CHROMATOGRAPHIC BEHAVIOR, SOLUBILITIES, AND PARTITION RATIOS OF ERGOSTEROL AND CALCIFEROL IN SOLVENT MIXTURES

By

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AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

Year 1952

Approved L. T. Ewing

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THESIS ABSTRACT

The chromatographic behavior of hexane-dioxane mixtures on Superfiltrol is discussed and the adsorption of dioxane from hexane solutions on Superfiltrol, calculated from chromatographic studies, is verified by data obtained by the normal method for determining adsorption from solution.

Chromatographic behavior of ergosterol and calciferol on Superfiltrol with eluting solvent mixtures of dioxane and hexane is discussed and an equation is deduced which relates the adsorbate retained by the chromatographic column to the quantity of the adsorbent, to the amount of adsorbate entered on the column, and to the volume of solution containing the adsorbate before adsorption. The chromatographic separation of calciferol from ergosterol is discussed and an 81% recovery of pure calciferol from a 50-50 mixture of calciferol and ergosterol is demonstrated utilizing superfiltrol as the adsorbent and a 1.96% dioxane in hexane eluting solvent mixture.

Solubilities are given for ergosterol and calciferol in ethanol-water mixtures and for ergosterol in hexane-ethanol mixtures and in hexane-dioxane mixtures. The solubility of non-electrolytes in binary solvent mixtures is discussed and the solubility of ergosterol and calciferol in ethanol-water mixtures is shown to be explainable by an equation derived from regular solution theory.

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Partition ratios are listed for ergosterol and calciferol in the two phase, liquid-liquid system, hexane-methanol-water. The change in the partition ratios with the amount of water in the system, is discussed as is the possibility of separating ergosterol and calciferol by counter-current extraction procedures utilizing a liquid-liquid system consisting of hexane and 95% methanol.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor D. T. Ewing for his experienced counsel and guidance during the course of this investigation.

Grateful acknowledgment is made to the Graduate School, Michigan State College, for its grant of a Graduate Council Fellowship and to the Chemistry Department for its financial assistance in the form of a Graduate Assistantship and a Research Fellowship sponsored by the State's Smelting and Refining Company, Lima, Ohio.

Acknowledgment is also made to Dr. O. D. Bird of Parke, Davis and Company for many valuable suggestions and to Miss H. LeDuc for technical assistance with the infrared absorption analyses.

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INTRODUCTION

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The use of mixed polar-non polar solvents in the chromatographic separation of calciferol, vitamin D_2, from irradiated ergosterol has received much attention in this laboratory during recent years. Carlson[1] found that ergosterol was more strongly adsorbed on Superfiltrol than calciferol was from mixtures of hexane and diethyl ether. Pinkerton[2] obtained a separation of calciferol from ergosterol on Superfiltrol using a mixture of hexane and diethyl ether as an eluting solvent. Burnett[3] found a satisfactory separation of calciferol from ergosterol on an "activated" alumina using a mixture of hexane and diethyl ether as the eluting solvent. Other studies[4] involving the use of mixtures of hexane ethanol and diethyl ether as eluting solvents and Superfiltrol as the adsorbent have been more or less inconclusive.

It will be noted that in every instance the solvent mixture has involved the use of diethyl ether as the polar constituent or as one of the polar constituents, and that ether is a somewhat undesirable solvent to handle, due to its volitility and consequently due to the difficulties encountered in maintaining a constant composition mixture of solvents.

Investigations were therefore undertaken to ascertain the feasibility of substituting a less volatile polar solvent for the diethyl ether. The investigations undertaken included the determination of the chromatographic behavior of ergosterol and calciferol in hexane-dioxane mixtures, the determination of the solubility of ergosterol and calciferol in ethanol-

water mixtures, the determination of the solubility of ergosterol in hexane-ethanol and hexane-dioxane mixtures, and the determination of partition ratios of ergosterol and calciferol in the two phase, liquid-liquid system: hexane, methanol, water.

EXPERIMENTAL

EXPERIMENTAL

Materials

Ethanol was treated to remove aldehydes by the method described by Wildeman[5] which consists of an alkaline silver nitrate oxidation of the aldehydes to the acids. After distillation, the ethanol was further treated with freshly amalgamated aluminium to remove water according to the method described by Wislicenus and Kaufmann,[6] followed by redistillation.

Methanol, C. P. anhydrous grade, was distilled, treated by refluxing with potassium hydroxide over aluminium chips to remove aldehydes, and redistilled.

Hexane was obtained by carefully fractionating a commercial product, taking the fraction between 67 and 69° C as hexane. To remove unsaturated hydrocarbons, the distillate was passed through an activated silica gel column, 4 cm. in diameter by 75 cm. in length. The hexane was chromatographed until it had an absorbancy of less than 0.015 at 230 mu. measured against water and showed no evidence of benzene in the spectrum.

p-Dioxane, practical grade, was purified by the method described by Fieser,[7] followed by redistillation.

The ergosterol, obtained from Winthrop Chemical Company, was recrystallized from a 50-50 (v/v) mixture of 95% ethanol and thiophene free benzene, as described by Huber, Ewing, and Kriger.[8] The resulting

ergosterol had an absorbancy which agreed with that reported by Huber, et al.[8]

Calciferol, Winthrop Chemical Company (N415EC) pure synthetic vitamin D_2, was used without further treatment and had an absorbancy in agreement with that reported by Huber, et al.[8]

The calciferol and ergosterol were stored under reduced pressure at -10° C in an atmosphere of CO_2.

Superfiltrol No. 63 was used as the adsorbent throughout this investigation.

Determination of the Chromatographic Behavior of Dioxane-Hexane Mixtures on Superfiltrol

Adsorption tubes were prepared by sealing a short piece of 3 mm. I. D. Pyrex glass tubing to one end of a convenient length of 7 mm. I. D. Pyrex glass tubing and by sealing a 15 cm. length of 35 mm. I. D. Pyrex glass tubing to the opposite end. These adsorption tubes were packed to a height of 6 cm. with 2 g. of Superfiltrol No. 63 following the method described by Ewing, Kingsley, Brown, and Emmett.[9]

Various solvent mixtures of hexane and dioxane were chromatographed and eluate fractions were collected with the aid of a Technicon Automatic Fraction Collector. The eluate fractions were analysed to determine the amount of dioxane by infrared absorbancy measurements utilizing a Beckman IR-2 (No. 108) infrared spectrophotometer, as described below.

The Determination of Dioxane in the Presence of Hexane by Infrared Absorbancy Measurements

Dioxane was found to have a different absorbancy at 11.35 microns than does hexane. A series of known composition mixtures of dioxane and hexane were used to establish a calibration of the absorbancy against the concentration of dioxane in hexane. These results are shown in Table I.

TABLE I

INFRARED ABSORPTION DATA FOR DIOXANE IN HEXANE SOLUTIONS AT 11.35 MICRONS

Volume % Dioxane in Hexane	Intensity I	Absorbancy $\log.(I_o/I)$
0	(I_o = 91.8)	-----
0.498	74.7	0.089
0.991	61.0	0.177
1.22	53.5	0.234
1.63	46.6	0.294
2.44	34.0	0.431

Agreement with Beer's Law was found throughout the concentration range 0 to 2.5% dioxane in hexane. (Fig. 1). All subsequent analyses were carried out within these absorbancy limits by dilution of an aliquot of the more concentrated solution.

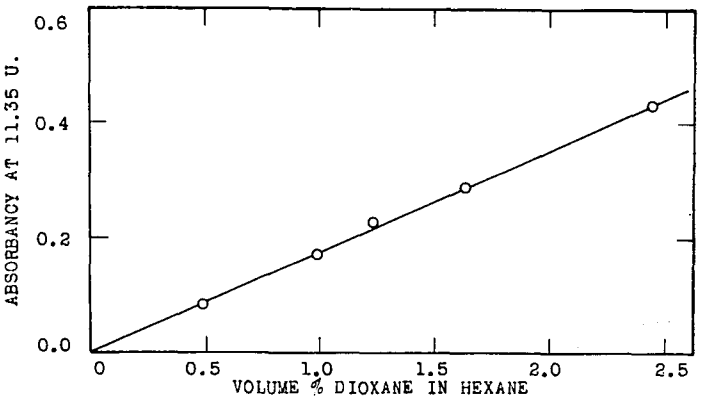


FIGURE 1

INFRARED ABSORBANCY CALIBRATION CURVE USED TO DETERMINE THE AMOUNT OF DIOXANE IN ELUATE SAMPLES.

The Determination of the Chromatographic Behavior of Calciferol and Ergosterol on Superfiltrol

Superfiltrol columns were prepared as described above and were "prewashed" with 25 ml. of the desired eluting solvent mixture. Ergosterol and/or calciferol in two ml. of the desired eluting solvent was entered on the Superfiltrol columns and was followed by an excess of the eluting solvent. Successive one ml. portions of the eluate were collected and analyzed by determining the ultra-violet absorbancy with the aid of a Beckman D. U. (No. 316) spectrophotometer.

