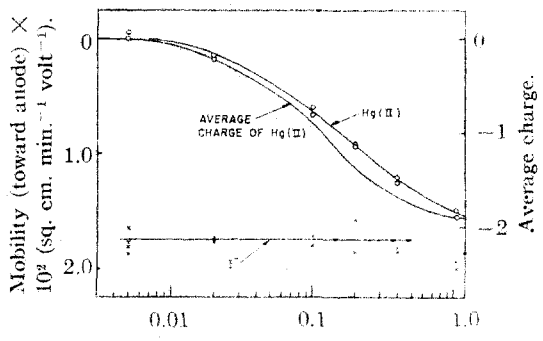

Fig. 1.—Filter paper electromigration of ions.

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).

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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.

(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).

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.

(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).

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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).

tained[3,5] by the elimination of acetamide from N-acetylcolchinol methyl ether (IA), and from the phosphorus pentoxide dehydration of a carbinol obtained by treatment of colchinol methyl ether (IB) with aqueous nitrous acid.[3,6] A seven-membered B-ring structure was also proposed by Tarbell[7] for iododeaminocolchinol methyl ether.

The ultraviolet absorption spectra of dihydrodeaminocolchinol methyl ether (IC; m. p. 97–97.5°; reported[3] 97°), N-acetylcolchinol methyl ether (Ia; m. p. 204–205°; reported[8] 199°; *Anal.* Calcd. $C_{21}H_{25}O_5N$; C, 67.89; H, 6.78. Found: C, 67.85; H, 6.61) and colchinol methyl ether (IB; m. p. of the hydrochloride 253–255° dec.; reported[8] about 254° dec.) are shown in Fig. 1. After acidification of the basic solution of colchinol methyl ether (addition of 1 ml. of 1 *N* sulfuric acid to 25 ml.) the absorption was virtually identical with that of I. This indicates that acetylamino- or amino-substitution on the B-ring does not significantly influence the ultraviolet absorption. The possibility that one of the nitrogen-containing compounds might have a six-membered B-ring (a 2,3,4,7-tetramethoxy-9,10-dihydrophenanthrene structure) is rather remote, in view of the close relationship shown here between the parent compound and the other members of the series. Accordingly, a seven-membered B-ring system appears to be established throughout the colchinol series. Colchicine can be hydrolyzed to trimethylcolchicinic acid and reconverted into colchicine by N-acetylation and O-methylation[9]; since the acetyl group of IA may be hydrolyzed without alteration of the B-ring, it would appear that the seven-membered B-ring is also present in colchicine.

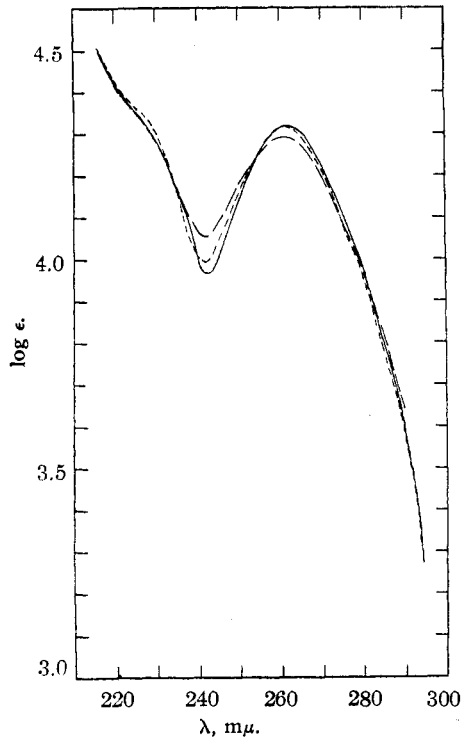
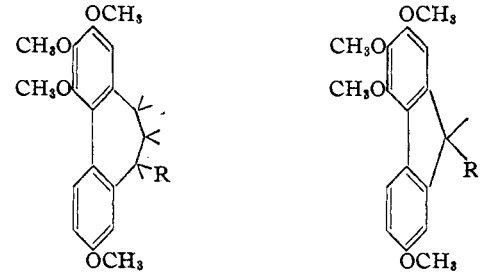


Fig. 1.— —— N-Acetylcolchinol methyl ether, 5.22 × 10^{-5} *M* in absolute ethanol; ····· colchinol methyl ether (hydrochloride, 2.60 × 10^{-5} *M* in 26:24 ethanol-water, 0.04 *M* in NaOH): ..... dihydrodeaminocolchinol methyl ether, 5.16 × 10^{-5} *M* in absolute ethanol.



