ring the reaction of the tertiary alcohol with hydrogen chloride was anomalous. The hydrogen chloride was passed into 2.65 g. of 1-methylcyclodecanol at room temperature for three hours. The alcohol liquefied, turned pink in color, and a small aqueous layer separated. After separation, drying over a granule of calcium chloride, and pumping to remove dissolved hydrogen chloride, it was noted that the product was pink in color and less viscous than the other tertiary chlorides. The product was low in chlorine, so a crystal of ferric chloride was added and the liquid was again treated with hydrogen chloride. However, even after this treatment the product contained only 35% hydrolyzable chloride. Nevertheless, the crude product underwent solvolysis smoothly, giving good first-order constants. Time did not permit a more detailed study of the phenomenon.

Di-*n*-amylmethylcarbinyl Chloride.—The action of *n*-amylmagnesium chloride on ethyl acetate yielded the tertiary alcohol (b.p. 84.5-86.5° at 2 mm., $n^{20}\mathrm{D}$ 1.4401). The alcohol was converted to the chloride with hydrogen chloride. The crude product contained 95% of the theoretical hydrolyzable chloride and was utilized directly for the rate measurements.

Acknowledgment.—We are indebted to the Office of Naval Research for the financial support which made this study possible, to Mrs. E. Kapper for synthesizing di-*n*-amylmethylcarbinyl chloride, to Dr. S. Siegel for a sample of the *p*-bromophenacyl ester of 1-methylcyclopropanecarboxylic acid, and to Drs. V. Prelog and M. Stoll for samples of several of the cycloalkanones.

LAFAYETTE, INDIANA RECEIVED AUGUST 13, 1951

[CONTRIBUTION FROM THE DIVISION OF DAIRY PRODUCTS RESEARCH LABORATORIES, UNITED STATES DEPARTMENT OF AGRICULTURE]

Chromatography of Methyl Stearate, Methyl Oleate, Methyl Linoleate and Methyl Linolenate: A Concept of Amplified Chromatographic Separations[1]

BY FLOYD E. KURTZ

More effective separations than previously reported of C_{18} esters, judged on the basis of completeness and time involved, have been obtained. A study of distribution coefficients of both single and mixed solutes and of certain column operating conditions has led to a concept of chromatography which predicts more effective separations than otherwise obtainable by the proper use of solutes of intermediate adsorption affinities. This prediction was verified experimentally. The similarities and differences between amplified chromatographic separations and those obtained by carrier displacement chromatography are briefly discussed.

The most effective chromatographic separations of the C_{18}-fat acids seem to be those of Riemenschneider, *et al.*,[2] who used silicic acid as adsorbent, petroleum ether for developing and took precautions to exclude air during the operation of the column. Pure methyl linoleate, first appearing in the eluate after 17 hours development, was isolated from the esters of the associated C_{18}-acids of tobacco seed oil in 27% yield. Similarly, from linseed oil, 98% methyl linolenate, first appearing in the eluate after 45 hours development, was isolated in 32% yield. Pure methyl linolenate was obtained by a repetition of this process on the best fractions. These separations were made for the purpose of obtaining samples of pure esters from natural products and did not reflect any extensive attempt to find optimum conditions.

Without differentiating between its components, Taylor has reported that air is held more tenaciously by silica gel than is water.[3] This indicates the difficulty that may be encountered in attempting to provide completely air-free conditions on a column. The extension of chromatography to the isolation of minor constituent unsaturated fat acids from mixtures would, therefore, appear to be favored by conditions affording maximum yields with a minimum time of development. The chromatographic process has been examined with this application in mind.

Materials. Solvents. **Petroleum Ether.**—Skellysolve F was distilled and the fraction of b.p. 35-50° collected. **Benzene.** A.C.S. thiophene-free grade benzene was washed repeatedly with water, dried over anhydrous potassium carbonate, filtered, distilled, and the fraction, b.p. 80°, collected. **Methanol.** A.C.S. 99.5% methanol was used as purchased.

Solutes. **Stearic Acid.**—Eastman Kodak Co. "Eastman" grade acid was crystallized three times from acetone; m.p. 68.5°, neut. equiv. 284.0 (calcd. 284.3). **Methyl stearate** was prepared from the purified stearic acid; m.p. 38.0°, acidity 0.2% (calcd. as stearic acid). **Methyl caproate** was prepared from Eastman grade caproic acid; b.p. 149.0-149.5°, $n^{20}\mathrm{D}$ 1.4048. **Methyl myristate** was prepared from Eastman grade myristic acid and a portion of uniform boiling point chromatographed on silica gel. A sample was selected for further work from the middle of a series with a uniform $n^{20}\mathrm{D}$ value of 1.4369. **Methyl oleate,** iodine value 85.6 (calcd. 85.7), $n^{20}\mathrm{D}$ 1.4522, which was prepared by the method of Wheeler and Riemenschneider[4] was used in obtaining the data of Fig. 1a and Tables I-III. That used in the remainder of the experiments was purchased from the Hormel Foundation, Austin, Minn.; iodine value 85.4, $n^{20}\mathrm{D}$ 1.4522. **Methyl linoleate,** iodine value 172.6 (calcd. 172.4), $n^{20}\mathrm{D}$ 1.4616 and **methyl linolenate,** iodine value 260.0 (calcd. 260.4), $n^{20}\mathrm{D}$ 1.4711, were purchased from the Hormel Foundation.

All melting points recorded above are corrected; boiling points are uncorrected. Each of the unsaturated esters was chromatographed and the refractive indices of the middle fractions, representing the major portion of each sample, were found to be identical with the values for the unchromatographed esters.

Adsorbents.—All adsorbents in Table I were used as purchased. **Silica gel.**—Standard silica gel, the type normally obtained from commercial sources, which was purchased from the Davison Chemical Corp., Baltimore, Md., was used throughout the investigation except as otherwise noted in Table I. For the data of Table I and of Fig. 1a the gel was used as purchased. Otherwise it was purified by extracting with acetone until no more soluble matter was obtained, drying with a current of air, washing with distilled water and activating at 175° for 18 hours. From this material the partially deactivated gels were prepared by contact with the vapors of the deactivating

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[1] Taken in part from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1951.