Solubility Determinations

Figure 2 shows the apparatus used to obtain the saturated solution samples for the solubility measurements. These depicted "Equilibrium tubes", which are modifications of those described by Craig and Post,[10] were immersed in a constant temperature water bath controlled to $\pm$ 0.05°. Approximately 1 ml. portions of the desired solvent, were shaken with excess solute, ergosterol or calciferol, for ten hours. The "equilibrium tubes" were inverted to filter the saturated solutions into the tared 5 ml. volumetric flasks. The saturated solution samples were weighed, diluted with ethanol, and the ultra-violet absorption spectrum of the diluted samples determined with a Beckman model D. U. (#316) spectrophotometer. The solubilities were calculated using the following equations:

$$C_e = \frac{A}{23.3 \times n} \qquad (1)$$

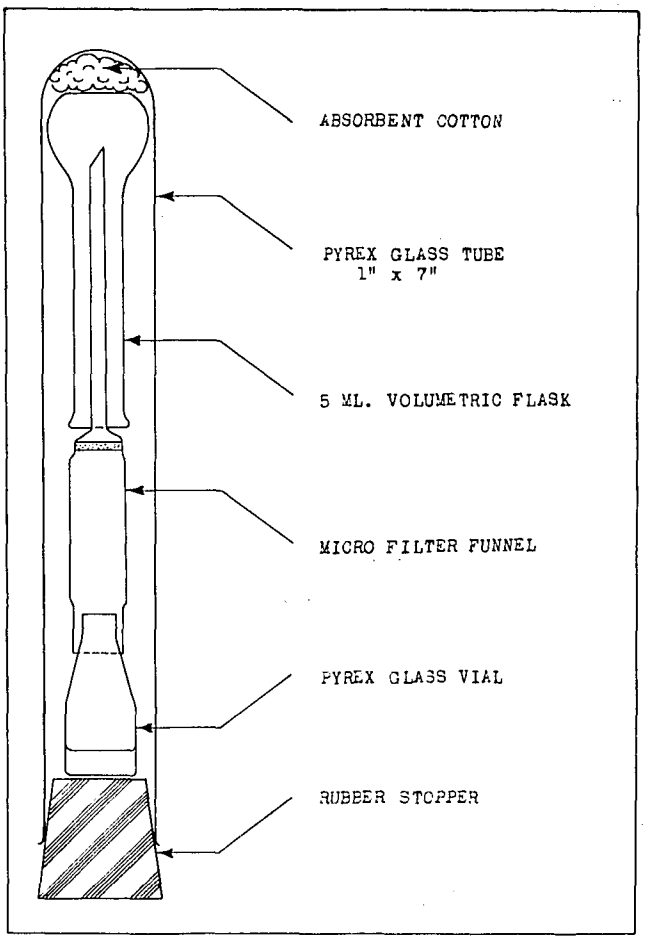


FIGURE 2

APPARATUS USED TO OBTAIN THE SATURATED
SOLUTIONS FOR SOLUBILITY DETERMINATIONS.

$$C_c = \frac{A}{36.8 \times n} \qquad (2)$$

where C_e and C_c equal the solubility of ergosterol and calciferol respectively in grams per 1000 grams of solution, A equals the absorbancy determined for the saturated solutions, n equals the weight of the saturated solutions in grams, 23.3 and 36.8 equal the absorbancy of an ethanol solution containing one gram per 1000 grams of solution for ergosterol and calciferol respectively. The absorbancy values were measured at wave lengths 282 mu. and 265 mu. for ergosterol and calciferol respectively.

Partition Ratio Determinations

Hexane was equilibrated with the various mixtures of methanol and water until equal volumes of the two phases, non-polar and polar, were obtained. The various phases were separated and divided into 10 ml. portions and ergosterol and/or calciferol were added to the non-polar phases. These solutions in the non-polar phases were subsequently equilibrated with three successive ten ml. portions of the polar phases and the concentration after each equilibration was determined from the ultra-violet absorbancy at 282 mu. for ergosterol and at 265 mu. for calciferol.

Data and Results

The solubility data for ergosterol and calciferol in ethanol-water mixtures at 15°, 25°, and 35° C are listed in Tables II through VII.

TABLE II

THE SOLUBILITY OF ERGOSTEROL IN ETHANOL-WATER MIXTURE AT 15° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
100	0.6771	54.0	3.21	
"	0.6975	48.2	3.03	3.15
"	0.7526	56.2	3.22	
80.1	0.6734	3.89	0.248	
"	0.8064	4.94	0.263	0.255
"	0.8725	5.33	0.263	
"	0.7838	4.47	0.245	
69.0	0.7514	1.32	0.0753	
"	0.7341	1.23	0.0723	0.0715
"	0.7639	1.19	0.0670	
64.5	0.8219	0.776	0.0407	
"	0.8402	0.767	0.0393	0.0402
"	0.8925	0.843	0.0407	
59.4	0.8923	0.444	0.0214	0.0207
"	0.8877	0.415	0.0201	

TABLE III

THE SOLUBILITY OF ERGOSTEROL IN ETHANOL-WATER MIXTURES
AT 25° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
100	0.7014	77.1	4.73	
"	0.7013	77.4	4.75	
"	0.7914	86.3	4.70	4.71
"	0.7604	83.7	4.74	
"	0.7617	82.5	4.68	
"	0.7451	81.0	4.69	
91.0	0.9627	38.4	1.72	
"	0.8705	34.2	1.69	
"	0.8902	35.0	1.69	1.71
"	0.9472	36.5	1.66	
"	0.7385	30.3	1.77	
"	0.7902	32.0	1.75	
82.0	0.8457	10.9	0.556	
"	0.8024	10.3	0.555	0.550
"	0.7976	10.0	0.540	
81.7	0.6287	7.78	0.532	
"	0.7234	8.96	0.535	0.535
"	0.7628	9.26	0.524	
"	0.6306	8.11	0.553	
70.3	0.7829	2.99	0.164	
"	0.8108	3.97	0.158	0.161
"	0.8221	3.08	0.161	
"	0.8173	3.07	0.162	
65.0	1.6651	3.42	0.0885	
"	1.6848	3.20	0.0817	0.0865
"	0.7924	1.64	0.0892	
49.7	1.0554	0.321	0.0131	
"	0.8912	0.238	0.0115	0.0131
"	0.9197	0.307	0.0141	
"	0.9438	0.295	0.0135	

TABLE IV

THE SOLUBILITY OF ERGOSTEROL IN ETHANOL-WATER MIXTURES
AT 35° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
100	0.6695	106	6.83	6.90
"	0.6458	103	6.87	
"	0.6138	99.2	6.95	
"	0.6139	98.8	6.93	
81.9	0.6758	14.0	0.890	
"	0.7264	14.4	0.855	0.878
"	0.7555	15.4	0.881	
"	0.7532	15.4	0.879	
66.3	0.8044	2.79	0.149	
"	0.7539	2.54	0.145	0.146
"	0.7994	2.64	0.143	
64.1	0.7083	1.77	0.108	
"	0.6973	1.82	0.112	0.111
"	0.7142	1.82	0.110	
"	0.5960	1.58	0.114	
59.7	0.6913	0.940	0.0584	
"	0.7115	0.921	0.0557	0.0580
"	0.7265	1.01	0.0602	
"	0.7286	0.967	0.0572	
53.6	0.9714	0.626	0.0278	
"	1.0231	0.674	0.0285	0.0282
"	1.0336	0.675	0.0283	
53.2	1.9559	1.49	0.0320	
"	2.0431	1.61	0.0329	0.0319
"	2.0382	1.46	0.0308	
49.7	1.9855	0.911	0.0198	
"	1.8933	0.840	0.0191	0.0194
"	1.9765	0.897	0.0196	
"	2.0784	0.929	0.0192	
48.2	1.7412	0.661	0.0163	0.0165
"	1.6741	0.649	0.0167	

TABLE V

THE SOLUBILITY OF CALCIFEROL IN ETHANOL-WATER MIXTURES AT 15° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
90.0	0.7021	1190	46.2	
"	0.7016	1190	46.5	46.4
"	0.7095	1210	46.4	
79.9	0.7415	285	10.5	
"	0.7390	276	10.2	10.2
"	0.7804	284	9.90	
66.1	0.7141	40.7	1.54	
"	0.7231	40.2	1.51	1.53
"	0.7040	40.0	1.54	
49.8	0.8576	4.20	0.133	
"	0.7355	3.85	0.142	0.138
"	0.7892	3.99	0.137	
37.5	0.8629	0.729	0.0162	0.0230
"	0.8570	0.730	0.0297	

TABLE VI

THE SOLUBILITY OF CALCIFEROL IN ETHANOL-WATER MIXTURES
AT 25° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
85.1	0.5821	921	43.1	
"	0.5510	863	42.5	42.8
"	0.5782	912	42.8	
75.3	0.6812	267	10.7	
"	0.5401	212	10.7	10.7
"	0.5728	222	10.6	
62.8	0.7329	48.8	1.81	
"	0.7458	50.3	1.83	1.83
"	0.7196	49.1	1.85	
46.7	0.6095	3.42	0.153	
"	0.5815	3.42	0.159	0.152
"	0.6199	3.47	0.152	
39.8	0.6722	1.71	0.0693	
"	0.6431	1.65	0.0699	0.0694
"	0.6827	1.73	0.0689	

TABLE VII

THE SOLUBILITY OF CALCIFEROL IN ETHANOL-WATER MIXTURES AT 35° ± 0.05° C

Wt. % Ethanol	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
81.1	0.7523	1190	43.0	
"	0.7540	1190	42.8	43.0
"	0.7601	1210	43.2	
66.1	0.7095	141	5.39	
"	0.7164	145	5.52	5.45
"	0.7109	143	5.45	
55.0	0.6728	25.3	1.02	
"	0.6461	25.4	1.07	1.04
"	0.6872	26.1	1.03	
39.0	0.5829	2.41	0.113	
"	0.4999	2.10	0.114	0.115
"	0.5692	2.47	0.118	
31.5	0.9867	1.47	0.0399	
"	1.0247	1.51	0.0400	0.0399
"	0.9799	1.44	0.0397	

The solubility data for ergosterol in ethanol-hexane and dioxane-hexane mixtures at 25°C are listed in Tables VIII and IX respectively.

