can be correlated with their r's at selected relative lengths α_e in accordance with the theoretical relationship between these two quantities.

AKRON 16, OHIO RECEIVED JULY 13, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, A. AND M. COLLEGE OF TEXAS, AND THE TEXAS AGRICULTURAL EXPERIMENT STATION]

Displacement Analysis of Lipids. II. Increased Separability of Fatty Acids by Depressed Solubility[1]

BY LENNART HAGDAHL[2] AND RALPH T. HOLMAN

Introduction

Displacement analysis, developed by Tiselius,[3] has been shown to be an effective technique in chromatographic investigations. The displacement development of a chromatogram by a substance which is adsorbed more strongly than any constituent of the sample, separates the constituents into zones, the least adsorbed constituent migrating first. Theoretically, with significant differences in adsorption, the separated constituents should be virtually quantitatively recoverable. Displacement development has been used successfully for the separation of sugars,[3,4] peptides[5] and amino acids.[5]

Considerable work has been done on the chromatographic separation of fatty acids by elution techniques,[6,7,8] but displacement separation of fatty acids has received little attention. Unfortunately, attempts by Claesson[9] to displace saturated fatty acids on charcoal by their higher homologs were unsuccessful, and he concluded that displacement analysis could not be applied to this homologous series with the adsorbents tried. He did, however, report group separation of saturated, unsaturated and branched fatty acids on silica gel by displacement.[10] After coupled filters and a mixer[11] were developed and added to the Tiselius–Claesson apparatus,[12] the improved performance of the instrument allowed the displacement separation of saturated fatty acids to be observed under conditions similar to those of Claesson's experiments. Thus, for example, the present authors[13] were able to separate lauric, myristic, palmitic and stearic acids by development using picric acid as displacer on Carbo Activ charcoal. The present report is a continuation of these investigations, extending the usefulness of displacement separation to the lower and higher fatty acids.

Methods

The fatty acids used in this investigation were Eastman Kodak Co. Organic Chemicals. Adsorption isotherms were made at 25° using 1-g. samples of adsorbent shaken ten minutes with 25 ml. of solutions of differing concentrations of fatty acid. After equilibration, the adsorbent was removed by filtration and aliquots of the filtrate were titrated.

The instrument used for the chromatographic experiments was a modified copy of the Tiselius–Claesson interferometric adsorption analysis apparatus[12] built by D. Milton Kvanbeck of Minneapolis. In all experiments the mixer and coupled filters developed by Hagdahl[11] were used. All frontal and displacement analyses were made using 1 part Darco G 60 charcoal and 2 parts Hyflo Supercel as adsorbent.

Experimental and Results

In order to continue in this country the studies begun at Uppsala[13] it was realized that a locally-available adsorbent was necessary. Therefore, investigations on the European charcoals were discontinued, and a search for an adsorbent available on the American market was begun. Adsorption isotherms were measured for a variety of representative adsorbents using 95% alcohol solutions of lauric and myristic acids. The adsorbents were evaluated as regards the shape of the isotherms and the difference between adsorption isotherms for myristic and lauric acids on a given adsorbent. The data thus gained are summarized briefly in Table I. According to the theory of displacement analysis put forth by Tiselius, the separability of substances can be predicted from an inspection of the respective isotherms.

Judging from the isotherms it appears that several of the adsorbents could be used for separation of fatty acids and are superior to Carbo Activ and Carboraffin Supra which were used in the preliminary studies.[13] From these, Darco G60 was chosen as a suitable adsorbent for separation of

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[1] Presented at the 115th A. C. S. meeting, San Francisco, March, 1949. This investigation was supported by grants from the Research Corporation and the National Dairy Council on behalf of the American Dairy Association.

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[3] Tiselius, *Arkiv. för Kemi Min. Geol.*, **16A**, 18 (1943).

[4] Tiselius and Hahn, *Kolloid Z.*, **105**, 177 (1943).