[2] R. W. Riemenschneider, S. F. Herb and P. L. Nichols, Jr., *J. Am. Oil Chemists' Soc.*, **26**, 371 (1949).

[3] R. K. Taylor, *Ind. Eng. Chem.*, **37**, 649 (1945).

[4] D. H. Wheeler and R. W. Riemenschneider, *Oil & Soap*, **16**, 207 (1939).

agent. The gel, unless otherwise noted, was of 200–325 mesh.

Methods. Iodine Value.—The Wijs method (30 min.) was used if the sample contained methyl linoleate or methyl linolenate. Otherwise, the Wijs or the Hanus method (30 min.) was used interchangeably.

Titrations with Alkali.—Except for the data of Table X, the acid content of a sample dissolved in 95% methanol was determined by the difference in the alkali requirements of the solvent and the solvent plus sample. The maximum contaminations recorded in Table X correspond to one extra drop of 0.01234 *N* sodium hydroxide solution which was sufficient, in each instance, to produce a deeper color than that of the blank.

Determination of *K* Values. First Method.—One hundred ml. of a solution of mixed esters was equilibrated with a weighed quantity of adsorbent at 20° for one hour. After gravity filtration, the solvent was removed under carbon dioxide, the esters were weighed and analyzed by determination of their iodine value. From these data the distribution of each ester between the adsorbent and the solution was calculated. In a number of instances the results were checked by analyses of the adsorbed esters which were eluted with acetone.

Second Method.—The filtration of the equilibrated esters without loss of solvent by evaporation was accomplished by connecting the flask in which they were contained through a filter tube provided with a sintered glass filter plate and two standard taper male joints to a similar flask, in which the air had been displaced with petroleum ether vapor, and plunging the second flask into cold water. Corrections for the solution retained mechanically by the filtration system were determined by carrying out this procedure with various amounts of completely deactivated gel. The values so obtained were used to correct the weights of recovered esters.

Third Method.—A solution of mixed esters of known concentrations was passed over a convenient weight of silica gel in a tube until the adsorbate was in equilibrium with the solution. The excess solution was removed with carbon dioxide pressure and the adsorbed esters recovered and analyzed. This method is extravagant in time and materials. It is also to be expected that, unless precautions were taken to eliminate oxygen, the more highly unsaturated esters would suffer considerable oxidation. With the methyl stearate–methyl oleate pair this method gave highly reproducible results and allowed determinations of *K* values for predetermined concentrations of each ester.

The comparisons in Table I were obtained in the following manner. A convenient weight of each adsorbent, depending upon its adsorption capacity, was added to an aliquot of the same solution. The $(K - 1)$ value for each adsorbent was determined and then multiplied by the multiplication factor required for raising to a value of 100 that value of silica gel on curve 1a, Fig. 1, which corresponded to the same total concentration of esters as that in the equilibrated solution.

The $(K - 1)$ values recorded in column 4, Table IV, are those obtained for the equilibrium concentrations in columns 1 and 2. To show the $(K - 1)$ values obtained with benzene–petroleum ether solutions, column 3, for the same total concentration of esters, *i.e.*, for the sum of the concentrations in columns 1 and 2 of the same line, the experimental $(K - 1)$ values obtained with the mixed solvents were plotted against total ester concentration as in Fig. 1 and from the resultant curves the desired values were obtained.

***K'* Values.**—The values in column 5, Table IV, were calculated from the data of Fig. 2 for the equilibrium concentration of each ester recorded in columns 1 and 2.

Chromatographic Separations. First Method.—A Pyrex tube, 23 mm. i.d., was provided with a sealed-in coarse grade sintered glass filter plate and a 2 mm. i.d. drip tube. It was packed with successive layers of anhydrous sodium sulfate, silica gel and anhydrous sodium sulfate and operated by maintenance of the solvent at a level sufficient to produce the desired flow-rate.

Second Method.—A 20-ml. test-tube, in the bottom of which a small hole had been drilled, was provided with a circle of hardened filter paper held over the hole by linen tape. The tube was filled with solvent and packed by means of centrifugal pressure for 15–20 minutes at 2000 r.p.m. (8 in. radius of rotation) after each addition of material. It was often found advisable to improve the planarity of a boundary by loosening a thin layer of material and recentrifuging. After adsorption of the solutes, the tube was connected to a reservoir of solvent and developed with the aid of an adjustable nitrogen pressure. It was found, by using dye solutions, that the most uniform movement of solutes appeared to take place on columns packed in this manner.

Following is a key to the experimental methods used in obtaining the data which are presented in the next section: Table I: first *K*-value method; Table II: third *K*-value method of equilibration; Table III: third *K*-value method; Table IV: second *K*-value method; Tables V–VII: second chromatographic method; Tables VIII–X: first chromatographic method; Fig. 1, curves 1a–5a, curves 1b–5b: first *K*-value method, second *K*-value method, respectively; Fig. 2: second *K*-value method without analysis.

Results and Discussion

Mathematical equations pertaining to partition chromatography[5], ion exchange chromatography[6] and discontinuous countercurrent distribution in tubes[7] which have been developed and verified experimentally, correlate the movement of solutes with their distribution coefficients and the number of theoretical plates in the system. These equations require constant distribution coefficients and, therefore, cannot be applied to separations, by elution chromatography, of the solutes studied here. This does not alter the basic importance of such values, however, in determining the effectiveness of a separation.