TABLE VIII

THE SOLUBILITY OF ERGOSTEROL IN ETHANOL-HEXANE MIXTURES AT 25° ± 0.05° C

Wt. % Hexane	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
100	0.4989	41.5	3.57	
"	0.4835	39.4	3.51	3.57
"	0.4594	38.6	3.61	
86.5	0.5244	242	19.8	
"	0.5275	247	20.1	20.0
"	0.4957	233	20.2	
78.9	0.5283	329	26.8	
"	0.5567	340	26.3	26.5
69.8	0.3800	314	35.6	
"	0.3517	287	35.0	34.9
"	0.4596	368	34.2	
62.9	0.4952	398	34.6	
"	0.5732	466	35.0	34.9
"	0.5547	454	35.2	
45.8	0.4988	310	26.7	
"	0.5409	344	27.4	27.6
"	0.5122	342	28.8	
33.1	0.3778	169	21.7	
"	0.4979	244	21.1	21.5
"	0.5702	288	21.7	
19.4	0.6113	198	13.9	
"	0.4740	153	13.9	13.8
"	0.5028	157	13.5	
14.4	0.5911	110	7.92	
"	0.5510	90.0	7.02	7.47
"	0.5809	101	7.48	
11.4	0.3852	97.7	10.9	
"	0.4010	104	11.1	10.9
"	0.3961	100	10.8	
0	(100% Ethanol - See Table III)			4.71

TABLE IX

THE SOLUBILITY OF ERGOSTEROL IN DIOXANE-HEXANE MIXTURES AT 25° ± 0.05° C

Wt. % Hexane	Wt. Sat. Solution Sample g	Absorbancy A	Solubility g per 1000 g solution	
			Observed	Average
100	See Table VIII			3.57
79.8	0.2602	201	31.0	
"	0.2446	178	31.3	31.1
"	0.3910	284	31.1	
62.0	0.3249	399	52.7	52.7
"	0.1955	240	52.7	
52.2	0.3476	496	61.2	61.1
"	0.3733	529	61.0	
39.5	0.1649	237	61.7	61.9
"	0.2179	314	62.0	
25.0	0.2051	257	53.7	53.7
"	0.3105	388	53.7	
0	0.1951	141	31.2	
(100%	0.3314	222	28.8	30.9
Dioxane)	0.4342	321	31.8	

The partition ratios for ergosterol and calciferol in the various two liquid phase systems consisting of various mixtures of hexane methanol and water are listed in Tables X and XI.

TABLE X

THE PARTITION RATIOS OF ERGOSTEROL IN VARIOUS HEXANE, METHANOL-WATER SYSTEMS

Volume % Water Added to Methanol	Concentration mg per 100 ml solution C		Partition Ratios $\frac{C_{non\text{-}polar}}{C_{polar}}$	
	non-polar	polar	Observed	Average
0	89.8	102.6	0.876	
"	41.9	47.8	0.876	0.876
"	19.5	22.3	0.875	
1	102.3	94.3	1.09	
"	53.2	49.0	1.09	1.09
"	27.6	25.4	1.09	
3	130.0	61.9	2.10	
"	88.1	41.9	2.10	2.10
"	59.8	28.5	2.10	
5	142.6	32.6	4.37	
"	116.0	26.6	4.37	4.37
"	94.5	21.6	4.37	
12	145.0	7.80	18.6	
"	137.7	7.40	18.6	18.6
"	130.7	7.02	18.6	
20	96.9	2.24	43.2	
"	94.7	2.19	43.2	43.2
"	92.6	2.14	43.3	

TABLE XI

THE PARTITION RATIOS OF CALCIFEROL IN VARIOUS HEXANE, METHANOL-WATER SYSTEMS

Volume % Water Added to Methanol	Concentration mg per 100 ml solution C		Partition Ratios $\frac{C_{non\text{-}polar}}{C_{polar}}$	
	non-polar	polar	Observed	Average
0	6.93	7.47	0.928	
"	3.33	3.59	0.928	0.927
"	1.60	1.73	0.926	
1	7.61	6.95	1.10	
"	3.98	3.63	1.10	1.10
"	2.08	1.90	1.09	
3	9.15	6.10	1.50	
"	5.49	3.66	1.50	1.50
"	3.29	2.19	1.50	
5	10.5	3.78	2.78	
"	7.70	2.77	2.78	2.78
"	5.65	2.03	2.78	
12	20.0	1.44	13.9	
"	18.6	1.34	13.9	13.9
"	17.3	1.24	13.9	
20	14.3	0.396	36.1	
"	13.9	0.385	36.1	36.1
"	13.5	0.374	36.1	

DISCUSSION

DISCUSSION

The Chromatographic Behavior of Dioxane-Hexane Mixtures

The approach to chromatographic equilibrium between the adsorbent and the various solvent mixtures of dioxane and hexane was determined by following the concentration of dioxane in the eluent. These data are listed in Table XII. In Fig. 3, the fraction, c/c_0, where c equals the concentration of dioxane in the samples of the eluate and c_0 equals the concentration of dioxane in the eluent, has been plotted against the volume of eluate. It will be noted that the slope of the center portion of the various curves decreases from left to right. This is interpreted as a difference in the rate of approach to chromatographic equilibrium for the various solvent mixtures.

The volume of eluate, which precedes the solution of the adsorbate is called the retardation volume,[11] v_r. The retardation volume is measured as that volume of liquid which has emerged from the column to the point where the adsorbate appears. In this work, the approach to chromatographic equilibrium differs for each solvent mixture, hence the point at which the adsorbate first appears is considered not a true measure of the retardation volume. However a useful value may be obtained by assuming instantaneous establishment of the equilibrium. For example, the data for the eluent containing 0.498% dioxane indicates that the dioxane concentration increases throughout a 9 ml. portion of the eluate

TABLE XII

THE CONCENTRATION OF VARIOUS ELUATE FRACTIONS OBTAINED BY CHROMATOGRAPHING VARIOUS SOLVENT MIXTURES OF DIOXANE AND HEXANE

Solvent / Ml Eluate	Concentration of Dioxane Volume %						
	16.7% Dioxane	9.09% Dioxane	3.85% Dioxane	1.96% Dioxane	1.48% Dioxane	0.991% Dioxane	0.498% Dioxane
1.0	0.010						
1.5	5.55						
2.0	16.1	0					
2.5	16.7						
3.0	16.7	6.02					
3.5							
4.0		8.95	0				
4.5			0.139				
5.0	16.7	9.09	1.56				
5.5			3.64				
6.0		9.09	3.78				
6.5			3.85				
7.0			3.85	0			
7.5				0.186			
8.0			3.85	1.26	0		
8.5				1.82			
9.0				1.96	0.062		
9.5							
10.0				1.96	0.312		
11.0					1.19		
12.0					1.44	0	
13.0					1.48	0.0901	
14.0						0.548	
15.0					1.48	0.915	0
16.0						0.962	
17.0						0.991	
18.0							0.0089
19.0						0.991	
21.0							0.125
24.0							0.467
27.0							0.498
30.0							0.498

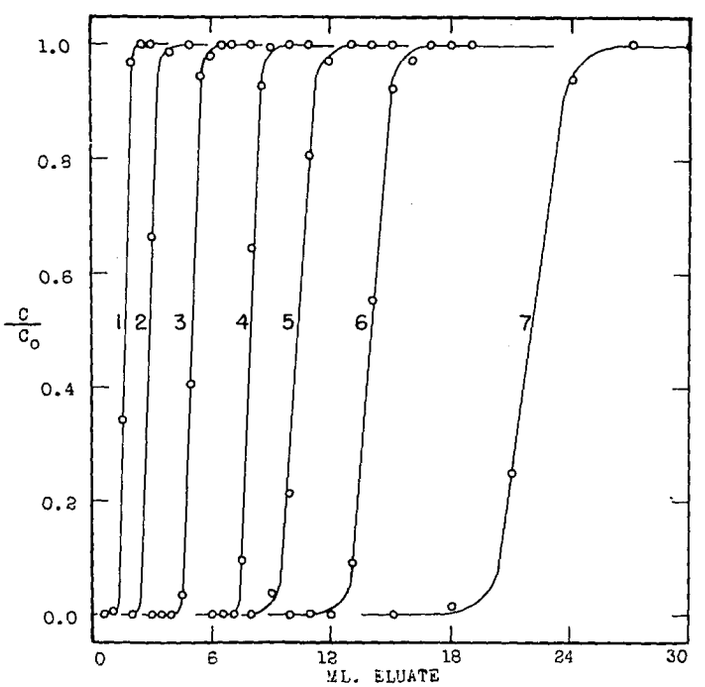


FIGURE 3

CHROMATOGRAPHIC BEHAVIOR OF DIOXANE-HEXANE MIXTURES ON SUPERFILTROL. C EQUALS THE CONCENTRATION OF ELUATE SAMPLES, C_o EQUALS THE CONCENTRATION OF SOLVENT MIXTURES (1) 16.7 %, (2) 9.09 %, (3) 3.85 %, (4) 1.96 %, (5) 1.48 %, (6) 0.99 %, AND (7) 0.498 % DIOXANE IN HEXANE.

before reaching equilibrium, i.e. the increase takes place from 15 ml. to 24 ml. of eluate. Therefore, the retardation volume was determined as follows:

$\frac{1.205}{3}$ = 0.401 = average c/c_o value for the three 3 ml. samples analyzed which contained some dioxane but less than the eluent.

$\frac{9}{0.401}$ = 3.609 ml. = the volume of solution of the eluent concentration, which contains the total amount of dioxane found in the 9 ml. portion from 15 ml. to 24 ml. of eluate.

24.0 - 3.6 = 20.4 ml. = the retardation volume.

The retardation volumes thus determined for the various solvent mixtures of dioxane and hexane are shown in Table XIII.

