Anal. Calcd. for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.98; H, 5.39; N, 33.44.

Diacetyl derivative, m.p. 137–142°; mixture m.p. with authentic 8-diacetamidocaffeine, 137–142°.

Reaction of 8-Caffeinylhydrazine with ethylene bromide. A stream of nitrogen was passed through a solution of 10 g. of 8-caffeinylhydrazine (0.045 m.) in 350 ml. of dimethylformamide as it was heated to reflux and while it was refluxing. To this refluxing solution was added 4.5 g. of ethylene bromide (0.024 m.) in 30 ml. of dimethylformamide over a period of 1.5 hr. A green precipitate formed after the addition was complete and the solution darkened. The solution was refluxed for 11 hr. and then cooled in an ice-salt bath. A mixture of green and brown precipitates was collected. The combined precipitate was treated with 500 ml. of boiling ethanol. The green precipitate (2.5 g.) remained undissolved and treatment of the ethanol filtrate with hexane gave 1.4 g. of a white precipitate. The green precipitate showed no N—H peak in the infrared and an acid solution of the compound exhibited a peak at 348 mμ in the ultraviolet. This compound was thought to be 1,2-bis(8-caffeinylazo)ethane, m.p. >320°.

Anal. Calcd. for $C_{18}H_{22}N_{12}O_4$: C, 45.95; H, 4.71; N, 35.73. Found: C, 45.99, H, 4.76; N, 35.22.

The tannish-white precipitate was identified as 8-aminocaffeine by its elemental analysis, the characteristic triplet peak it exhibited in the N—H region of the infrared and conversion to the known 8-diacetamidocaffeine. Yield was 15%.

Anal. Calcd. for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.59; H, 5.55; N, 32.67.

Diacetyl derivative, m.p. 143–146°; mixture m.p. with authentic 8-diacetamidocaffeine, 142–145°.

When this reaction was repeated using phenol as a solvent 8-aminocaffeine was obtained in 43% yield. No 1,2-bis(8-caffeinylazo)ethane was found.

Anal. Calcd. for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 46.37; H, 5.80; N, 32.98.

Diacetyl derivative m.p. 143–145°; mixture m.p. with authentic 8-diacetamidocaffeine, 142–145°.

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Versatility and Temperature Range of Silicone Grease as Partitioning Agent for Gas Chromatography

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Although high vacuum silicone grease has been used rather extensively as partitioning agent in gas chromatography, and column packings containing this material are readily available commercially, there appears to have been published no description of the processing we have found necessary in order to realize the full potential of this partitioning agent. It may be claimed, with some justification, that a properly prepared column containing high vacuum silicone grease as partitioning agent easily surpasses all others as regards temperature range and variety of compounds that may be satisfactorily chromatographed. Chief limitation to the separations possible is that resolution is based largely on differences in vapor pressure. Major structural differences and differences in functional groups do have significant effects on retention times; however, subtle structural differences and degree of unsaturation usually have little effect on retention times. Even this limitation sometimes becomes an asset when separations on silicone grease are combined with separations on other agents where minor structural differences have larger effects on solubility in the partitioning agent.

There is considerable variation between different commercial[1] lots of high vacuum silicone grease, as regards its performance as a partitioning agent before being conditioned or "cured" at high temperature. Some lots have initially given such extremely long retention times and broad chromatography bands as to be of little use. In all cases, the grease must be heated for a period of at least 50 hours at temperatures above 300° in order to bake out volatile materials. All lots of grease which have been examined have become highly satisfactory as a partitioning agent after being cured as described in the Experimental section. A typical, but by no means maximum, variation between two lots of silicone grease is illustrated in Table I.