In this paper K is defined as equal to k_1/k_2, k_1 representing the distribution coefficient of the more strongly adsorbed solute between the adsorbent and the liquid phase and k_2 that of the less strongly adsorbed solute when the equilibrium is established in the presence of both solutes and K' is defined as equal to k_1'/k_2' in which the primes indicate that the constants are calculated from adsorption isotherms of single solutes. Although these values furnish a measure of the separability of two solutes on a column, their comparative separability under different conditions is related more directly to the ratio of the corresponding $(K - 1)$ or $(K' - 1)$ values. The relationships between a number of variables and the $(K - 1)$ or $(K' - 1)$ values of the C_{18}-esters are given below.

The relative selectivity of various adsorbents is shown in Table I. For separating the C_{18}-esters

TABLE I

COMPARISON OF VARIOUS ADSORBENTS[a]

Adsorbent	Relative $(K - 1)$ value
Standard silica gel	100
High-temperature silica gel	62
Silicic acid (Merck)	31
Alumina (Fisher)	90
Alumina (according to Brockmann)	86
Magnesium oxide	12
Darco	70
Coconut charcoal	24
Wood charcoal	22
Nuchar	14
Norit-A, neutral	2

[a] Relative $(K - 1)$ values determined in the system: methyl oleate–methyl stearate–petroleum ether–adsorbent.

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(5) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(6) S. W. Mayer and E. R. Tompkins, THIS JOURNAL, **69**, 2866 (1947).

(7) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).

the choice of an adsorbent seemed to lie between one of the aluminas and standard silica gel. It was found, however, that a considerable proportion of esters adsorbed on alumina could not be removed while adsorption on silica gel was completely reversible, as shown in Table II.

TABLE II

REVERSIBILITY OF ADSORPTION ON SILICA GEL[a]

Experiment[b] No.	Vol. of wash solution, ml.	Methyl oleate in adsorbed esters, %
1	0	...
1	25	12.4
1	100	21.3
1	200	22.5
1	300	22.8
2	0	100.0
2	300	23.1
2	400	23.0

[a] Equilibrium composition of adsorbed esters obtained by washing silica gel (2 g.) with a petroleum ether solution of 0.21 g. of methyl oleate and 1.86 g. of methyl stearate per 100 ml. at a flow-rate of 60 ml./hr. [b] Experiment 1, no initial adsorbate on silica gel. Experiment 2, 0.2 g. of methyl oleate adsorbed per g. of silica gel prior to washing.

The dependence of ($K - 1$) values on the equilibrium concentrations of the esters is illustrated by the data in Table III. Since the data were reproducible with considerable precision it was decided to correlate them by means of an empirical equation. For this purpose, from an analogy with the Freundlich equation, $q/x = kc^n$, the equation, $q_1/q_2 = a(c_1{}^m/c_2{}^n)$, was chosen. From the data of Table III, and expressing c in mg./100 ml., the following relationships were obtained

$$q_1/q_2 = 3.192\ (c_1{}^{0.8976}/c_2{}^{0.9556}) \qquad (1)$$

$$K = 3.192\ (c_2{}^{0.0444}/c_1{}^{0.1024}) \qquad (2)$$

TABLE III

RELATIONSHIP OF ($K - 1$) VALUES TO EQUILIBRIUM CONCENTRATION OF ESTERS

Concn. of methyl oleate, mg./100 ml.	Concn. of methyl stearate, mg./100 ml.	($K - 1$)
848	856	1.16[a]
187	1573	1.59[a]
18.7	157.3	1.96[b]

[a] The equilibrium was obtained by washing 2 g. of silica gel with 400 ml. of a petroleum ether solution of mixed esters at a flow-rate of 60 ml./hr. [b] The equilibrium was obtained by washing 2 g. of silica gel first with 15 ml. of the solution used in the preceding experiment and then with 800 ml. of this solution diluted with petroleum ether to the recorded concentration.

These relationships were tested with a solution containing 161 mg. of methyl oleate and 415 mg. of methyl stearate per 100 ml. The adsorbed esters contained 49.5% methyl oleate in comparison with a calculated value of 49.0%. It is likely that more extensive and accurate data might indicate that additional terms were needed to express completely the relationships.

Equations of the type of (1) and (2) can be utilized in reaching certain conclusions regarding the chromatographic process. They indicate no limit to the enrichment of methyl stearate during either the development or the predevelopment adsorption stage of a separation, but a limit, during either stage, to the enrichment of methyl oleate. This limit can be calculated for the adsorption stage from equation (1) and for the development stage from equation (2). Since, from equation (2), high K values are favored both by a low concentration of esters and by a low ratio of methyl oleate to methyl stearate, the limiting c_1/c_2 ratio, corresponding to a K value of 1, would depend upon the total concentration of the esters. The equilibrium c_1/c_2 ratio, for a K value of 1, can be shown, from equation (2), to equal $3.192^{22.5221}/c_1{}^{1.3063}$. If c_1 were 6 mg./100 ml., as was obtained for methyl oleate in its separation from methyl stearate (see Table V), the equilibrium c_1/c_2 ratio would be about 2×10^{10}. While this extrapolation is far beyond that warranted by the accuracy of the data, it seems justifiable to conclude that the limiting ratio is large enough to permit the isolation of what would normally be considered pure methyl oleate. It is also of interest to obtain an indication of the rate of enrichment at a lesser ratio, say 10^4, for which component 1 would still be considered pure. For $c_1 = 6$ mg./100 ml. K would be equal to 1.9 which compares favorably with the value of 2.2 calculated for the preadsorption concentrations of 696 mg./100 ml. of methyl oleate and 692 mg./100 ml. of methyl stearate (Table V). It can be concluded that the limitation deduced from equation (2) still permits the complete separation, from a practical viewpoint, of the two esters because of both an adequate rate of enrichment and an adequate limiting ratio.