TABLE XIII

THE CHROMATOGRAPHIC ADSORPTION OF DIOXANE FROM HEXANE SOLUTIONS ON SUPERFILTROL

Solvent Composition Volume % Dioxane in H Hexane	Retardation Volume ml.	Specific Retardation Volume ml.per g.	$\frac{X}{m}$ ul.per g
0.498	20.4	10.2	50.4
0.991	13.4	6.70	67.0
1.498	9.60	4.80	72.0
1.960	7.69	3.85	77.0
3.850	4.65	2.33	93.0
9.090	2.39	1.20	119.5
16.670	1.35	0.680	135.0

The significance of these "calculated" retardation volumes is readily seen from Fig. 4, where the amount of dioxane adsorbed per gram of Superfiltrol was calculated directly from the specific retardation volumes. To check the validity of the calculation of the amount of dioxane adsorbed per gram of Superfiltrol from the specific retardation volumes, the adsorption of dioxane from hexane by Superfiltrol was determined by normal equilibrium methods. These results are also shown in Fig. 4 and substantiate the values determined from the retardation volumes in the low concentration region. The adsorption values determined by normal equilibrium methods for solutions containing more than 4% dioxane, were unreliable due to inherent errors in the method. Normal equilibrium methods involve subtracting the concentration of the solution after adsorption from the concentration of the solution before adsorption and for the solutions containing more than 4% dioxane involves subtracting one large number from another large number. Since the adsorption of dioxane is small, the actual error for such an adsorption value becomes large. The determination of the retardation volumes, however, were not subject to this inherent error and the calculated adsorption values from solutions containing more than 4% dioxane were reproduced with high precision.

The Chromatographic Behavior of Ergosterol and Calciferol on Superfiltrol in the Presence of Mixtures of Dioxane and Hexane

In the initial phase of this study, the adsorbate was added to the chromatographic column in a hexane solution from which both calciferol

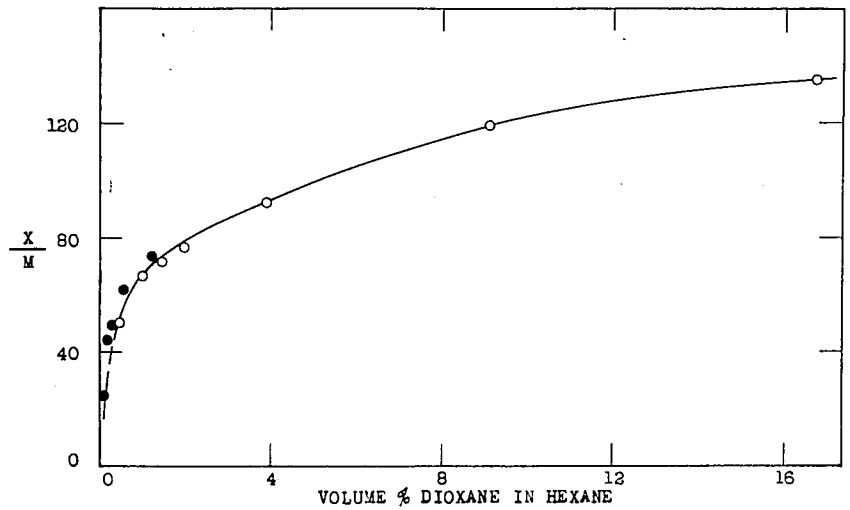


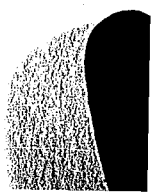
FIGURE 4

ADSORPTION OF DIOXANE FROM HEXANE SOLUTIONS ON SUPERFILTROL; o CALCULATED FROM CHROMATOGRAPHIC RETARDATION VOLUMES AND ● DETERMINED BY NORMAL EQUILIBRIUM METHODS.

and ergosterol are strongly adsorbed on Superfiltrol,[12] since strong adsorption yields a narrow initial zone of ergosterol or calciferol. Subsequent elution of the ergosterol or calciferol thus entered onto the Superfiltrol column, indicated however that this procedure was not feasible for these adsorbates. From Fig's. 5 and 6 one can readily see that both ergosterol and calciferol undergo decomposition during the adsorption or desorption processes on Superfiltrol. Subsequent investigation indicated that these adsorbates could be added to the column in a two ml. portion of the eluting solvent without serious decomposition being evidenced.

The eluate concentration history data are listed in Tables XIV and XV. The elution of ergosterol and/or calciferol proceeded differently for each different eluent solvent mixture. The concentration history curves for calciferol and for ergosterol are given in Figures 7 and 8 respectively. It will be noted that the general shape of these curves undergoes a change from an asymetrical bell to a symetrical bell as the concentration of dioxane in the eluting solvent mixture decreases.

DeVault's theory[13] and Weiss' theory[14] of chromatography explain these observations on the basis of a change in the adsorption isotherm of these adsorbates as the amount of dioxane in the solvent mixture changes. Vermeulen and Hiester[15] have shown that the variation in symmetry of the concentration history curves in the case of ion-exchange chromatography is explainable on the basis of variations in the equilibrium



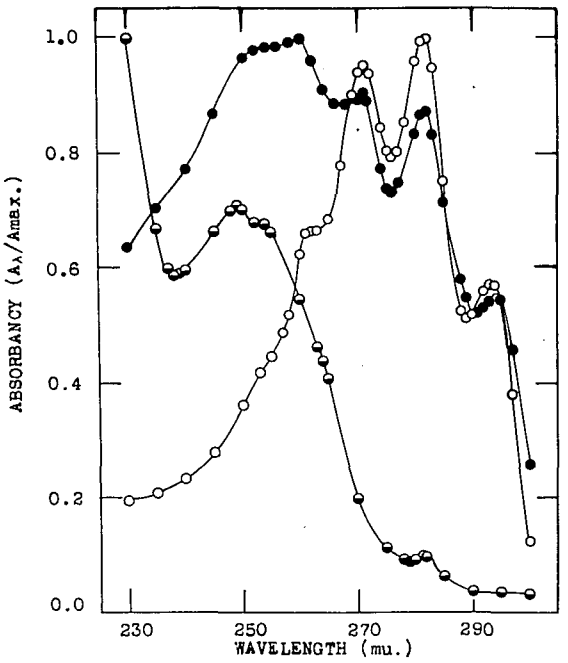


FIGURE 5

DECOMPOSITION OF ERGOSTEROL ON SUPERFILTROL
○ PURE ERGOSTEROL AND ELUATE FRACTIONS OBTAINED WHEN ERGOSTEROL WAS ADDED TO A SUPERFILTROL COLUMN IN HEXANE SOLUTION AND ELUTED WITH SOLVENT MIXTURES OF ⊖ 0.99% DIOXANE AND ● 16.7% DIOXANE IN HEXANE.

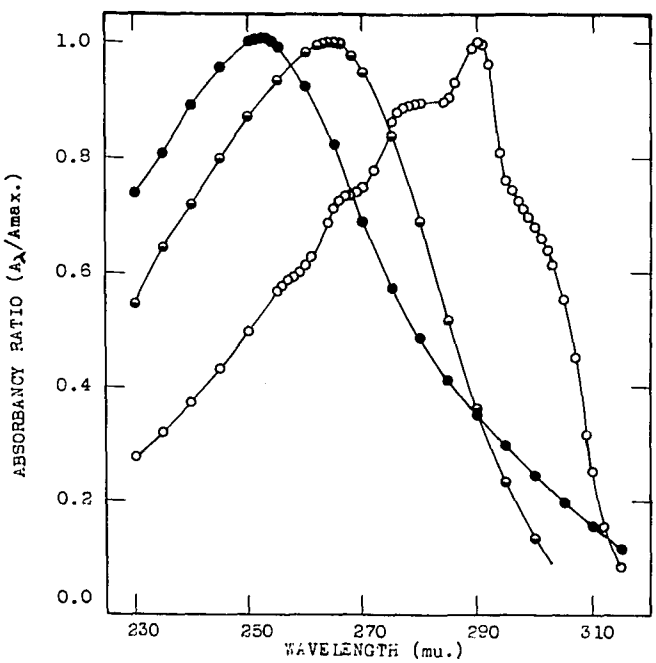


FIGURE 6

DECOMPOSITION OF CALCIFEROL ON SUPERFILTROL.
◒ PURE CALCIFEROL, AND ELUATE FRACTIONS OBTAINED WHEN CALCIFEROL WAS ADDED TO A SUPERFILTROL COLUMN IN HEXANE SOLUTION AND ELUTED WITH SOLVENT MIXTURES OF ● 3.85 % DIOXANE AND ○ 16.7 % DIOXANE IN HEXANE.

TABLE XIV

THE ELUATE CONCENTRATION HISTORY DATA FOR CALCIFEROL CHROMATOGRAPHED ON SUPERFILTROL WITH VARIOUS MIXTURES OF HEXANE-DIOXANE AS ELUTING SOLVENTS

Ml. Eluate	Solvent → Mg Calciferol Eluted Per Ml Eluate				
	9.09% Dioxane	3.85% Dioxane	1.96% Dioxane	0.991% Dioxane	0.498% Dioxane
1	0				
2	0.808	0			
3	1.008	0.0388			
4	0.126	0.638	0		
5	0.0266	0.816	0.0056		
6	0.0068	0.199	0.0712		
7		0.0368	0.560		
8		0.0136	0.622		
9		0.0084	0.288	0	
10		—	0.0870	0.0102	
11			0.0276	0.1214	
12			0.0128	0.296	
13				0.316	
14				0.226	
15				0.1316	
16				0.0674	
17				0.0312	0
18				0.0146	0.0066
19					0.0252
20					0.0536
21					0.0678
22					0.0636
23					0.0498
24					0.0344
25					0.0224
26					0.0134
27					0.0078

TABLE XV

THE ELUATE CONCENTRATION HISTORY DATA FOR ERGOSTEROL CHROMATOGRAPHED ON SUPERFILTROL WITH VARIOUS MIXTURES OF HEXANE-DIOXANE AS ELUTING SOLVENTS