[5] Tiselius, The "Svedberg," Almquist and Wiksells, Stockholm (1944).

[6] Kaufman and Wolf, *Fette u. Seifen*, **50**, 519 (1943).

[7] Cassidy, THIS JOURNAL, **62**, 3076 (1940); **63**, 2735 (1941).

[8] Dutton, *J. Phys. Chem.*, **48**, 179 (1944).

[9] Claesson, *Arkiv för Kemi Mineral. Geol.*, **23A**, 1 (1946).

[10] Claesson, *Rec. trav. chim.*, **T65**, 9 (1946).

[11] Hagdahl, *Acta Chem. Scand.*, **2**, 574 (1948).

[12] Tiselius and Claesson, *Arkiv. för Kemi Mineral. Geol.*, **15B**, 18 (1942).

[13] Holman and Hagdahl, *Arch. Biochem.*, **17**, 301 (1948).

TABLE I

ADSORPTION ISOTHERMS OF LAURIC AND MYRISTIC ACIDS ON VARIOUS ADSORBENTS

Adsorbent	Fatty acid	Equilibrium concentrations, mg./g. 0.25%	0.50%	0.75%	1.00%	1.25%
Darco G60	C_{14}	59	82	91	97	99
	C_{12}	38	59	70	78	81
Nuchar grade XXX	C_{14}	68	92	109	115	116
	C_{12}	42	61	73	79	80
Nuchar grade C500	C_{14}	95	128	145	154	159
	C_{12}	74	99	117	128	135
Nuchar C190	C_{14}	117	154	176	189	195
	C_{12}	90	117	133	144	154
Nuchar C115	C_{14}	108	133	148	159	169
	C_{12}	72	102	117	130	141
Nuchar WA	C_{14}	28	35	36	37	38
	C_{12}	22	29	31	32	32
Cliffchar Airfloat	C_{14}	0	0	0	0	0
	C_{12}	0	0	0	0	0
Cliffchar Activated	C_{14}	76	86	92	95	97
Carbon	C_{12}	65	76	83	87	89
Norit A	C_{14}	74	95	105	112	116
	C_{12}	52	73	80	84	86
Charcoal (5-690)	C_{14}	106	140	154	161	164
(Fisher)	C_{12}	74	100	114	123	129
Activated carbon Grade P W (Columbia)	C_{14}	164	195	213	227	237
	C_{12}	93	125	150	170	181
Activated charcoal	C_{14}	62	74	81	85	91
(Merck)	C_{12}	43	56	64	70	76
Carboraffin Supra	C_{14}	35	55	72	85	95
(Lurgi, Prag)	C_{12}	22	36	47	57	68
Carbo Activ	C_{14}	27	35	37	37	37
(Merck)	C_{12}	18	26	30	30	31
Carbon Black G	C_{14}	0	...	...	...	...
(Fisher)	C_{12}	0	...	...	...	...
Silica Gel S-157	C_{14}	0	...	...	...	...
(Fisher)	C_{12}	0	...	...	...	...
Silica Gel T-200 22-08-	C_{14}	0	...	...	...	...
x1926 (Davison)	C_{12}	0	...	...	...	...

acids of medium chain length in alcohol. This charcoal has been previously used by Cassidy[7] in studies of the adsorption and separation of the higher fatty acids and by Cheldelin and Williams[14] in their studies of the adsorption of the lower acids. Dutton[8] also used this charcoal for separation of oleic and stearic acids.

In the course of determining adsorption isotherms on the various fatty acids, it was observed that adsorption of fatty acids from 95% alcohol was greater than from absolute alcohol. Therefore, the effect of water concentration of the solvent was investigated, and it was found that adsorption of the poorly-adsorbed short-chain acids could be increased markedly by the use of aqueous alcohol as solvent.