The effect of an additional component on the separability of methyl oleate from methyl stearate

TABLE IV

SEPARABILITY OF ESTER-PAIRS UNDER VARIOUS CONDITIONS[a]

Concentration of ester, mg./100 ml.		$K - 1$	$K - 1$	$K' - 1$
Component 1 Me oleate	Component 2 Me stearate	Petroleum ether–benzene solution	Petroleum ether soln.	Petroleum ether soln.
25	48	..	1.73	1.05
193	235	..	1.12	0.25
386	427	..	0.88	.12
54	373	..	1.57	4.32
193	235	..	1.12	0.25
352	79	..	0.71	(−) .66
Me linoleate	Me oleate	3% benzene–97% petroleum ether		
19	40	1.10	1.59	0.84
175	238	0.85	1.33	.37
364	434	.55	0.92	.23
54	372	.75	1.15	4.45
175	238	.85	1.33	0.37
316	87	.93	2.03	(−) .64
Me linolenate	Me linoleate	6% benzene–94% petroleum ether		
165	223	1.04	1.40	0.35
354	408	0.83	0.85	.16
50	347	.86	1.28	5.37
165	223	1.04	1.40	0.35

[a] The first group under each ester-pair illustrates the effect of concentration on their separability with the ratio of the two esters relatively constant—the second group, the effect of their ratio with concentration relatively constant.

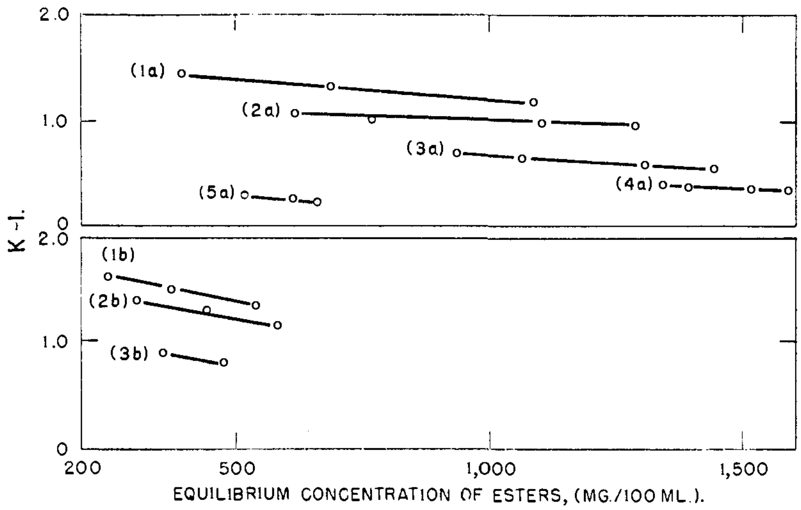

Fig. 1.—Effect of partial deactivation on (K − 1)-values in the system: silica gel–methyl oleate–methyl stearate–solvent.

Curve No.	Solvent	Adsorbent
1a	Petroleum ether	Fully activated silica gel
2a	Petroleum ether	Silica gel deactivated with 4.0% water
3a	Petroleum ether	Silica gel deactivated with 12.0% water
4a	Petroleum ether	Silica gel deactivated with 12.0% methanol
5a	Benzene	Fully activated silica gel
1b	Petroleum ether	Fully activated silica gel
2b	Petroleum ether	Silica gel deactivated with 1.4% water
3b	Petroleum ether	Silica gel extracted with hydrochloric acid and reactivated

is illustrated in Fig. 1, the data of which are strictly comparable only within each section. Water and methanol, which are adsorbed more strongly than either of the esters, and benzene, with an adsorption affinity between that of methyl stearate and the solvent, decrease the selectivity of the gel as shown by lower K values, and in proportion to the amount present. Smaller concentrations of benzene also lower the K values as seen in Table IV. Curve 3b in Fig. 1 was obtained with a gel which had been pretreated with 30% hydrochloric acid and could not be restored to its original activity. The partial loss of activity is likely due to adsorbed chlorides and represents the effect of a very strongly held additive.

The effect of temperature on K values was found to be small. Compared with an arbitrary (K − 1) value of 100 at 25°, the value at 14° was found to be 107 and at 2° to be 111. Relative to actual separations it is to be expected that this trend would be opposed by a lower column efficiency at lower temperatures.

The data in Table IV extend the study of the relationship between solute concentration and (K − 1) values to other esters. It is to be noted that, in contrast to the trend found with methyl stearate and methyl oleate, the (K − 1) values increase directly with an increase in the ratio of the more highly adsorbed to the less highly adsorbed solute. Due to the analytical problems involved with the more highly unsaturated esters and their greater susceptibility to oxidation, it is felt that, pending confirmatory data, this trend should be accepted with reservation. As recorded here, it indicates a limitation to the enrichment of the less strongly adsorbed solute which, as before, would still permit a complete separation. The (K' − 1) values in column 5 afford a measure of the relative movement of pure solutes on a column. The data emphasize the fact that these values have a much greater dependence on the c_1/c_2 ratio than do the (K − 1) values. Within a thirtyfold increase in this ratio, the separability of pure solutes decreased to zero from values higher than those of the corresponding mixed solutes.

The preceding paragraphs have been concerned with the separability of solutes as related to the equilibrium values of their distribution coefficients. The discussion that follows concerns the relationship of their separability to certain factors involved in the practical operation of a column. For ease and accuracy of analysis of relatively small volumes of eluate the solute pair methyl oleate–stearic acid was employed in this phase of the investigation.