Ml. Eluate	Solvent → Mg. Ergosterol Per Ml. Eluate: 9.09% Dioxane	3.85% Dioxane	1.96% Dioxane	0.991% Dioxane
1	0			
2	0.434	0		
3	0.912	0.0114		
4	0.300	0.0382		
5	0.0338	0.392		
6	0.0142	0.646		
7	0.0082	0.323		
8		0.0856		
9		0.0330	0	
10		0.0174	0.0218	
11		0.0112	0.1068	
12		0.0074	0.246	
13			0.262	
14			0.162	
15			0.0796	
16			0.0406	
17			0.0208	
18			0.0120	
19			0.0076	
20				0
21				0.0046
22				0.0070
23				0.0146
24				0.0324
25				0.0516
26				0.0606
27				0.0560
28				0.0442
29				0.0340
30				0.0248
31				0.0172
32				0.0116
33				0.0078
34				0.0052

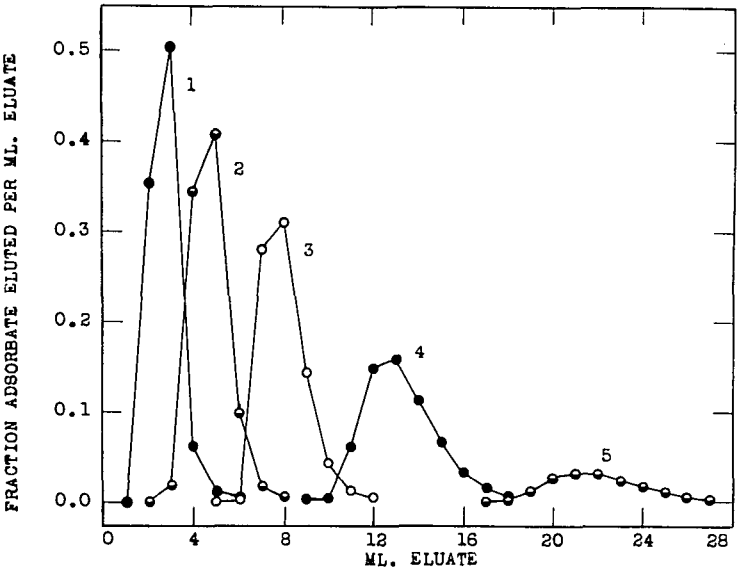


FIGURE 7

ELUTION CONCENTRATION HISTORY CURVES FOR CALCIFEROL FROM A SUPERFILTROL COLUMN ELUTED BY SOLVENT MIXTURES (1) 9.09 %, (2) 3.85 %, (3) 1.96 %, (4) 0.99 %, AND (5) 0.498 % DIOXANE IN HEXANE.

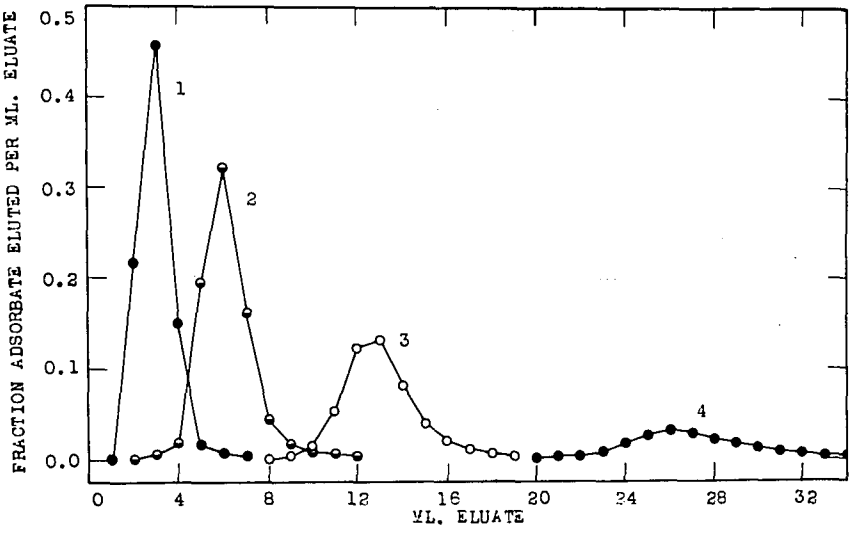


FIGURE 8

ELUTION CONCENTRATION HISTORY CURVES FOR ERGOSTEROL FROM A SUPER-FILTROL COLUMN ELUTED BY SOLVENT MIXTURES (1) 9.09 %, (2) 3.85 %, (3) 1.96 %, AND (4) 0.99 % DIOXANE IN HEXANE.

parameter.* In so far as adsorption chromatography may be compared to ion-exchange chromatography, one might consider the change from asymetrical to symetrical concentration history curves, as being indicative of an approach to trace conditions as the concentration of dioxane in the eluting solvent decreases, since the amount of calciferol or ergosterol in solution at a given instance is decreased due to stronger adsorption.

The threshold volumes for calciferol and for ergosterol when eluted by these solvent mixtures have been determined by the usual method consisting of an extrapolation of the central portion of the elution curves (Fig's. 9 and 10). It is apparent (Table XVI) that the threshold volumes for calciferol and ergosterol increase with a decrease in the amount of dioxane in the eluent solvent mixtures.

TABLE XVI

THE THRESHOLD VOLUMES AND THE RECOVERY OF CALCIFEROL AND ERGOSTEROL WHEN CHROMATOGRAPHED WITH VARIOUS MIXTURES OF HEXANE-DIOXANE ON SUPERFILTROL

Solvent Composition % Dioxane	Threshold Volume		% Adsorbate Recovered	
	Calciferol	Ergosterol	Calciferol	Ergosterol
9.09	1.32	1.55	95.2	85.2
3.85	3.12	4.39	90.0	78.0
1.96	6.05	10.5	83.0	48.2
0.991	10.7	23.2	60.8	18.6
0.498	18.8	----	17.2	----

*The equilibrium parameter depends upon the equilibrium exchange between a trace ion and the carrier ion and also depends upon the relative concentration of the trace ion and the carrier ion in the case of ion-exchange chromatography. Thus Vermeulen and Hiester have shown that the symmetry of the concentration history curve is a criterion for trace condition.

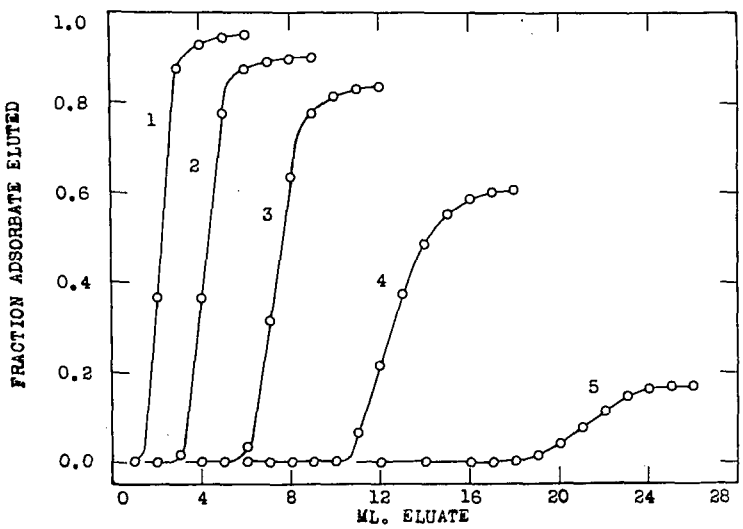


FIGURE 9

THE CHROMATOGRAPHIC ELUTION OF CALCIFEROL FROM SUPER-FILTROL COLUMNS BY SOLVENT MIXTURES (1) 9.09 %, (2) 3.85 %, (3) 1.96 %, (4) 0.99 %, AND (5) 0.498 %, DIOXANE IN HEXANE.

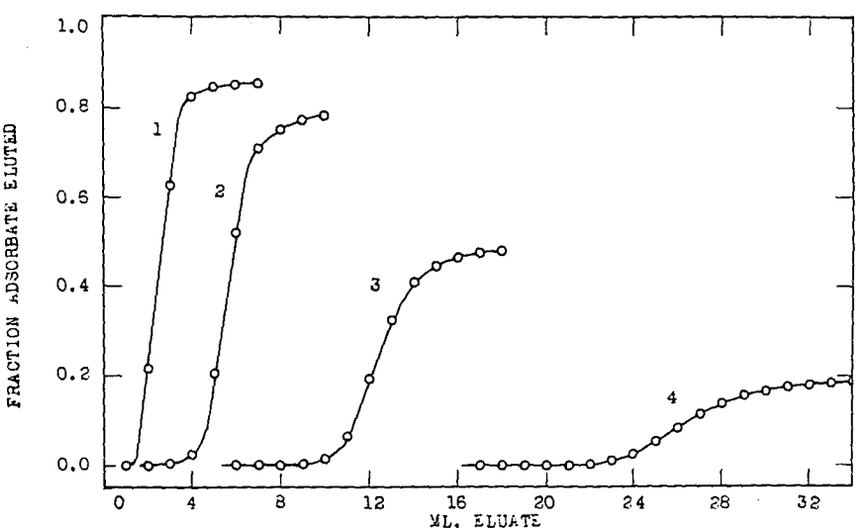


FIGURE 10

THE CHROMATOGRAPHIC ELUTION OF ERGOSTEROL FROM SUPERFILTROL COLUMNS BY SOLVENT MIXTURES (1) 9.09 %, (2) 3.85 %, (3) 1.96 %, AND (4) 0.99 %, DIOXANE IN HEXANE.

In Fig. 11, the log of the threshold volumes have been plotted against the log of the concentration of dioxane in the eluent. It appears that the following relationship holds:

$$\log V_t = n \log C + k_1 \qquad (3)$$

where V_t = the threshold volume, C = the concentration of dioxane, n and k_1 = constants independent of the solvent composition.