The dependence of adsorption upon solubility was suggested by Lundelius[15] from his experiments on the adsorption of iodine from various solvents. Sata[16] has shown that an inverse relationship exists between the solubility of picric acid in water–alcohol mixtures and its adsorption, and Landt and Knop[17] have described a similar phenomenon with propionic and valeric acids in aqueous alcohol solutions.

In Fig. 1 the effect of alcohol concentration in the solvent is shown. Reducing the alcohol from 100 to 40% increases the adsorption of caprylic acid more than 10 times. It is to be noticed also, that as the adsorption increases, the isotherms gradually change from nearly a straight line in 100% alcohol, to rather sharp curves when the water content of the solvent becomes considerable. This is an advantage in favor of separation of the fatty acids by displacement analysis, provided the isotherms for related acids are similarly curved.

From the straight-line character of the isotherms of caprylic acid in absolute and 90% alcohol, it can be predicted that displacement separation of this acid and its closely related acids should not be feasible, although separation should be achieved by elution. Inasmuch as displacement analysis is the better technique, attempt was made to separate caprylic acid from its lower homologs by increasing the water content of the solution.

Isotherms for butyric, caproic and caprylic acids in 95 and 50% alcohol solutions are shown in Fig. 2. The isotherms indicate that increasing the water content of the solvent increases the adsorption of the higher acid far more than that of the others, in effect, spreading the isotherms. This phenomenon has been demonstrated by Wiegner, *et al.*,[18] who found that addition of salt to water increased the adsorption of acetic, propionic and butyric acids and spread their isotherms.

In Fig. 2 the dotted line drawn from the origin to the point on the caprylic acid isotherm at which 1% caprylic acid is in equilibrium with the charcoal, cuts the caproic acid isotherm at about 0.25% caproic acid, but does not pass through the butyric acid isotherm. This is interpreted to indicate that if 1% caprylic acid were used as displacer for caproic and butyric acids, caproic acid would emerge from the column in an equilibrium concentration of about 0.25%, whereas, butyric acid would be eluted and emerge as a free peak. This prediction was tested by displacement analysis experiments, and frontal analyses were made to test separability also.

In the frontal analysis experiments 0.5% butyric, 0.5% caproic and 0.5% caprylic acids were dissolved in 50% aqueous alcohol and in absolute alcohol. Each was then pressed into a 10.5-cc. filter and the emerging fronts were observed interferometrically. The results shown in Fig. 3A and 3C for absolute alcohol and 50% alcohol, respec-

(14) Cheldelin and Williams, THIS JOURNAL, **64**, 1513 (1942).

(15) Lundelius, *Kolloid Z.*, **26**, 145 (1920).

(16) Sata, *ibid.*, **49**, 275 (1929).

(17) Landt and Knop, *Z. Elektrochem.*, **37**, 645 (1931).

(18) Wiegner, Magasanik and Virtanen, *Kolloid Z.*, **28**, 51 (1921).

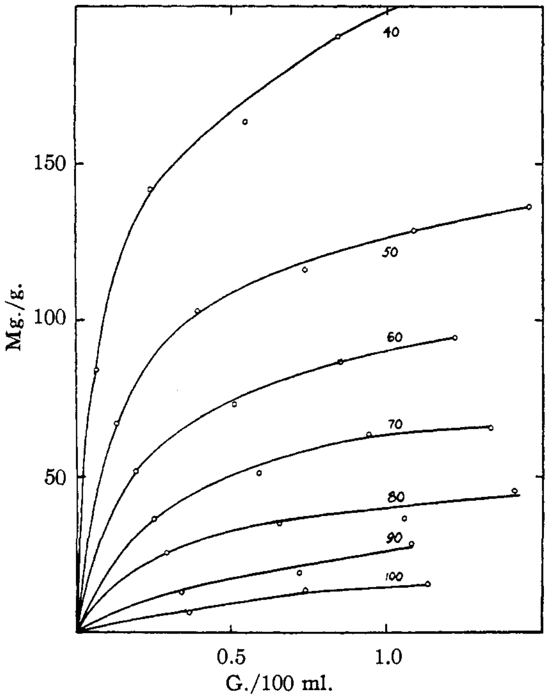

Fig. 1.—Adsorption of caprylic acid on Darco G60 in various concentrations of aqueous ethanol; per cent. alcohol indicated by each isotherm.