If conditions were such that initially a very incomplete separation was obtained, a decrease in the flow-rate during development, a decrease, with the total quantity of solute constant, of the ratio of component 1 to component 2, a decrease in the particle size of the adsorbent, or an increase in the ratio of adsorbent to solute resulted, as expected, in an improved separation. It was noteworthy, however, that as conditions were made progressively more favorable a limit to the separations, in which

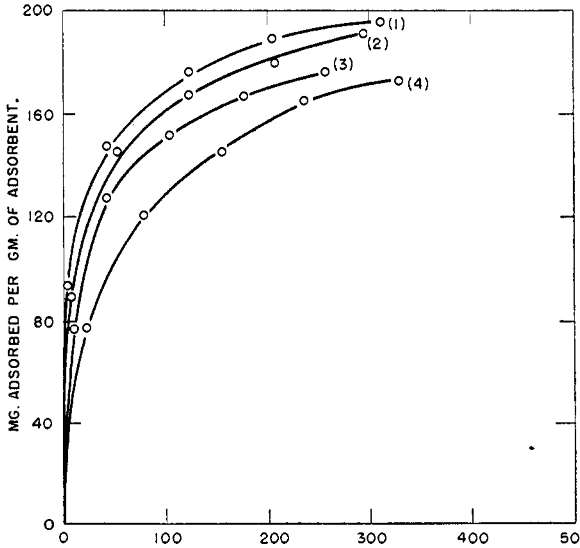

Fig. 2.—Adsorption isotherms at 20° in the system: silica gel–petroleum ether–ester: 1, methyl linolenate; 2, methyl linoleate; 3, methyl oleate; 4, methyl stearate.

the unseparated solutes were still a significant proportion of the total, was rather quickly reached. This behavior is discussed further in the section on amplified separations.

In comparison with the concentration previously used, a 33-fold dilution of the solutes, prior to adsorption on a column, resulted in a separation which was both less complete and less rapid. This result is in conflict with various recently published recommendations of the use of dilute solutions, a view supported by the relationship, shown in this paper, between K values and solute concentration. The following is offered as an explanation of the above stated result and in an attempt to harmonize the conflicting views to be found on this subject in the literature. It can be stated that the minimum requirement for a complete separation is the simultaneous appearance in the eluate of the trailing edge of component 2 and the leading edge of component 1. This condition is satisfied if $v_2/v_1 = x_a/(x_a - x_1)$ in which x_a represents the length of the column of adsorbent, x_1 that of the adsorbed band of component 1 at the completion of the predevelopment adsorption, v_2 the average rate of travel of the trailing edge of component 2 and v_1 that of the leading edge of component 1 during development. With increasing values of $x_a/(x_a - x_1)$ relative to v_2/v_1 a mixed solute zone would appear and become progressively larger. When dealing with concentrations less than necessary to saturate the adsorbent, dilution would increase both ratios. In general, one would expect v_2/v_1 to increase fairly uniformly while the increase in $x_a/(x_a - x_1)$ would be critically dependent, for any system, upon its original value. In separations employing a sufficiently high adsorbent–solute ratio, dilution could, conceivably, favorably affect the degree of separation obtained. In separations employing a minimum adsorbent–solute ratio, as was used by the author to minimize the required development, dilution would be expected to affect the separations adversely.

Relative to a given separation, one obvious way to handle larger quantities of solutes, without loss of efficiency or increase of experimental time, is to operate several columns of the same dimensions in parallel. By varying the dimensions of a single column, equally efficient separations were obtained by increasing both the flow-rate and the column-length, with a constant diameter, by the same ratio as that of the solutes being separated. When, however, the increased quantity of adsorbent was used in a wider tube, stearic acid, the more strongly adsorbed solute, contaminated the methyl oleate from its initial appearance in the eluate.

The separations of the C_{18}-esters, summarized in Tables V–VII, reflect the use of the most selective adsorbent examined by the author and the use of centrifugally packed columns. Otherwise, no attempt was made to ascertain and use the most favorable combination of variables since the data presented up to this point indicated that, at best, the separations would be less complete than if obtained by the amplified method which is discussed in the next section. From a use of the refractive index, reproducible through the fourth decimal place, of a chromatographed pure ester as a standard and a knowledge of the shapes of the elution curves of mixtures, it is considered likely that the correct refractive index of a single fraction represents a purity of 99–100%, while that of a member of a series with the same value represents a purity nearer the upper limit. The iodine value of lot 19, Table V, the only single fraction with a correct refractive index, indicated a purity of 99.7%. Using the word "pure" as thus indicated, the recovery of

TABLE V

SEPARATION OF METHYL STEARATE FROM METHYL OLEATE[a]

Lot No.	Vol. of eluate, ml.	Wt. of esters, mg.	Analysis 0.01017 *N* $Na_2S_2O_3$	Wt. of Me stearate mg.	Wt. of Me oleate, mg.
1	300	49.8	0.00	49.8	0.0
2	75	18.5	.00	18.5	.0
3	75	14.7	.00	14.7	.0
4	75	11.2	.47	10.5	.7
5	75	9.6	.43	8.9	.7
6	75	7.5	.65	6.5	1.0
7	75	6.7	.64	5.8	0.9
8	75	5.8	.50	5.1	.7
9	75	4.9	.57	4.0	.8
10	82	4.3	.94	2.9	1.4
11	102	5.5	1.36	3.5	2.0
12	75	3.2	1.12	1.5	1.7
13	75	3.3	1.37	1.2	2.1
			n^{20}D		
14	75	3.5	1.4495	1.2	2.3
15	75	3.7	1.4502	1.0	2.7
16	110	6.8	1.4504	1.6	5.2
17	98	5.9	1.4506	1.2	4.7
18	100	6.7	1.4517	0.4	6.3
19[b]	50	99.2	1.4522	.3[c]	99.9

[a] 138.3 mg. of methyl stearate plus 139.1 mg. of methyl oleate were dissolved in 20 ml. of petroleum ether and adsorbed on 3.00 g. of silica gel, 3.6 cm. high by 1.3 cm. wide. The column was developed with the same solvent at 280 ml./hr. [b] Benzene was added to the column. [c] Calculated from an iodine value of 85.4.

