under the mild conditions described by Ott and Kramer,[6] yielded a colorless liquid on fractional distillation of the product; b.p. 52–53° at 1.8 mm., $n^{17}\text{D}$ 1.4670, identical in these properties as well as infrared spectrum with a sample of methyl ester prepared from II (R = H) by reaction with diazomethane. The bromoacid III (R = H) and the bromoester III (R = CH_3) gave results entirely similar to the above when treated, respectively, under hydrogenolysis conditions with palladium–hydrogen and zinc–acetic acid. The acid IV (R = H) obtained *via* palladium hydrogenolysis was identical with the apocamphane-1-carboxylic acid described by Bartlett and Knox[10]; m.p. 218° after recrystallization from methanol–water and petroleum ether, amide[14] m.p. 185° (lit.[10] 184–185°). The ester IV (R = CH_3) obtained from the zinc hydrogenolysis was recovered as a colorless liquid, b.p. 55° at 0.4 mm, $n^{25}\text{D}$ 1.4690, which when saponified gave the pure acid IV, m.p. 219–220° on recrystallization from petroleum ether, which showed no depression of m.p. on admixture with the acid sample resulting from the palladium reaction and had an identical infrared spectrum.

We are presently studying the circumstances required for the occurrence or absence of rearrangement to correlate such factors with details of the mechanism of related hydrogenolysis reactions.

Support of this research by the U. S. Rubber Co. is gratefully acknowledged.

(14) Identical with a sample kindly provided by Dr. P. D. Bartlett.

DEPARTMENT OF CHEMISTRY
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RECEIVED NOVEMBER 20, 1957

SEPARATION OF POLYUNSATURATED FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY

Sir:

The partial separation of methyl esters of high molecular weight fatty acids by gas chromatography has been reported.[1–4] This analysis has great importance to the study of fat nutrition and metabolic processes involving fats. The above mentioned work included the separation of methyl oleate and methyl stearate and the partial separation of methyl oleate and methyl linoleate. Separation of methyl linoleate from methyl linolenate and other polyunsaturates has not been reported. The above work was done at 197 to 256° and used, as the partition medium, Apiezon M or L vacuum grease.[4] The retention times under conditions optimum for good resolution were of the order of 2 to 3 hours for the C_{18} fraction.

We wish to report the complete separation of the methyl esters of the commonly known fatty acids including the polyunsaturated materials. This includes the complete separation of the methyl esters of the following acids: palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, and erucic. The time required for an analysis has been much shortened, evidenced by the fact that methyl stearate has an emergence time of 27 minutes compared to approximately two hours in the previous work.

Using the column and conditions described below, the fatty esters in each carbon number class emerge from the column in times which increase with the number of double bonds in the compound. Thus, the order of emergence is reversed compared to that obtained with Apiezon L and other non-polar partition media. Table I gives the retention volumes for those materials for which data have been obtained thus far.

Work currently in progress indicates that further improvements in resolution are possible and that the retention times can be reduced further without seriously harming resolution.

Plots of the logarithm of the retention volume *versus* the number of carbon atoms in the fatty acid chain or the number of double bonds in the fatty acid chain of a given length, give straight lines which allow the prediction of approximate retention volumes for some of the less abundant fatty esters such as methyl arachidonate (methyl eicosatetraenoate) and methyl eicosapentaenoate.

Experimental conditions: column, copper $^1/_4''$ o.d., 12 ft. in length; partition medium, 23.1 g. of packing, consisting of 30% Reoplex 400 Plasticizer[5] on Johns–Mansville Celite 545[6] (80–100 mesh); detector, Gow-Mac Filament Thermal Conductivity Cell (Model 9285 - Pretzel geometry); flow rate, 75 ml. per minute of helium measured at column exit and at room temperature; column and cell temperature, 240°; pressure drop across column, 30 p.s.i.g.; sample size, 10 microliters.

TABLE I

Compound	Retention vol. (ml.) (V'_R)[7]
Methyl caprylate	140
Methyl caprate	245
Methyl laurate	420
Methyl myristate	710
Methyl palmitate	1220
Methyl palmitoleate	1340
Methyl stearate	2000
Methyl oleate	2180
Methyl linoleate	2520
Methyl linolenate	3040
Methyl arachidate	3400
Methyl erucate	(6000)

It is planned to determine retention volumes under similar conditions on this and other polar partition media for these and other known fatty acid esters and report a more complete list of data in a forthcoming publication.

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(1) A. T. James and A. J. P. Martin, *Biochem. J.*, **63**, 144 (1956).

(2) A. T. James, *Fette, Seifen, Anstrichmittel*, **59**, 73 (1957).

(3) William Insull, Jr., and A. T. James, American Chemical Society Meeting, New York City, September, 1957.

(4) S. R. Lipsky, private communication.

(5) Geigy Chemical Company, Dyestuffs Division, Yonkers, New York.

(6) The Celite was acid washed, neutralized, rinsed and dried according to Lipsky.[4]

(7) V'_R values are calculated for a flow rate of 75 ml./min. and under these conditions V_R for air is 94 ml.