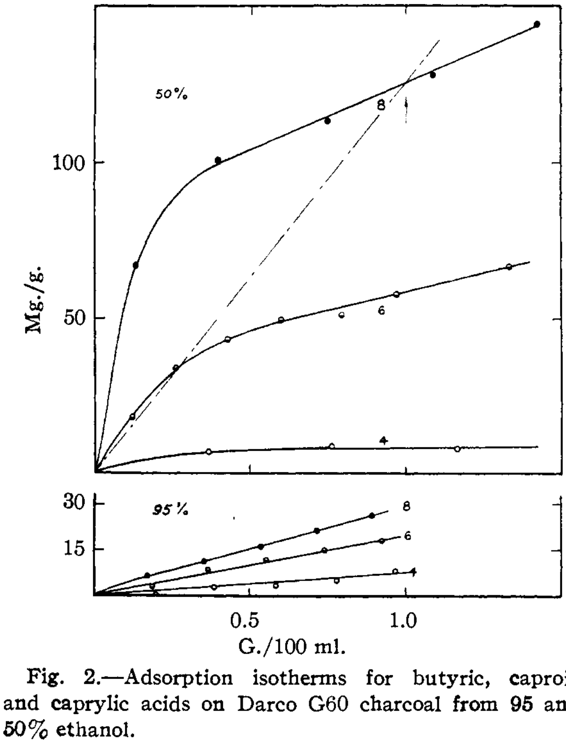

Fig. 2.—Adsorption isotherms for butyric, caproic and caprylic acids on Darco G60 charcoal from 95 and 50% ethanol.

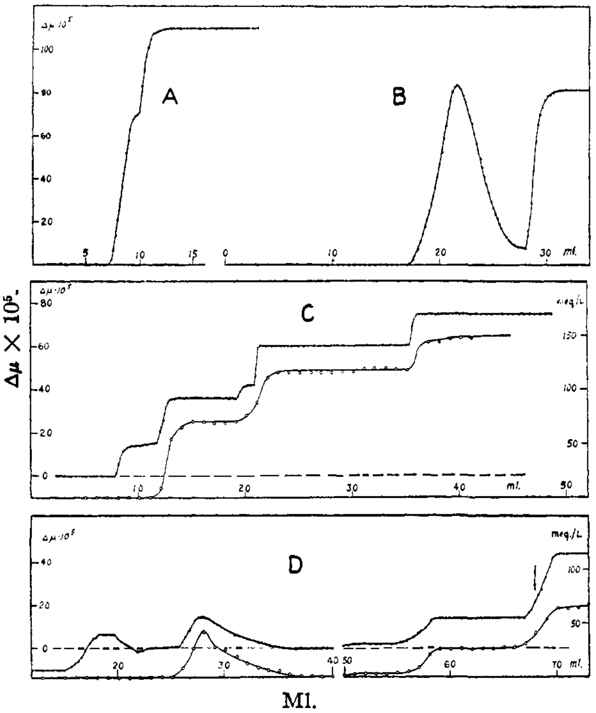

Fig. 3.—Frontal and displacement analyses of butyric, caproic and caprylic acids, adsorbent 1 Darco G60 + 2 Hyflo Supercel: A, frontal analysis, filter 10.5-cc., 0.5% of each acid in absolute alcohol; B, displacement analysis, filter 26.4-cc., sample 40 mg. of caproic acid + 20 mg. of butyric acid (displacer 1.0% caprylic acid) in absolute alcohol; C, frontal analysis, same as A except solvent 50% alcohol; D, displacement analysis, same as C except solvent 50% alcohol.

tively, are striking. The three acids show scarcely any separation in absolute alcohol, whereas in 50% alcohol the three acids emerge in well-defined steps which were verified titrimetrically. It is clear that some type of verification is advisable in frontal analyses of this type, for an artifact appeared in the form of a well-defined initial step. In this case, it is probable that the step represents displaced alcohol. Another unexpected step appeared between butyric and caproic acids. This is probably due to an impurity in the caproic acid.