IA, R = $NHCOCH_3$
IB, R = NH_2
IC, R = H

IIA, R = CH_2CH_2OH
IIB, R = H

The absorption spectrum of Cook's carbinol[6] (Fig. 2) indicates that this alcohol is not derived from the ring system represented by I. A rearrangement very likely occurred during the nitrous acid reaction on IB, but it is not yet possible to assign a structure to the carbinol. The

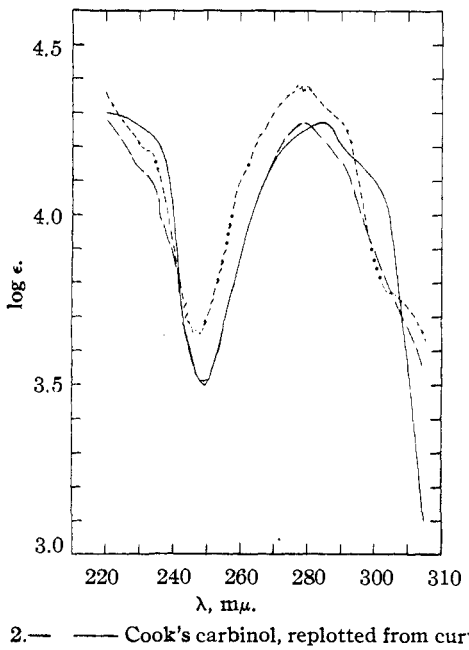


Fig. 2.— —— Cook's carbinol, replotted from curve of ref. 6; ------- 2,3,4,7-tetramethoxy-9-(β-hydroxyethyl)-fluorene, 0.60 × 10^{-5} *M* in absolute ethanol: ..... 2,3,4,7-tetramethoxyfluorene, 1.08 × 10^{-5} *M* in absolute ethanol.

(5) Cook, Graham, Cohen, Lapsley and Lawrence, *J. Chem. Soc.*, 322 (1944).

(6) Cohen, Cook and Roe, *ibid.*, 194 (1940).

(7) Tarbell, Frank and Fanta, THIS JOURNAL, **68**, 502 (1946).

(8) Windaus, *Sitzungsber. Heidelberg. Akad. Wiss., Math.-Nat. Kl.*, A, 18 Abh. (1914); 16 Abh. (1919).

(9) Johanny and Zeisel, *Monatsh.*, **9**, 865 (1888).

spectra of 2,3,4,7-tetramethoxy-9-(β-hydroxyethyl)-fluorene (IIA) (m. p. 100–101°; *Anal.* Calcd. for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 69.05; H, 6.74) and 2,3,4,7-tetramethoxyfluorene (IIB) (m. p. 97–97.5°; *Anal.* Calcd. for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.30; H, 6.65) are included for comparison.

There is a considerable body of evidence[10] indicating that non-planarity in biphenyls, induced by blocking effects of substituted groups, results in decreased and changed absorption characteristics over the range 240–260 mμ. Very few compounds related to IC are available for study, but the spectral characteristics found here in the colchinol series suggest that the three-membered bridge does not introduce a major hindrance to the assumption of co-planarity by the A-C rings in the colchinol series.

Following the submission of this communication, Rapoport, Williams and Cisney confirmed Cook's structure for dihydrodeaminocolchinol methyl ether by synthesis; abstract 59, Division of Organic Chemistry, Phila. Meeting of the American Chemical Society, April 9–14, 1950. In presenting the paper, Dr. Rapoport announced conclusions on the basis of spectral data that racemic colchinol methyl ether had been synthesized and except for configuration was identical with the natural material.

