodide ions remained practically constant and independent of chloride concentration that of

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[1] This document is based on work performed for the Atomic Energy Committee at the Oak Ridge National Laboratory.

[2] H. Cremer and A. Tiselius, *Biochem. Z.*, **320**, 273 (1950).

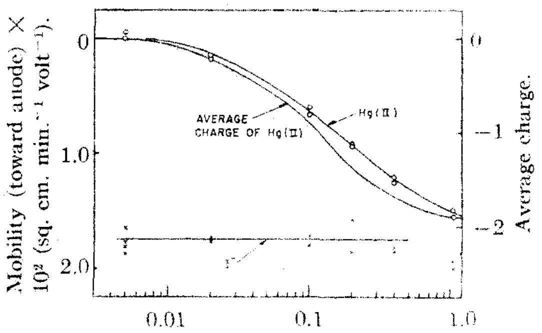

Fig. 1.—Filter paper electromigration of ions.

Hg(II) was practically zero at low *M* Cl^- and increased rapidly in the region 0.1 to 1 *M* Cl^-. The mobility of Hg(II) is roughly paralleled by the calculated values of the average charge of the Hg(II) ions using the data of Sillén.[3] Thus, if data on the relative mobility of complex ions of different charge can be obtained, this technique may become generally applicable for rapid determination of estimates of stability constants of complex ions.

Acknowledgment.—We are indebted to Dr. H. H. Shain for a number of valuable discussions and for informing us of the details of his work on the continuous separation of ions by filter-paper electromigration.

(3) L. G. Sillén, *Acta Chem. Scand.*, **3**, 539 (1949).

OAK RIDGE NATIONAL LABORATORY KURT A. KRAUS
OAK RIDGE, TENNESSEE GILBERT W. SMITH

RECEIVED JULY 20, 1950

PAPER CHROMATOGRAPHY OF STEROIDS[1]

Sir:

The separation of cholesterol and cholestenone has been achieved by using paper impregnated with "Quilon"[2] as the stationary phase and simple primary alcohols as solvents. In effect, the stationary phase consists of the stearic acid residues.

Use of paper impregnated with rubber latex,[3] silicic acid[4] and alumina[5] in paper chromatography has been reported. Of these, alumina paper was tried and found to give erratic results. The method of Zaffaroni and co-workers[6] for the paper chromatography of steroids using paper saturated with formamide or propylene glycol as the stationary phase and a hydrocarbon solvent was also tried. In these experiments the steroids were found to move with the front. Using ordinary paper (Whatman No. 1), cholesterol was found either to move with the solvent front or remain at the origin.

For ease of location, tritiated cholesterol was used and the material located by scanning the paper with a windowless counting tube designed to locate weakly radiating substances on paper.[7]

The presence of cholesterol at the points of high activity was confirmed by the red color developed after papers treated with a solution of silicotungstic acid were dried.[8] Cholestenone gave an olive green color with this reagent, but only when the steroid was present in relatively large amounts. Cholestenone was most easily detected by the yellow color obtained with a reagent consisting of a solution of iodine and potassium iodide in water.[9]

The most satisfactory solvents, to date, have been methanol, ethanol and ethanol–water 8:2. The latter solvent gives the best separation of cholesterol and cholestenone. The results are collected in Table I.

TABLE I

Solvent	Cholesterol (R_f)	Cholestenone (R_f)
Methanol	0.56	0.77
Ethanol	.92	.97
80% Ethanol	.52	.86

All experiments were carried out as descending chromatograms using 1.5 × 15 inch strips of the impregnated paper. The paper was usually wet to a distance of about 25 cm. from the origin. R_f values were measured from the farthest point of the origin and the foremost point of the colored or active zone.

Projected work includes widening the range of usable solvents, development of supplementary color reactions and extension of this method to other steroids.

(1) The work described in this paper was sponsored by the Atomic Energy Commission.

(2) Stearato chromic chloride, generously supplied by E. I. du Pont de Nemours and Co., Inc.

(3) Boldingh, *Experientia*, **4**, 270 (1948).

(4) Kirchner and Keller, THIS JOURNAL, **72**, 1867 (1950).

(5) Datta and Overell, *Biochem. J.*, **44**, xliii (1949).

(6) Zaffaroni, Burton and Keutmann, *Science*, **111**, 6 (1950).

(7) Gray, Ikeda, Benson and Kritchevsky, *Rev. Sci. Inst.*, in press.

(8) Montignie, *Bull. soc. chim.*, **51**, 690 (1932).

(9) Munier and Macheboeuf, *Bull. soc. chim. Biol.*, **31**, 1144 (1949).

RADIATION LABORATORY AND
DEPARTMENT OF CHEMISTRY DAVID KRITCHEVSKY
UNIVERSITY OF CALIFORNIA MELVIN CALVIN
BERKELEY 4, CALIFORNIA

RECEIVED JULY 31, 1950

COLCHICINE. NATURE OF THE B-RING[1,2]

Sir:

The structure of deaminocolchinol methyl ether has been established by J. W. Cook[3,4] as 9,12,13,14-tetramethoxy-3,4,5,6-dibenzocycloheptatriene-1,3,5. This compound, together with isodeaminocolchinol methyl ether, may be ob-

(1) The work carried out at the University of Pennsylvania was aided by a Grant-in-Aid from the American Cancer Society recommended by the Committee on Growth of the National Research Council.

(2) This investigation was supported (in part) by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(3) Barton, Cook and Loudon, *J. Chem. Soc.*, 176 (1945).

(4) Buchanan, Cook, Loudon and MacMillan, *Nature*, **162**, 692 (1948).