UCRL- 1749

UNIVERSITY OF CALIFORNIA — BERKELEY

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

RADIATION LABORATORY

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

UCRL-1749
Unclassified-Chemistry Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

UNCLASSIFIED

PAPER CHROMOTOGRAPHY OF STEROIDS

David Kritchevsky and Martha R. Kirk

May 15, 1952

Berkeley, California

PAPER CHROMATOGRAPHY OF STEROIDS[1]

by

David Kritchevsky and Martha R. Kirk

ABSTRACT

The preparation and use of "Quilon" (stearato chromic chloride) impregnated paper for the reverse phase paper partition chromatography of steroids is described. The R_f values for a number of steroids in a variety of solvents are reported. Separation of cholesterol from epicholesterol, ergosterol and 7-dehydrocholesterol has been achieved. Several other separations are also reported.

For publication in the Journal of the American Chemical Society

---

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

PAPER CHROMATOGRAPHY OF STEROIDS[1]

by

David Kritchevsky and Martha R. Kirk

Through the use of impregnated filter paper it has been possible to achieve separation of various steroid mixtures by paper chromatography. The corticosteroids have been separated using papers treated with propylene glycol (2,3), formamide (2,3) of alumina (4,5); the estrogens on paper treated with alumina (4,5), glycerol (6), ethylene glycol (6) or capryl alcohol (6); and the androgens on papers impregnated with alumina (4,5) or a silicone (7). Recently, Neher and Wettstein (8) have reported the separation of weakly polar steroids on paper treated with phenyl cellosolve. The successful separation of cholesterol and cholestenone on paper impregnated with "Quilon" (stearato chromic chloride) has already been reported. (9). This method

---

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

(2) A. Zaffaroni, R. B. Burton and E. H. Keutmann, Science, 111, 6 (1950).

(3) A. Zaffaroni, R. B. Burton and E. H. Keutmann, J. Biol. Chem., 188, 763 (1951).

(4) I. E. Bush, Nature, 166, 445 (1950).

(5) I. E. Bush, Biochem. J., 50, 370 (1952).

(6) R. J. Boscott, Biochem. J., 48, xlvii (1951).

(7) T. H. Kritchevsky and A. Tiselius, Science, 114, 299 (1951).

(8) R. Neher and A. Wettstein, Helv. Chim. Acta, 35, 276 (1952).

(9) D. Kritchevsky and M. Calvin, J. Am. Chem. Soc., 72, 4330 (1950).

has also been used for the separations of Vitamins D_2 and D_3 from a mixture of sterols (10). This report covers the results obtained by application of this method to a number of steroids. The R_f values obtained with twenty-one steroids using a variety of solvents are tabulated in Tables I, III, IV, and V. Each R_f value represents the average of at least six separate chromatograms.

In the case of the weakly polar steroids, the separation of cholesterol from 7-dehydrocholesterol, ergosterol and epicholesterol has been accomplished. Stigmasterol and ergosterol have also been separated. Any two steroids whose R_f values are sufficiently far apart may be separated by this system, and, in the case of a mixture of cholesterol and testosterone, separation has indeed been carried out. The separations are summarized in Table II.

In the case of the corticosteroids and the androgens, the R_f values are reproducible but no resolution could be achieved with the solvent systems used. The salient feature of the data concerning these compounds is that the addition of water to the anhydrous solvent gives a higher R_f value rather than the lower one which might be expected. Several of the weakly polar steroids exhibit the expected lowering of R_f upon dilution of the methanol.

The only compounds which did not show R_f values in the neighborhood of 0.80 when methanol was the solvent were compounds that had a long hydrocarbon side chain and no conjugation in the ring system. Cholestenone, ergosterol and 7-dehydrocholesterol have a conjugated system in the A ring and all give R_f values similar to those obtained with the other classes of steroids used. In the case of

---

(10) R. B. Davis, J. M. McMahon and G. Kalnitsky, J. Am. Chem. Soc., 74, 000 (1952).

ergosterol, chromatography in methanol-water gave higher R_f values than those obtained using pure methanol. Variations in the side chain do not appear to effect the R_f values as may be seen from the values for cholesterol and stigmasterol.

It is possible that the systems under investigation are exceedingly sensitive to small changes in solvent composition and that the mixtures we have used have bracketed the area of greatest sensitivity. It may be seen from Table III that the R_f value of cortisone in methanol-water 9:1 is greater than it is in either methanol-water 95:5 or 85:15.