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RECEIVED APRIL 6, 1950

(10) (a) Pickett, Groth, Duckworth and Cunliffe, THIS JOURNAL, **72**, 44 (1950); (b) O'Shaughnessy and Rodebush, *ibid.*, **62**, 2906 (1940); (c) Williamson and Rodebush, *ibid.*, **63**, 3018 (1941); (d) Jones, *ibid.*, **63**, 1658 (1941).

(11) Research Associate.[2]

(12) American Cancer Society Postdoctoral Fellow.

(13) American Cancer Society Research Assistant.

(14) National Institutes of Health Predoctoral Fellow.

OCCURRENCE OF THE CITRIC ACID CYCLE IN TUMORS[1]

Sir:

In a survey of possible substrates which may contribute to the respiration of tumor tissues it has been found that fatty acids, such as palmitic or acetic, are oxidized by surviving slices of tumors about as readily as they are by normal tissues. The procedure used was to measure the radioactivity of the respiratory carbon dioxide produced by the tissue in the presence of 0.001 *M* sodium palmitate labeled in the carboxyl carbon with C^{14}, as described previously for normal tissue.[2] The results apply to three transplanted mouse tumors: a rhabdomyosarcoma and a mammary adenocarcinoma, both of which have been studied extensively in this Institute; and a hepatoma kindly supplied by Dr. Julius White of the National Cancer Institute. The pertinent data as given in Table I suggest that fatty acid oxidation may represent a major source of energy in tumors.

TABLE I

OXIDATION OF PALMITIC AND ACETIC ACIDS BY MOUSE TUMORS *in vitro*

Approximately 2 g. of tumor slices was used in 20 ml. of Ca-free Ringer phosphate. Experiments run 3–3.5 hours in oxygen at 38°.

Tissue	Substrate	Respiratory CO_2[a] μM.	Relative sp. Act.
Hepatoma	Palmitate,	450	4.5
Mammary	0.001 *M*	300	4.4
carcinoma	0.01 *M* Acetate, COOH-labeled	320	12.5
	0.01 *M* Acetate, CH_3-labeled	300	14.8
Rhabdomyosarcoma	Palmitate, 0.001 *M*	250	6.7
	0.01 *M* Acetate, COOH-labeled	210	4.0
	0.01 *M* Acetate, CH_3-labeled	210	4.4
Normal rat liver	Palmitate,	212	7.0
Normal rat kidney	0.001 *M*	425	7.5

[a] Relative specific activity is defined as: (sp. act. of resp. CO_2 (measured as $BaCO_3$) × 100)/(sp. act. of fatty acid (measured as $BaCO_3$)).

TABLE II

ACTIVITY OF QUINIDINE CITRATE

Experimental conditions as in Table I. Activities are counts per minute per 5 sq. cm. dish at "infinite thickness." Approximately 100 mg. of carrier citrate added in each experiment.

Tumor	Substrate	Quinidine citrate Amt. mg.	Quinidine citrate Activity cts./min.	Estimated activity of metabolic citrate Counts/min.
Mammary adeno-carcinoma	Acetate	151	293	4×10^4
	Palmitate	213	74	1×10^4
Hepatoma	Palmitate	135	138	.....

The citric acid cycle is the only process now known for the complete oxidation of fatty acids in normal tissues. Confirmation for the occurrence of this process in these tumors was obtained by isolating pure, radioactive quinidine citrate as a product of the oxidation of the labeled fatty acids (Table II). In the experiments with the mammary tumor 0.2 *M* *trans*-aconitate was added to cause accumulation of citrate[3]; in the experiment with hepatoma 0.01 *M* citrate was added to "trap" metabolic citrate. From the amounts of citrate expected in the presence of *trans*-aconitate and the amount of carrier added, it can be estimated that the metabolic citrate was of high specific activity and could have arisen

(1) Aided by grants from the U. S. Atomic Energy Commission, the American Cancer Society and the U. S. Public Health Service.

(2) Weinhouse, Millington and Volk, *J. Biol. Chem.*, **184**, 191 (1950).

(3) Advantage was taken of the observation of Saffran and Prado, *J. Biol. Chem.*, **180**, 1301 (1949), that *trans*-aconitate is an inhibitor of aconitase and causes accumulation of citrate in respiring tissues *in vitro*. This procedure has provided further evidence for the citric acid cycle in tumors, and will be reported separately.