It may also be noted in Table XVI that the amount of calciferol and ergosterol eluted decreases with the concentration of dioxane in the eluent solvent. In Fig. 12, the log of the adsorbate not recovered is plotted against the log of the concentration of dioxane in the eluent solvent mixtures. A relationship similar to Equation (3) may also be stated,

$$\log A_R = m \log C + k_2 \qquad (4)$$

where A_R = the adsorbate not recovered, C = the concentration of dioxane, m and k_3 = constants independent of the solvent composition. Combining Equations (3) and (4) yields

$$\log A_R = a \log V_t + b \qquad (5)$$

where $a = \frac{m}{n}$ and $b = k_2 - m\, k_1$. Further, the threshold volume, according to Weil-Malherbe[16] is dependent on the amount of adsorbent, s, the quantity of adsorbate, m_o, and on the initial volume, v_o, the volume of the solution of the adsorbate before adsorption. The general relationship is given as follows;

$$V_t = K s^{1/\alpha} / m_o^{\beta} + \gamma v_o \qquad (6)$$

where K, α, β, and γ are constants. Substituting into Equation (5) we obtain the expression,

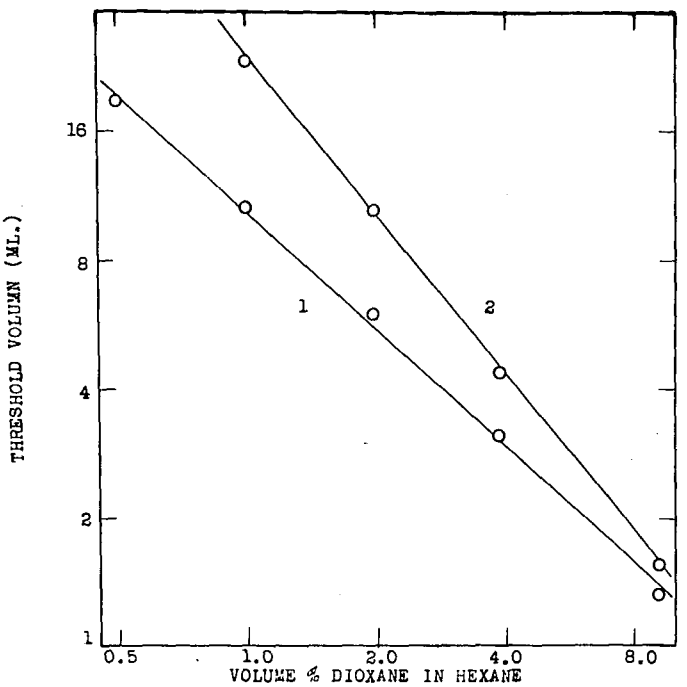


FIGURE 11

THE THRESHOLD VOLUMES FOR (1) CALCIFEROL AND (2) ERGOSTEROL ON SUPERFILTROL FOR VARIOUS ELUENT MIXTURES OF DIOXANE IN HEXANE.

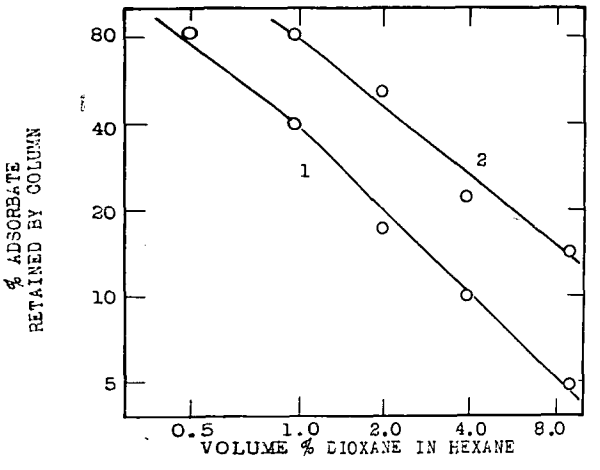


FIGURE 12

THE RETENTION OF (1) CALCIFEROL AND (2) ERGOSTEROL BY SUPERFILTROL COLUMNS WHEN ELUTED BY SOLVENT MIXTURES OF DIOXANE IN HEXANE.

$$\log A_R = a \log [Ks^{1/\alpha} / m_o^{\beta} + \gamma v_o] + b \quad (7)$$

which relates the quantity of adsorbate retained by the column to the quantity of adsorbent, the amount of adsorbate entered on the column, and the volume of solution containing the adsorbate before adsorption.

The Chromatographic Separation of Calciferol from Ergosterol

It was apparent, from the preceding considerations that a quantitative recovery of calciferol and/or of ergosterol was not possible if an "equilibrium" elution method were employed. Consequently, a two step elution proceedure was attempted. After eluting a column, to which ergosterol had been added, in the normal manner, with 40 ml. of eluting solvent containing 0.99% dioxane, the column was further eluted with 10 ml. of solvent containing 16.7% dioxane. The absorption curve for the eluate thus obtained is shown in Fig. 13. By comparing this absorption curve with that of pure ergosterol (Fig. 5), it is apparent that the ergosterol thus treated, has undergone decomposition during the process of adsorption or desorption. Attempts to overcome this difficulty were unsuccessful.

The separation of calciferol from ergosterol was investigated utilizing a solvent mixture of hexane and dioxane which would yield the best recovery of pure calciferol. A comparison of the concentration history curves for calciferol and for ergosterol indicates that the best solvent mixture is that containing 1.96% dioxane in hexane. In Fig. 14, the concentration history curves for ergosterol and for

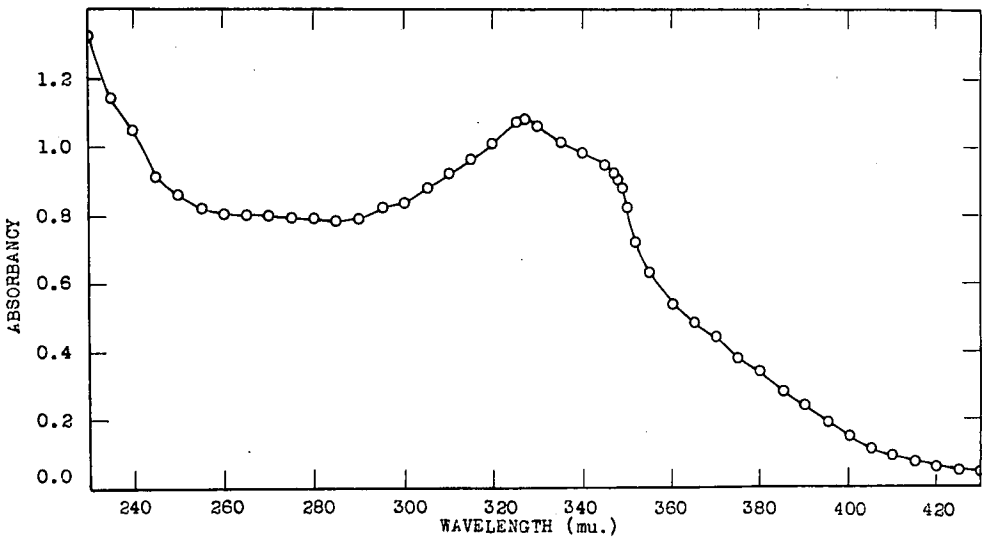


FIGURE 13

ABSORPTION CURVE FOR THE RESIDUE ELUTED BY A SOLVENT MIXTURE CONTAINING 16.7 % DIOXANE IN HEXANE FROM A SUPERFILTROL COLUMN WHICH HAD PREVIOUSLY BEEN SUBJECTED TO ELUTION OF ERGOSTEROL BY A SOLVENT MIXTURE OF 0.99 % DIOXANE IN HEXANE WHERE 81.4 % OF THE ERGOSTEROL WAS RETAINED BY THE COLUMN.

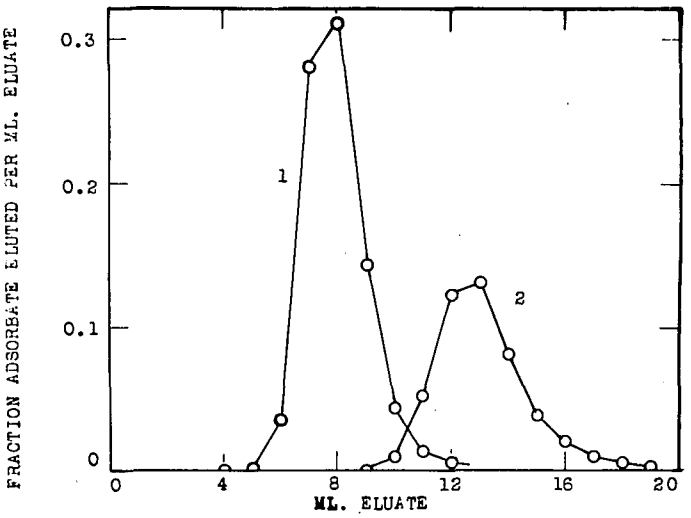


FIGURE 14

THE CONCENTRATION HISTORY CURVES FOR (1) CALCIFEROL AND (2) ERGOSTEROL OBTAINED BY ELUTION FROM SUPERFILTROL BY A SOLVENT MIXTURE OF 1.96 % DIOXANE IN HEXANE.

calciferol, obtained by elution with the solvent mixture containing 1.96% dioxane in hexane, are illustrated. If the elution of calciferol or ergosterol were not affected by the presence of the second adsorbate, one would expect the recovery of pure calciferol to be 77.3%, since eluate fractions from 10 to 12 ml. would contain a mixture of calciferol and ergosterol. The recovery of pure calciferol was found to be dependent on the amount of ergosterol in the mixture to be separated. These observations are illustrated in Figure 15. Whereas the recovery of pure calciferol decreased with an increase in the amount of ergosterol in the mixture to be separated, the total recovery of calciferol increased with an increase in the ergosterol present in the mixture. From Equation (5) it can be shown that an increase in the total amount of calciferol recovered should be coupled with a decrease in the threshold volume. Thus, the threshold volume for calciferol should experience a decrease with an increase in the amount of ergosterol in the mixture to be separated. In Fig. 16 this is seen to be the case.