The displacement analyses were carried out using 40 mg. of caproic acid and 20 mg. of butyric acid displaced by 1.0% caprylic acid in 50% alcohol and in absolute alcohol on a 26.4-cc. filter. In absolute alcohol the isotherms for these three acids are nearly straight lines. A line drawn from a point on the caprylic acid isotherm does not cut the other two isotherms, hence displacement is not possible. In the attempted displacement in absolute alcohol shown in Fig. 3B, butyric and caproic acids wandered as a single free peak and no apparent separation was obtained. Displacement in 50% alcohol gave very good separation of the acids. As predicted, butyric acid emerged as an eluted free peak, and caproic acid emerged as a step in an equilibrium concentration of about 0.3%. The over-all recovery up to the point indicated by the arrow was 94.5%. Thus, by using

50% alcohol as solvent, the useful range of displacement analysis has been extended down to the four-carbon acid. Caution should be exercised here also, because artifacts, probably due to a displaced solvent, are apparent when the interferometric observation is supplemented with titrimetric observations. The same impurity appeared in the displacement diagram as appeared in the frontal analysis, and in the same relative position.

To learn if the advantage gained by mixed solvents extends to higher acids also, the separation of lauric, myristic and palmitic acids was also investigated. Displacement separation of 80 mg. of myristic acid and 40 mg. of lauric acid dissolved in 8 ml. of absolute alcohol was attempted on a 10.5-cc. filter, using 1.0% palmitic acid in absolute alcohol as displacer. The result shown in Fig. 4A indicates that the acids were only partially separated under these conditions. Reduction of the sample size to one-half still did not enable the lauric acid to come into equilibrium with the adsorbent (Fig. 4B). By using 85% alcohol, which is near the limit for solubility of 1% palmitic acid, the adsorption was increased and the same filter size permitted the separation of the larger amounts of the acids into well-defined zones, observed both interferometrically and titrimetrically (Fig. 4C). The recoveries in these 3 experiments were 94, 95.5 and 95.6%, respectively, indicating that increased adsorption did not increase losses in this instance.