TABLE VI

SEPARATION OF METHYL OLEATE FROM METHYL LINOLEATE

Lot No.	Vol. of eluate, ml.	Wt. of esters, mg.	Analysis n^{20}D	Wt. of Me linoleate, mg.	Wt. of Me linoleate, mg.
1	100	0.0	....	..	..
2	100	14.7	1.4522	14.7	0.0
3	100	41.8	1.4522	41.8	.0
4	100	32.7	1.4522	32.7	.0
5	100	23.4	1.4522	23.4	.0
6	100	20.1	1.4526	19.2	.9
7	100	13.0	1.4529	12.2	1.0
8	200	24.3	1.4547	17.8	6.5
9	200	19.6	1.4569	9.8	9.8
10	200	14.6	1.4579	5.7	8.9
11	300	21.0	1.4589	5.6	15.4
12	300	34.4	1.4602	5.1	29.3
13	300	25.7	1.4616	0.0	25.7
14[b]	300	38.1	1.4616	.0	38.1
15	300	30.3	1.4616	.0	30.3
16	300	24.0	1.4621	..	..

[a] 202.3 mg. of methyl oleate plus 191.3 mg. of methyl linoleate were dissolved in 20 ml. of petroleum ether and adsorbed on 4.00 g. of silica gel, 4.8 cm. high by 1.3 cm. wide. The column was developed with 2% benzene in petroleum ether at 500 ml./hr. [b] The benzene in the solvent was increased to 3%.

TABLE VII

SEPARATION OF METHYL LINOLEATE FROM METHYL LINOLENATE[a]

Lot. No.	Vol. of eluate, ml.	Wt. of esters, mg.	Analysis $n^{20}\text{D}$	Wt. of Me linoleate, mg.	Wt. of Me linolenate, mg.
1	100	0.0	....	..	..
2	100	27.2	1.4615	27.2	0.0
3	100	40.6	1.4616	40.6	.0
4	100	31.7	1.4616	31.7	.0
5	100	23.7	1.4616	23.7	.0
6	100	17.8	1.4619	17.2	.6
7	100	14.6	1.4630	12.5	2.1
8	100	11.6	1.4640	8.7	2.9
9	200	21.4	1.4660	11.6	9.8
10	200	18.0	1.4672	7.5	10.5
11	300	24.8	1.4687	6.5	18.3
12	300	30.4	1.4697	4.7	25.7
13	300	30.0	1.4711	0.0	30.0
14	300	28.8	1.4711	.0	28.8
15	400	35.0	1.4711	.0	35.0
16	300	9.3	1.4711	.0	9.3
17	300	4.0	....	..	..

[a] 202.2 mg. of methyl linoleate plus 199.3 mg. of methyl linolenate were dissolved in 20 ml. of petroleum ether and adsorbed on 4.00 g. of silica gel, 4.8 cm. high by 1.3 cm. wide. The column was developed with 4% benzene in petroleum ether at 500 ml./hr.

pure esters varied from 49–71% and the appearance, in the eluate, of the more strongly adsorbed pure ester of each pair occurred within 4–6.5 hours.

A Concept of Amplified Chromatographic Separations.—Schwab and Jockers have reported instances of increased separability of inorganic ions in the presence of a third ion which is adsorbed between them.[8] Strain has stated[9] that similar effects have been observed in separations of extracts of plant and animal materials. Tiselius suggested the use of solutes of intermediate adsorption affinities, in displacement chromatography, for separating the contiguous zones of pure solutes he stated were obtained in this process.[10] He has since implemented[11] this suggestion in separations of amino acids and peptides. In neither paper did he discuss the existence of zones of mixed solutes; in the latter he stated that the close contact of the zones in displacement chromatography caused some difficulty in properly cutting the fractions. The new procedure, named "carrier displacement chromatography," has been applied by Holman to the separation of saturated fatty acids.[12] His data, however, show a continuous elution of acids instead of the expected acid-free zones. Synge and Tiselius[13] have mentioned the possibility that some phenomena observed in the elution of adsorption columns with successively different solvents might be interpreted as resulting from this type of displacement.

In view of the vast literature of chromatography which has appeared in the 13 years which elapsed between the observations of Schwab and Jockers and the presentation by Tiselius and Hagdahl of the method of carrier displacement chromatography it appears likely that there has been no general understanding of the potential usefulness of solutes of intermediate adsorption affinities for improving separations. The concept of amplified chromatographic separations which was developed by the author prior to his contact with the work of Tiselius and Hagdahl[11] supplements their work and the earlier concept of Tiselius[10] in several ways. It is based on the quantitative relationships presented earlier in this paper, it considers the nature of the zones of mixed solutes and it deals with elution chromatography in which the action of an intermediately adsorbed solute is somewhat different from that of a similar solute in displacement chromatography.

During a separation of two solutes, A and C, of which A is the more strongly adsorbed, movements in zones of mixed solutes are governed by the relationships found for K values, in zones where pure solutes have separated by those for K' values. Since, as deduced from equations (1) and (2), K values of the magnitude reported in this paper permit a complete separation of the two solutes, the typical appearance of mixed solutes in the eluate must have a basis other than inadequate K values. There are a number of conditions which could lead to a zone of mixed solutes through mixing, either within the column or in the eluate. For simplicity, the discussion here is limited to the effect of a non-planar top surface of the adsorbent but it could, without difficulty, be extended to include other adverse conditions. Figure 3a represents a complete separation within a column of solutes A and C. However, due to the non-planar A/C interface, a mixture of both solutes would appear in the eluate unless it were possible for C to draw away from A to such an extent that all of it was lower in the column than the lowest boundary of A. This, however, is not possible. Movement of the tail of C is restricted to decreasing in concentration until $K' - 1$ is zero. Since K' values vary inversely with concentration, it is conceivable that, with dilute solutions of solutes having large K' values, the A/C ratio at their common boundary would become large enough that the separation would be considered complete. However, with K' values of the magnitude found for the esters studied in this paper, an appreciable mixed solute zone would appear at all reasonably high concentrations.

The effect of progressively more favorable conditions, noted earlier, can be rationalized on the basis of the above discussion since they would be expected to produce a comparatively rapid improvement in a separation as long as they contributed to movement of the solutes toward the positions shown in Fig. 3a, but not thereafter. This view stresses the extravagance of prolonged development as a means of greatly increasing the yields of pure solutes. The optimum development would seem to be that producing a degree of separation improvable only through lowering the concentrations of the solutes at the A/C interface. At this point, the more economical procedure would be to isolate the mixed solutes and rechromatograph them.