Summary

1. p-Dioxane was determined in the presence of hexane by infrared absorption at 11.35 micron and agreement with Beer's Law throughout the concentration range 0 to 2.5% dioxane in hexane was found.

2. An equilibrium between Superfiltrol and eluting solvent mixtures of dioxane and hexane was observed and the time of establishment of the equilibrium was found to be dependent on the amount of dioxane in the solvent mixture.

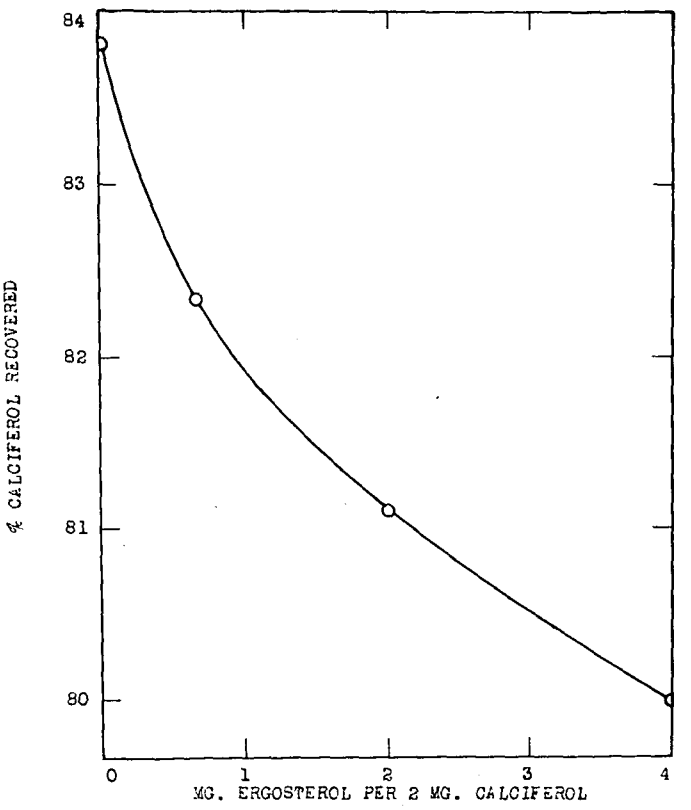


FIGURE 15

THE EFFECT OF ERGOSTEROL ON THE RECOVERY OF CALCIFEROL FROM A SUPERFILTROL COLUMN WHEN ELUTED BY A SOLVENT MIXTURE OF 1.96 % DIOXANE IN HEXANE.

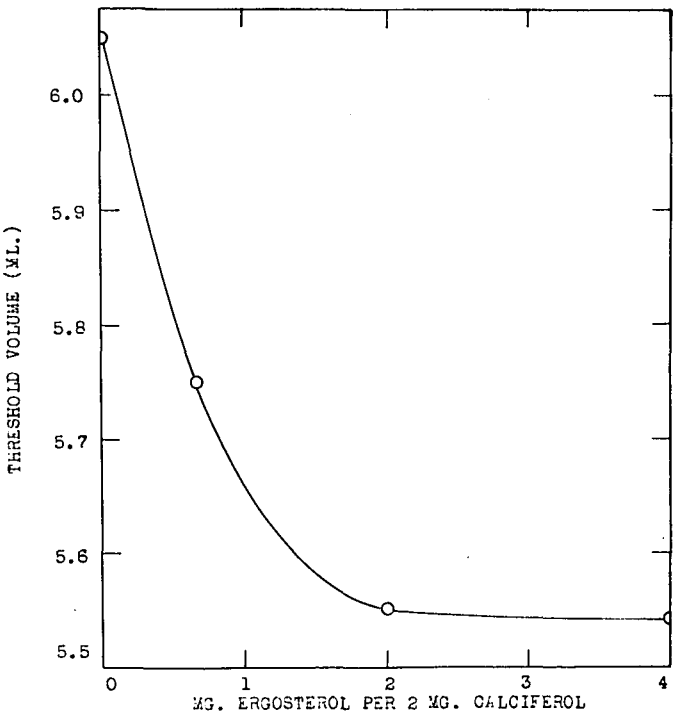


FIGURE 16

THE EFFECT OF ERGOSTEROL ON THE THRESHOLD VOLUME OF CALCIFEROL ELUTED FROM A SUPERFILTROL COLUMN BY A SOLVENT MIXTURE OF 1.96 % DIOXANE IN HEXANE.

3. The adsorption of dioxane from hexane solutions by Superfiltrol has been calculated from the chromatographic equilibrium studies, and has been shown to agree with the adsorption curve determined by normal procedure.

4. The chromatographic behavior of calciferol and ergosterol with eluting solvent mixtures of hexane and dioxane has been studied and an equation has been deduced relating the amount of adsorbate retained by the column to the quantity of adsorbent, the amount of adsorbate, and the volume of the solution containing the adsorbate before adsorption.

5. The amount of ergosterol present in a mixture of calciferol and ergosterol was found to influence the recovery of pure calciferol. The total recovery of calciferol was found to be increased by an increase of ergosterol in such a mixture.

6. The separation of calciferol from ergosterol has been shown to be dependent on the amount of ergosterol in the mixture to be separated. A mixture containing 2 mg. of both calciferol and ergosterol was chromatographed to give an 81% recovery of pure calciferol.

The Solubility of Non-electrolytes in Solvent Mixtures

Solubility studies of non-electrolytes in solvent mixtures has been the subject of a recent paper by Gordon and Scott[17] in which they showed that regular solution theory[18] predicts enhanced solubility where the solubility parameter of the solute is between those of the solvents. It was the purpose of this study to show how the theory, with a few added assumptions, may be used to explain other than enhanced solubility.

The regular solution theory of Hildebrand,[18] applied to the solubility of solids in liquids, relates the solubility to the thermodynamic properties of the pure components, i.e. to the melting temperature of the solute, the heat of fusion of the solute and the "solubility parameters" of the solute and solvent. The solubility parameter is defined as the square root of the "internal pressure" or "cohesive energy density" and for a given substance, is usually calculated from the molar volume and the molar energy of vaporization,[19] thus

$$\delta = \left(\frac{E^V}{V} \right)^{1/2} \qquad (8)$$

The ideal solubility, in mole fraction X^i, is given by the equation

$$\log X^i = \frac{-\Delta H^f}{2.3\ R} \left(\frac{1}{T} - \frac{1}{T_m} \right) \qquad (9)$$

where ΔH^f is its heat of fusion and T_m is its melting temperature.

If the solution is not ideal, the deviations from ideality are related to the partial molal heat of mixing ΔH_1, of the supercooled liquid solute with the solvent

$$\log X_1 = \log X^i - \frac{\Delta H_1}{2.3\ RT} \qquad (10)$$

The theory further relates ΔH_1 to the molal volume V_1, the volume fraction of the solvent ϕ_o, and the solubility parameters δ,

$$\Delta H_1 = V_1\ \phi_o^2\ (\delta_1 - \delta_o)^2 \qquad (11)$$

where the subscripts $_1$ and $_o$ refer to the solute and solvent respectively.

For a three component system involving two solvent components, the theory[20] leads to equations which are identical with Equations (10) and (11) if ϕ_0 is taken as the total volume fraction of solvents 2 and 3 and δ_0 is taken as the volume fraction average of δ_2 and δ_3.

$$\delta_0 = \frac{\delta_2\phi_2 + \delta_3\phi_3}{\phi_2 + \phi_3} \qquad (12)$$

$$\phi_0 = \phi_2 + \phi_3 \qquad (13)$$

When the solubility parameter of the solute δ_1, has a value between δ_2 and δ_3, the theory[20,21] predicts a maximum solubility. If the solution is not ideal the activity, a_1, of the solute in the saturated solution is substituted for X^i and the combination of equations (10) and (11) thus becomes

$$\log X_1 = \log a_1 - \frac{1}{2.3RT} V_1 \phi_0^2 (\delta_1 - \delta_0)^2 \qquad (14)$$

and the maximum solubility is therefore reached where $\delta_1 = \delta_0$.

If the volume fractions are considered proportional to the concentrations of components then

$$\frac{\phi_2}{\phi_3} = \frac{X_2}{X_3} = \frac{C_2}{C_3} \qquad (15)$$

and

$$\phi_2 = kX_2;\ \phi_3 = kX_3;\ \phi_2+\phi_3 = k(X_2 + X_3) \qquad (16)$$

where X_2 and X_3 are the mole fractions of the solvent components and k is a constant considered independent of the concentration of the solvent

components. Substituting into Equation (12) for ϕ_2, ϕ_3 and $\phi_2 + \phi_3$ according to Equation (16) yields

$$\delta_c = \frac{\delta_2 X_2 + \delta_3 X_3}{X_2 + X_3} \qquad (17)$$

Consider the case where the solute is but slightly soluble throughout in all mixtures of the solvents, then $X_1 \ll X_2 + X_3$ and $X_2 + X_3 \cong 1$. Equation (17) therefore becomes

$$\delta_c = \delta_2 X_2 + \delta_3 X_3 \qquad (18)$$

and

$$\phi_c = \phi_2 + \phi_3 = k(X_2 + X_3) = k \qquad (19)$$
$$X_2 = 1 - X_3$$

Substituting according to Equations (18) and (19) into Equation (11) and rearranging gives

$$\Delta H_1 = -V_1 (a' + b' X_3 + d' X_3^2) \qquad (20)$$

where

$$a' = -k^2(\delta_1 - \delta_2)^2$$
$$b' = +2k^2(\delta_2^2 - \delta_1 \delta_2 + \delta_1 \delta_3 - \delta_2 \delta_3)$$
$$d' = -k^2(\delta_2^2 + \delta_3^2 - 2\delta_2 \delta_3)$$

but $X_3^2 = X_3 - X_2 X_3$, therefore

$$\Delta H_1 = -V_1 [a' + (b' + d') X_3 - d' X_2 X_3] \qquad (21)$$

By definition the solubility parameter equals the square root of the energy of interaction (per cc.).