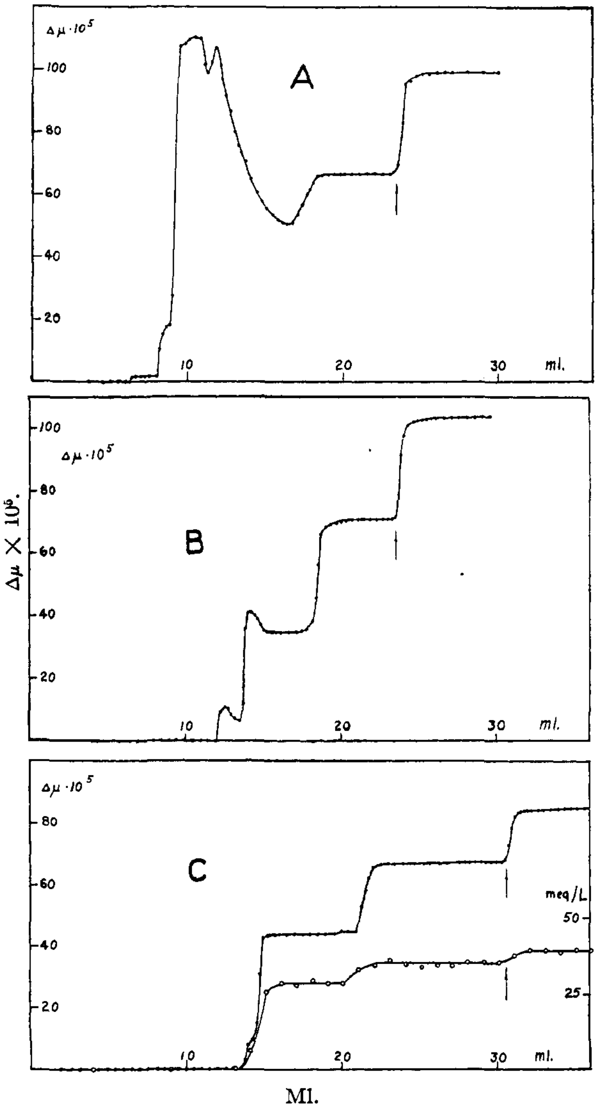

Fig. 4.—Displacement separation of lauric and myristic acids, filter 10.5-cc., adsorbent 1 Darco G60 + 2 Hyflo Supercel, displacer 1% palmitic acid: A, sample, 40 mg. of lauric acid + 80 mg. of myristic acid in absolute alcohol; B, sample, 20 mg. of lauric acid + 40 mg. of myristic acid in absolute alcohol; C, sample, 40 mg. of lauric acid + 80 mg. of myristic acid in 85% alcohol.

Discussion

In the experiments presented, adsorption and separation have been increased as the solvent has been altered in the direction of lower solubility for the solute. It appears that by using a solvent nearly saturated with the solutes, the equilibrium conditions shift in the direction of greater adsorption. Thus it appears that the solubility of the substances to be separated is an important factor in determining the degree of adsorption.[15,16,17] If this is the case, changes in solubility induced by changes in temperature should also affect the adsorption. That such is the case is shown in Fig. 5 where isotherms of caprylic, capric and lauric acids show increased adsorption on Dargo G60 as the

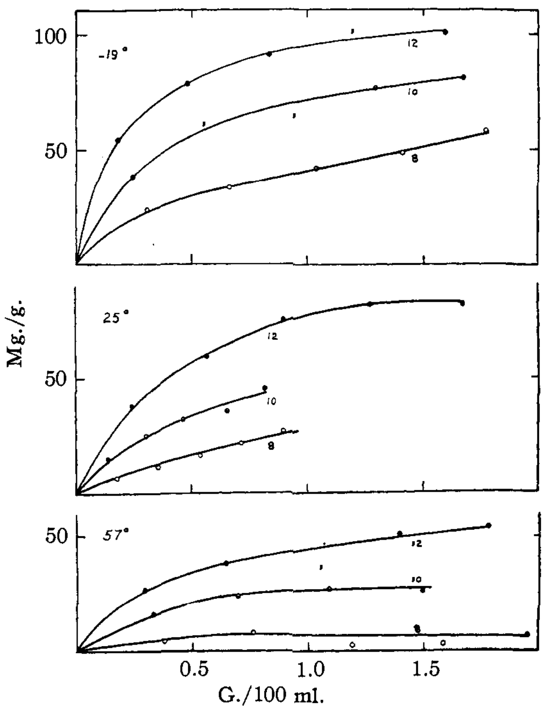

Fig. 5.—Effect of temperature upon isotherms of caprylic, capric and lauric acids.

temperature changes from 57 to −19°. This effect has also been observed by Wöhler and Wenzel.[19] This increase in adsorption by decreased temperature might also lend itself to improving separations of difficultly separable substances.

It cannot be stated whether the effects observed here are due to solubility *per se* or to some property which varies with solubility. It is likely that the phenomenon of increased adsorption of fatty acids with depressed solubility is the same phenomenon of increased adsorption of acids by addition of salts to the solvent as described by Wiegener, *et al.*,[18] and referred to by Tiselius as "salting out adsorption."[20] It may also be that the effect is related to surface tension phenomena as suggested by Wiegener, *et al.* The effects of changing solvents in chromatography to alter adsorption of various substances is common knowledge, and it may be that the results reported here are but an extension of that principle.