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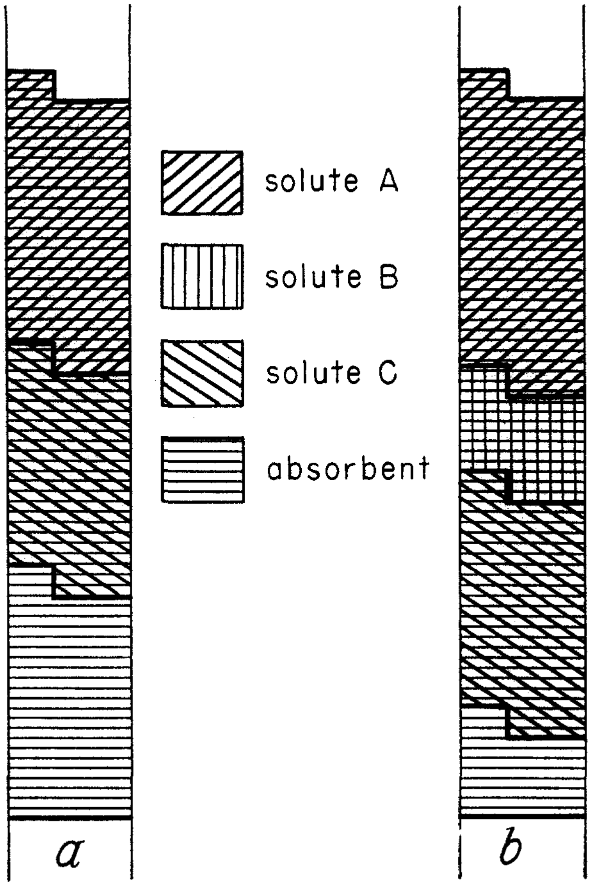

Fig. 3.—Representation of a concept of amplified chromatographic separations.

TABLE VIII

AMPLIFIED SEPARATION OF METHYL STEARATE FROM METHYL OLEATE WITH METHYL MYRISTATE AS THIRD SOLUTE[a]

Lot No.	Vol. of eluate, ml.	Wt. of solute, mg.	Analysis n^{30}D
1	500	104	Solid
2	100	44	1.4384
3	200	72	1.4375
4	200	53	1.4373
5	200	39	1.4371
6	200	33	1.4369
7	200	27	1.4369
8	200	21	1.4370
9	200	19	1.4369
10	280	22	1.4369
11[b]	12	134	1.4422

[a] 501 mg. of methyl myristate, 95 mg. of methyl oleate and 103 mg. of methyl stearate were dissolved in 40 ml. of petroleum ether and adsorbed on 7.00 g. of silica gel, 2.8 cm. high by 2.3 cm. wide. The column was developed with the same solvent at 200 ml./hr. [b] The petroleum ether was replaced with acetone.

TABLE IX

AMPLIFIED SEPARATION OF METHYL OLEATE FROM STEARIC ACID WITH METHYL CAPROATE AS THIRD SOLUTE ADDED TO SOLUTION PRIOR TO DEVELOPMENT[a]

Lot No.	Vol. of eluate, ml.	Wt. of solute, mg.	Contaminant, mg.
		Methyl oleate	Stearic acid[b]
1	500	51	0.0
2	500	59	.0
3	500	59	.0
4	500	19	.0
5	500	3	.0
6	1200	2	.0
		Stearic acid	Methyl oleate[c]
7[d]	100	0	0
8[e]	100	68	10.8
9	100	34	6.7
10	100	53	0.0
11	100	37	.1
12	100	6	.1
13	100	1	.1

[a] 202.7 mg. of stearic acid plus 211.1 mg. of methyl oleate plus 1.9954 g. of methyl caproate were dissolved in 100 ml. of petroleum ether and adsorbed on 24 g. of silica gel, 9.4 cm. high by 2.3 cm. wide. The column was developed with the same solvent at 30° at 350 ml./hr. [b] Determined by maintenance of color of neutralized alcoholic phenolphthalein solution when added to combined lots 1–6. [c] Determined by addition of each sample to 1 ml. of Wijs solution (30 min.) and titration with 0.0201 *N* thiosulfate solution. [d] The solvent was replaced with 3% AcOH in petroleum ether. [e] The solvent was replaced with 0.5% AcOH in petroleum ether.

The expected action of a third solute, adsorbed between A and C, is represented in Fig. 3b. B would enable C to draw away from A by establishing *K* value conditions. Its initially greater eluting effect on C than on A would then be increased by its greater concentration at the B/C than at the B/A interface. If easily removable, it would then be of practical value in effecting their separation.

For a qualitative test of this concept methyl myristate, known to have an intermediate adsorption affinity, was chosen as the third solute for separating methyl oleate from methyl stearate. Prior separations, without the third solute, had all been characterized by a continuous elution of the esters, confirming the deductions made from a consideration of their *K* and *K'* values. The data of Table VIII show that an intermediate zone, represented by over 1000 ml. of eluate, free from both methyl stearate and methyl oleate, was obtained before the development was terminated by the addition of acetone.