$$\delta_i = C_{ii}^{1/2} \qquad (22)$$

Substituting $C^{1/2}$ for δ the constant d′ becomes

$$d' = -k^2(C_{22} + C_{33} - 2C_{32}^{1/2} C_{33}^{1/2}) \qquad (23)$$

and the term $C_{22}^{1/2} C_{33}^{1/2}$ equals C_{23}.[22] Thus Equation (23) becomes

$$d' = -k^2(C_{22} + C_{33} - 2C_{23}) \qquad (24)$$

If the energy of interaction between unlike solvent molecules is considered the arithmetic mean of the energies of interaction between like molecules, it is apparent that d′ equals 0 and Equation (21) becomes

$$\Delta H_1 = - V_1 (a' + b'X_3) \qquad (25)$$

Substituting Equation (25) into Equation (14) and assuming that V_1 is independent of the concentration of the solvent components and is essentially constant, the equation

$$\log X_1 = a + bX_3 \qquad (26)$$

$$a = \log a_1 + \frac{V_1 a'}{2.3RT}$$

$$b = \frac{V_1 b'}{2.3RT}$$

predicts an exponential change of the solubility with the change of the solvent composition.*

---

* It should be noted that Equation (26) is in essentially the same form as that of Stromberg,[23] which was derived on the basis of Zhukhovitsky's regular solution theory.[24]

The Solubility of Ergosterol and Calciferol in Solvent Mixtures.

Ergosterol was found to have a greater solubility in hexane-dioxane mixtures and in hexane-ethanol mixtures, than it does in either pure hexane, pure dioxane, or pure ethanol. The maximum solubility in hexane-dioxane mixtures was found to be between 40 and 52% by weight hexane and in hexane-ethanol mixtures was found to be between 63 and 69% by weight hexane. (Table XVII).

The theory predicts this enhanced solubility where the solubility parameter of the solute is between those of the two solvents and predicts the maximum solubility where $\delta_1 = \delta_0$. Since the solubility parameters for hexane and dioxane are 7.3 and 10.0 respectively,[25] and since ergosterol shows enhanced solubility in hexane-dioxane mixtures, the solubility parameter for ergosterol must be between these values. Also, since ergosterol experiences enhanced solubility in hexane-ethanol mixtures, its solubility parameter must be between those of hexane and ethanol. Further, since the solubility of ergosterol is nearly the same in hexane and in ethanol but much greater in dioxane, one can assign relative values to ergosterol and ethanol, thus

$$\delta_{\text{hexane}} = 7.3 < \delta_{\text{ergosterol}} < \delta_{\text{ethanol}} < 10.0 = \delta_{\text{dioxane}}.$$

The solubility of both ergosterol and calciferol in ethanol-water mixtures was found to decrease exponentially with an increase of water (Fig. 17) and follows the form of Equation (26). Apparently, therefore, the assumptions used in the derivation of Equation (26) may be considered

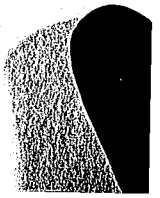
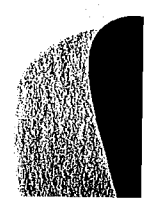


TABLE XVII

THE SOLUBILITY OF ERGOSTEROL IN MIXED SOLVENTS AT 25° ± 0.05°C

Solvent Mixture X / Y	Solubility g per 1000 g solution		
Wt. % Solvent Component Y	Ethanol Water	Ethanol Hexane	Dioxane Hexane
0	4.71	4.71	30.9
9.0	1.71		
11.4		10.9	
14.4		7.47	
18.0	0.550		
18.3	0.535		
19.4		13.8	
25.0			53.7
29.7	0.161		
33.1		21.5	
35.0	0.0865		
39.5			61.9
45.8		27.6	
50.3	0.0131		
52.2			61.1
62.0			52.7
62.9		34.9	
69.8		34.9	
78.9		26.5	
79.8			31.1
86.5		20.0	
100		3.57	3.57

valid for this solvent system, and the energy of interaction between unlike molecules of ethanol and water may be considered approximately equal to the arithmetic mean of the energies of interaction between like molecules of ethanol and of water.



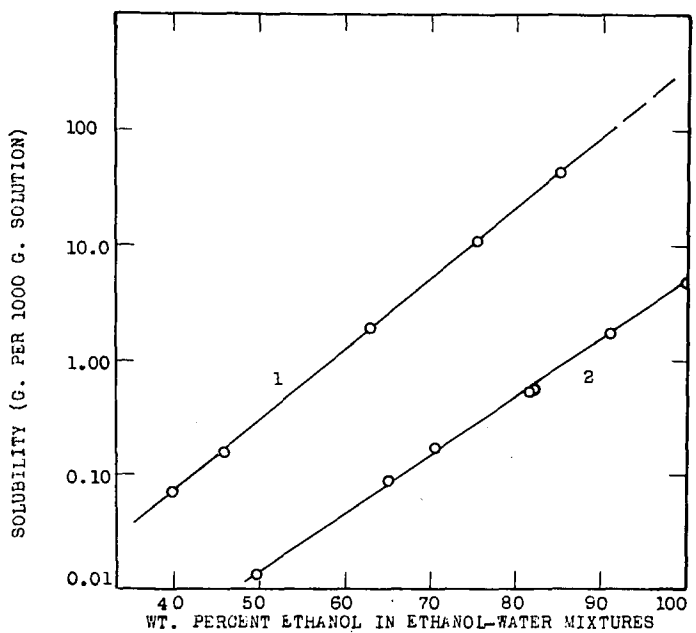


FIGURE 17

THE SOLUBILITY BEHAVIOR OF (1) CALCIFEROL AND (2) ERGOSTEROL IN ETHANOL-WATER MIXTURES AT 25° C.

The Effect of the Addition of Water on the Partition Ratios of Ergosterol and Calciferol in the Liquid-Liquid System: Hexane-Methanol.

Partition ratios of both ergosterol and calciferol were found to be independent of their concentrations over the concentration range studied. The partition ratios, however are influenced by the addition of water to the hexane-methanol system. Considering the partition ratios as being the ratio between the concentration of ergosterol or calciferol in the non-polar phase and the concentration in the polar phase, one can readily see that an increase of water to the hexane-methanol system causes an increase in these ratios, i.e. the water may be said to "force" the ergosterol or calciferol from the polar phase into the non-polar phase.

It was also found that the change in the partition ratios of ergosterol and calciferol with the addition of water to the hexane-methanol system, was not equivalent, i.e. the partition ratios of ergosterol increased more rapidly with the addition of water than did the partition ratios of calciferol. This fact lead to a consideration of the problem of separating calciferol from ergosterol by counter-current distribution. According to Craig[26] the ratio K_a/K_b is a measure of the ease of separating component a from component b by liquid-liquid extraction methods. The separation factor, $\beta = K_e/K_c$, where K_e and K_c equal the partition ratios for ergosterol and calciferol respectively, is plotted against the amount of water added to the methanol phase in Fig. 18. It is apparent that the system produced by adding 5% water

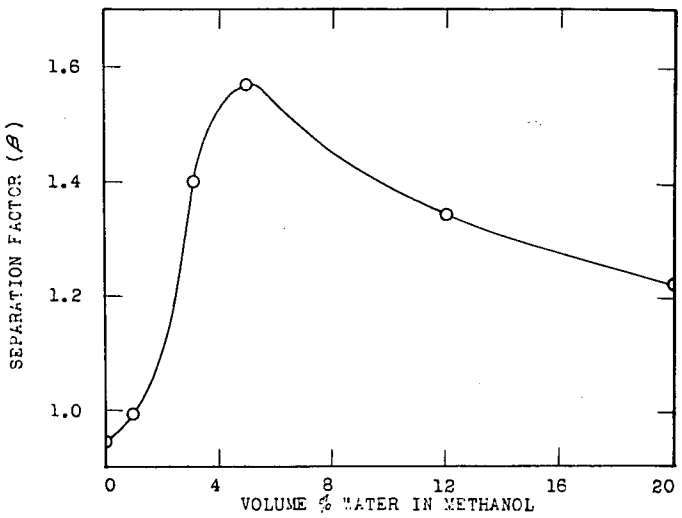


FIGURE 18

VARIATION IN THE SEPARATION FACTOR FOR CALCIFEROL AND ERGOSTEROL WITH THE QUANTITY OF WATER IN THE METHANOL WHICH WAS EQUILIBRATED WITH HEXANE TO FORM THE TWO PHASE LIQUID-LIQUID SYSTEM

(by Volume) to the methanol phase yields the best separation possibilities.*

Summary

1. The solubility of ergosterol in dioxane-hexane mixtures and ethanol-hexane mixtures has been determined and explained on the basis of existing regular solution theory.

2. The solubility of ergosterol and calciferol in ethanol-water mixtures at three temperatures has been determined and an explanation of their solubility behavior has been derived from the existing regular solution theory.

3. An approximation of the solubility parameters for ethanol and for ergosterol has been made, based on the solubility behavior of ergosterol.

4. The partition ratios of ergosterol and calciferol in the two phase liquid-liquid system, hexane-methanol have been determined showing the change in their partition ratios with a change in the amount of water added to the system.

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* Calculations, based on the work of Bush and Densen[27] indicates that a 50-50 mixture of ergosterol and calciferol would yield, after 100 extractions, a mixture containing approximately 85% ergosterol and a mixture containing approximately 85% calciferol using this system.

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