The spreading of isotherms by altering the character of the solvent should be useful in separating hitherto difficultly separable substances. In the other direction, this principle can be extended to include members of this homologous series only slightly soluble in alcohol. In this latter case the solubility can be increased by mixing alcohol with a solvent in which the solute is more highly soluble. For example, separation of behenic acid from palmitic and stearic acids has been accomplished by using chloroform–alcohol in which behenic acid is sufficiently soluble.

It appears from these experiments that a higher homolog is the displacer of choice. The most suitable displacer seems to be that member of the homologous series which is next lower in solubility to the substances to be displaced. By using that displacer in a solvent mixture which just dissolves the desired concentration of the displacer, one automatically chooses suitable conditions of solubility for the sample.

Summary

1. Adsorption isotherms for lauric and myristic acids in 95% alcohol on a series of varied adsorbents are given.
2. The depression of solubility of fatty acids in alcohol by admixture with water, or by lowering temperature increases adsorption and spreads isotherms. The lowered solubility caused by addition of water increases separability of fatty acids as shown in frontal and displacement analysis. The use of isotherms to predict the character of displacement diagrams for fatty acids has been verified.
3. The best displacer found thus far for a fatty acid is its nearest homolog of lower solubility in a solvent which just dissolves the desired concentration of displacer.

COLLEGE STATION, TEXAS RECEIVED APRIL 18, 1949

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(19) Wöhler and Wenzel, *Kolloid Z.*, **53**, 273 (1930).

(20) Tiselius, *Arkiv för Kemi, Mineral. Geol.*, **26B**, 1 (1948).

[CONTRIBUTION FROM THE S. A. M. LABORATORIES OF THE MANHATTAN PROJECT]

The Rearrangement of Chlorofluorocarbons by Aluminum Chloride[1]

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The reaction of aluminum chloride with chlorofluoromethanes and ethanes to replace fluorine by chlorine has been reported by Henne,[5,6] who found in attempting Friedel–Crafts reactions that only fluorine-free condensed products were formed. Similarly, benzotrifluoride yielded only benzotrichloride by reaction with aluminum chloride, but from 1,1,2-trichlorotrifluoroethane the intermediate chlorine replacement product tetrachloro-1,1-difluoroethane was isolated in small amounts. This last reaction was developed by Miller as a synthetic procedure[7] and is of general interest as illustrating a method for the preparation of chlorofluoro compounds with structures not directly obtainable by the progressive replacement of chlorine by fluorine.[8] Relatively mild reaction conditions were found to be necessary in order to avoid the complete replacement of fluorine by chlorine observed by Henne and his associates.

In the present work which was originally undertaken to extend the fluorine replacement reaction to other chlorofluorocarbons, the synthesis of tetrachloro-1,1-difluoroethane from 1,1,2-trichlorotrifluoroethane was re-examined and more efficient conditions for the preparation of this material developed. In addition, it was found that the reaction involved not only replacement of fluorine by chlorine but rearrangement as well. The recovered low-boiling fraction which boiled at the same point as the starting material, 1,1,2-trichlorotrifluoroethane was found to be largely 1,1,1-trichlorotrifluoroethane. Under the conditions described below the reaction proceeded as shown

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[1] This paper was largely based on work done for the Manhattan Project under Contract No. W-7405-Eng-50 at S. A. M. Laboratories, Columbia University and under contract No. W-7405-Eng-26, Supplement No. 4 at S. A. M. Laboratories Carbide and Carbon Chemicals Corporation, New York City.

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(5) Henne and Leicester, THIS JOURNAL, **60**, 864 (1938).

(6) Henne and Newman, *ibid.*, **60**, 1697 (1938).

(7) Miller, *ibid.*, **62**, 993 (1940).

(8) Locke, Brode and Henne, *ibid.*, **56**, 1726 (1934).