Small quantities of chromic chloride complexes with several other acids have been made available to us by the duPont Company. The complexes of furoic, salicylic and p-aminobenzoic acids were applied to the paper in the usual way, but the methanol seemed to wash much of the material out of the paper, a wide green zone appearing at the front in every experiment. The R_f values obtained in these experiments were very erratic. Glucono chromic chloride gave a paper stable to the solvent and the R_f values obtained with several compounds are listed in Table V. The R_f value of cholesterol on this paper is considerably higher than that for paper impregnated with stearato chromic chloride. The other R_f values are in the range that they were with the original Quilon paper, but show a somewhat greater variation in values.

Investigations involving smaller increments of change in the methanol-water ratios are underway using papers treated with stearato- and glucono chromic chloride respectively.

EXPERIMENTAL

Impregnation of Paper: Impregnation of the filter paper used (Whatman No. 1) could be carried out in either of two ways to give paper having the same chromatographic characteristics. A solution that was 2% in "Quilon" and 2% in the "neutralizer" solution suggested for use with this material (11) was sprayed on the paper and the paper dried at 100-110°C, or the paper was dipped into a trough containing the same solution, allowed to drain and dried in a similar fashion. In either of these operations the paper was supported on a rectangular wooden frame. Use of a solution that was 4% in "Quilon" and neutralizer gave a paper on which the R_f value of cholesterol was not altered. When a solution containing "Quilon" alone was used, the solvent seemed to wash the material from the paper and a green zone could be observed in the region of the solvent front. This paper gave erratic results.(*)

To determine the extent of impregnation of the paper by these methods, various samples of the treated paper were ashed and the residues assayed for chromium with the following results (12):

Sample	Cr(mg/25 cm.^{2})
Dipped, sol'n A	-0.26; 0.23
Dipped, sol'n B	0.24; 0.24
Sprayed, sol'n B	0.24; 0.22

Solutions A and B refer to solutions 2% in both "Quilon" and neutralizer which were prepared at different times.

---

(11) duPont Product Information Bulletin, "Quilon", January, 1950

(12) Analyses by Mr. V. Tashinian of the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

(*) The other chromic chloride complexes were all mixed with "neutralizer" prior to treatment of the paper.

Chromatography: Reagent grade solvents were used throughout. All ratios of solvents represent percentages by volume. In all cases 10 γ of material was applied to a spot 1-1/2 cm. in diameter on a strip of paper about 4 cm. wide. The papers were suspended from metal troughs in large test tubes (7 x 50 cm.) which contained a few cc. of the solvent mixture and which were sealed with rubber stoppers during the course of chromatography. Generally two papers were suspended from each trough. Descending chromatography was used throughout, the solvent being allowed to run 25-35 cm. from the origin. The papers were allowed to dry thoroughly before testing for the presence of the material being chromatographed. R_f values were measured from the foremost point of the origin and the leading portion of the spot.

As the precentage of water in the solvent was increased the time required for the solvent to run the specified distance was also greatly increased. The upper limit of dilution for methanol is about 20%. Methanol-water 75:25 takes very long to run and at lower temperatures does not wet the paper. Methanol-water 7:3 will not wet the paper. The dilution limit is somewhat higher with the higher alcohols.

Detection of Steroids: The methods available for the detection of steroids on "Quilon" treated paper have already been reported (13). In general, iodine vapor was used for detection of the androgens and progesterone; silicotungstic acid for cholesterol, epicholesterol, cholestanol, stigmasterol and cholestenone; antimony pentachloride for sitosterol, ergosterol and 7-dehydrocholesterol; and triphenyl tetrazolium chloride (3) for the corticosteroids. In some cases, several methods were used for

(13) D. Kritchevsky and M. R. Kirk, Arch. Biochem. Biophys. 35, 346 (1952).

detection of the same steroids and the same R_f values were obtained. With cholesterol-4-C^{14} the color tests were further confirmed by radioautography (14).

Acknowledgment: The authors are deeply grateful to Prof. Melvin Calvin for many helpful discussions during the course of this work. Thanks are also due the E. I. duPont de Nemours and Company, Inc. for their generous gifts of the various impregnating agents; to the Ciba Pharmaceutical Products, Inc. for the androgens; to Dr. E. C. Kendall for cortisone; to the Upjohn Company for the other corticosteroids; to Prof. W. G. Dauben for cholestanol and cholestenone; to Dr. J. F. Eastham for cholesterol-4-C^{14}; and to the Chemical Specialties Company, Inc. for the progesterone and pregnenolone used in these experiments.

---

(14) A. A. Benson, J. A. Bassham, M. Calvin, T.C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., 72, 1710 (1950).