As before, extensive experiments had shown that separations of methyl oleate and stearic acid were characterized by a zone of mixed solutes which could not be eliminated by the use of more favorable conditions. Methyl caproate, with an intermediate adsorption affinity and volatile on a steam-bath, permits a quantitative test of the concept. The results obtained with a solution of the three solutes are recorded in Table IX. A middle zone, free from A and C, again was obtained. However, a significant amount of C remained associated with A. Table X records the results obtained by adsorbing a mixture of methyl oleate and stearic acid on a column, carrying out a predevelopment with controlled amounts of methyl caproate in petroleum ether and continuing the development with the solvent alone. The eluate was filtered through a fine-grade sintered glass filter to ensure freedom from silica gel. The methyl oleate recovered from the eluate was shown to be free from methyl cap-

TABLE X

AMPLIFIED SEPARATION OF METHYL OLEATE FROM STEARIC ACID WITH METHYL CAPROATE AS THIRD SOLUTE ADDED TO DEVELOPING SOLVENT

Expt. No.	Lot No.	Vol. of eluate, ml.	Wt. of methyl oleate, mg.	Contamination, % stearic acid
1[a]	1	500	55.8	<0.1
	2	500	40.6	
	3	500	0.3	
2[b]	1	500	73.0	< .1
	2	500	25.3	
	3	500	0.0	
3[c]	1	500	96.1	< .1
	2	500	4.2	
	3	500	0.0	
4[d]	1	1000	100.6	< .07
	2	500	0.0	
	3	500	0.0	

[a] 102.8 mg. of methyl oleate plus 104.2 mg. of stearic acid were dissolved in 100 ml. of petroleum ether and adsorbed on 12.0 g. of silica gel, 4.7 cm. high by 2.3 cm. wide. Predevelopment with 200 ml. of 0.5% methyl caproate in petroleum ether and development with petroleum ether at 25° at 350 ml./hr. [b] Same solute and adsorbent as in (a). Predevelopment with 300 ml. of 0.5% methyl caproate in petroleum ether and development with petroleum ether at 28° at 350 ml./hr. [c] Same solute and adsorbent as in (a). Predevelopment with 500 ml. of 0.5% methyl caproate in petroleum ether and development with petroleum ether at 25° at 400 ml./hr. [d] 102.2 mg. of methyl oleate plus 100.9 mg. of stearic acid. The other details of solution and adsorbent the same as in (a). Predevelopment with 1000 ml. of 0.25% methyl caproate in petroleum ether and development with petroleum ether at 25° at 400 ml./hr.

roate, $n^{20}\text{D}$ 1.4048, by its correct value of $n^{20}\text{D}$ 1.4522. The best recovery, 98.4% in 2.5 hours, does not appear to be the limit obtainable by this method.

In comparing amplified chromatographic separations with those obtained by carrier displacement chromatography it can be said that in each type the separation of the tail of C from the head of A is initiated by the presence of B which permits the movement of both B relative to A and of C relative to B to proceed according to the relationships found for *K* values. Thereafter, the situation is different. In carrier displacement chromatography the beneficial effect of B on the separation is restricted to its maintenance of *K* value conditions. In amplified chromatographic separations the greater concentration of B at the B/C than at the B/A interface produces an amplified movement of C relative to A, hence the use of this work in describing the process. The data recorded in Table IX indicate the amplifying effect of B may be large and, therefore, of potential value in effecting otherwise difficult separations. Pending a fuller investigation of the relationships, it appears from the data of Tables IX and X that it is desirable to employ a uniform concentration of solute B in the preliminary stages of a separation. This can be accomplished, either as in carrier displacement chromatography, through the use of a displacing agent in the development of a solution of the three solutes or, as in Table X, by a predevelopment of A and C with a solution of B. Presumably when, in a separation, A and C had reached positions in the column corresponding to those in Fig. 3a it would be safe to utilize the amplifying effect of B to speed the further separation of C from A.

WASHINGTON 25, D. C.

RECEIVED APRIL 14, 1951

[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Optically Active 1-Cyclohexenyl- and 1-Cyclopentenylmethylcarbinols[1]

BY JAMES ENGLISH, JR., AND VINCENT LAMBERTI

1-Cyclohexenylmethylcarbinol and 1-cyclopentenylmethylcarbinol have been prepared in optically active form through the brucine salts of their hydrogen phthalates. The mutarotation of these compounds in the liquid state has been found due to ether formation.

Substituted allyl alcohols have been the subject of considerable study by a number of workers,[2] particularly from the point of view of their rearrangements. Kenyon[3] and coworkers reported the preparation and resolution of several alkyl-substituted allyl alcohols and observed the anomalous mutarotations of these substances. Kenyon, Partridge and Phillips[4] reported the retention of optical activity of α-phenyl-γ-methylallyl alcohol during its rearrangement. Prior to the recent publications of Braude, *et al.*,[5] no examples of allylic alcohols in which the allylic system is part of a ring are reported. These workers studied the rearrangement in acid solution of some cyclohexenyl- and cyclopentenyl-substituted carbinols in optically inactive form. All of these rearrangements seem, in the light of recent work,[2] to have been acid catalyzed, whether or not acid was deliberately added.

Since a similar spontaneous change in rotation was observed by Kögl[6] in the crystalline auxins **a** and **b**, it seemed of interest to prepare some simple models in which the allylic system is located on a ring as in the structures proposed for the auxins. The structures first chosen were 1-cyclohexenylmethylcarbinol and 1-cyclopentenylmethylcarbinol. The method of synthesis used is indicated below.

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[1] Taken from the dissertation of Vincent Lamberti presented to the Graduate School of Yale University in partial fulfilment of the requirement for the Ph.D. degree.

[2] E. A. Braude, *Ann. Repts. on Progress. Chem.* (*Chem. Soc. London*), **46**, 126 (1950).

[3] H. W. J. Hills, J. Kenyon and H. Phillips, *J. Chem. Soc.*, 576 (1936); B. C. Platt, *ibid.*, 316 (1941); R. S. Airs, M. P. Balfe and J. Kenyon, *ibid.*, 20 (1942); C. L. Arcus and J. Kenyon, *ibid.*, 312 (1938).

[4] J. Kenyon, S. M. Partridge and H. Phillips, *ibid.*, 207 (1937).

[5] E. A. Braude and W. F. Forbes, *ibid.*, 1755 (1951).

[6] F. Kögl, C. Konigsberger and H. Erxleben, *Z. physiol. Chem.*, **244**, 266 (1936).