TABLE I

R_f values of the weakly polar steroids

	CH_3OH	$CH_3OH \cdot H_2O$ 9:1	C_2H_5OH	$C_2H_5OH \cdot H_2O$ 8:2
Cholesterol	.56	.31	.92	.52
Epicholesterol	.80	--	--	.97
Cholestanol	.63	--	--	.56
Stigmasterol	.52	.27	--	.53
Sitosterol	.65	--	--	.54
Cholestenone	.82	--	.97	.86
7-dehydrocholesterol	.88	--	--	.94
Ergosterol	.84	.90	--	.95

TABLE II

SEPARATIONS IN METHANOL

Compounds	R_f Values
Cholesterol/ergosterol	0.52/0.84
Cholesterol/7-dehydrocholesterol	0.54/0.88
Cholesterol/epicholesterol	0.56/0.81
Cholesterol/testosterone	0.54/0.77
Stigmasterol/ergosterol	0.47/0.85

TABLE III

R_f Values of Corticosteroids

	CH_3OH	$CH_3OH.H_2O$ 95:5	CH_3OH-H_2O 9:1	CH_3OH-H_2O 85:15	CH_3OH-H_2O 8:2	C_2H_5OH
Cortisone	.75	.81	.86	.80	.80	.88
Desoxycorticosterone	.75	---	.85	---	---	.86
17-Hydroxycorticosterone	.73	---	.86	---	---	.88
Dehydrocorticosterone	.74	---	.83	---	---	.85
Corticosterone	.79	.83	.83	.80	.82	.83

	$C_2H_5OH-H_2O$ 8:2	CH_3OH-NH_4OH 9:1	$CH_3OH-NH_4OH-H_2O$ 85:5:15	$CH_3OH-HCOOH$ 99:1	$CH_3OH-HCOOH-H_2O$ 80:1:19
Cortisone	.94	.77	.94	.69	.91
Desoxycorticosterone	.95	.81	.97	.78	.92
17-Hydroxycortocosterone	.89	.79	.94	.73	.92
Dehydrocorticosterone	.95	.76	.95	.74	.92
Corticosterone	.91	.76	.97	.69	.92

TABLE IV

R_f Values of Androgens and Progestational Hormones

	CH_3OH	CH_3OH-H_2O 9:1	$CH_3OH-CHCl_3$ 9:1	$CH_3OH-CHCl_3$ 95:5	$CH_3OH-CCl_4$ 95:5
Dehydroisoepi-androsterone	.75	.87	.86	.82	.81
Dehydroisoepi-androsterone acetate	.76	.85	.87	.85	.86
Testosterone	.78	.83	.87	.83	.81
Testosterone Propionate	.81	.92	.87	.90	--
Androsterone	.80	.94	--	--	--
Progesterone	.80	.95	.97	--	--
Pregnandiol	.78	--	--	--	--
Pregnenolone	.75	--	--	--	--

	$CH_3OH-C_6H_6$ 99:1	$CH_3OH-C_6H_6$ 95:5	$CH_3OH-CHCl_3$ 1:9	CH_3OH-NH_4OH 95:5	C_2H_5OH
Dehydroisoepi-androsterone	.80	.87	1.00	.80	.97
Dehydroisoepi-androsterone acetate	.80	.88	1.00	.82	1.00
Testosterone	.82	.84	1.00	.82	1.00
Testosterone Propionate	.81	.85	1.00	.79	1.00
Androsterone	--	--	--	--	1.00
Progesterone	--	.86	.95	.78	--
Pregnandiol	--	--	--	--	--
Pregnenolone	--	--	--	--	--

TABLE IV (Cont'd)

R_f Values of Andorgens and Progestational Hormones

	$C_2H_5OH-H_2O$ 8:2	$C_2H_5OH-H_2O$ 7:3	$i-C_3H_7OH$	Dioxane
Dehydroisoepi-androsterone	.96	.96	.94	.99
Dehydroisoepi-androsterone acetate	.93	.97	.95	.98
Testosterone	.91	.98	.93	1.00
Testosterone Propionate	.97	1.00	.94	.96
Androsterone	.94	.95	--	.99
Progesterone	--	.98	.95	--
Pregnandiol	--	--	--	--
Pregnenolone	--	--	--	--

TABLE V

R_f Values on Glucono Chromic Chloride (Methanol Solvent)

Compound	R_f
Cholesterol	0.90
Progesterone	0.86
Cortisone	0.76
Desoxycorticosterone	0.80
17-Hydroxycorticosterone	0.75
Dehydrocorticosterone	0.85
Corticosterone	0.83