TABLE I

CHROMATOGRAPHY OF METHYL ESTERS OF FATTY ACIDS ON SILICONE GREASE[a]

Partitioning Agent	Temp. for Chromatog.	Retention Times (min.) for Esters of Acids		
		C_{16}	C_{17}	C_{18}
Lot I, uncured	320°	6.9	8.5	10.4
Lot II, uncured	305°	4.9	6.0	7.5
Lot I, cured[b]	310°	5.1	6.3	7.7
Lot II, cured[b]	305°	3.1	3.8	4.6

[a] The partitioning agents were prepared as described in the Experimental section. Chromatography was in a Pyrex glass column, 8 mm. × 2.5 m.; helium flow was approximately 190 ml./min. [b] Lot I was cured by heating for 8 days at 325–335°; Lot II similarly for 5 days.

The cured silicone grease packing is stable indefinitely when used at temperatures below about 275°. Packings used for several thousand hours have shown essentially constant retention times and unimpaired resolving power. At temperatures near 300°, there is a slow decrease in retention times, and after two thousand hours or longer, resolving power becomes impaired. Most samples of cured grease may be used at 325° for more than 200 hours before the resolving power deteriorates

(1) The silicone grease to which reference is made in this report was purchased from the Dow Corning Corporation, under the name "High Vacuum Grease," during the period 1956–1959.

TABLE II

GAS CHROMATOGRAPHY ON HIGH VACUUM SILICONE GREASE

Compound	Column Temp., °C.	Column Length,[a] m.	Retention Time,[b] min.:sec.
6-Hexadecanone[c]	190°	2	9:50
	220°	4	33:30
6-Heptadecanone[c]	190°	2	14:10
	220°	4	46:00
Dodecyl alcohol	200°	2	1:50
Tetradecyl alcohol	200°	2	2:45
Hexadecyl alcohol	200°	2	4:32
Octadecyl alcohol	200°	2	7:40
Methyl stearate	285°	3	13
Methyl tetracosanoate	285°	3	57
	305°	3	36
Methyl hexacosanoate	285°	3	95
	305°	3	54
Methyl 4-methyloctadecanoate[d]	200°	2	33
	260°	4	40
Methyl nonadecanoate[d]	200°	2	39
	260°	4	47
Dimethyl suberate	155°	2	4:10
Dimethyl sebacate	155°	2	8:26
Methallyl bromide	60°	2	2:00[e]
Crotyl bromide	60°	2	2:50[e]
Nitrobenzene	110°	2	8:40
Nitrosobenzene	110°	2	3:00
Aniline	110°	2	4:50
N-Phenylhydroxylamine	110°	2	[f]
cis-Dimethylsuccinic anhydride	145°	2	5:50
	145°	3	17:10
trans-Dimethylsuccinic anhydride	145°	2	4:50
	145°	3	13:30
Ester I	190°	1.5	15:55[g]
Ester III	190°	1.5	13:15[g]
Dibutyl ether	85°	2	9:35
Butyl alcohol	85°	2	3:40
Butyl acetate[h]	85°	2	6:10
Butyl propionate	85°	2	10:55
Butyl butyrate	85°	2	20:00

[a] Columns were made of Pyrex glass, 8 or 9 mm. o.d., except for the 3- and 4-m. columns, which were 15 mm. o.d. A spiral column of 20-mm. tubing gave relatively poor resolution. [b] Rate of helium flow was 145–160 ml./min. for the 8- and 9-mm. columns, 160–190 ml./min. for the 15-mm. columns, except as otherwise noted. Pressure required to give these rates of gas flow was in the range 12–22 cm. of mercury. [c] In the 2-m. column at 190°, recorder tracing barely touched baseline between the hexadecanone and heptadecanone bands; in the 4-m. column at 220°, tracing was at baseline between the bands for 7 min. [d] Nonadecanoate and 4-methyloctadecanoate boil about 3.5° apart at 3 mm. pressure. In the 2-m. column, tracing did not quite reach baseline between bands; in the 4-m. column, tracing touched baseline between bands. [e] Helium flow rate in these runs was about 30 ml./min. [f] Apparently *N*-phenylhydroxylamine disproportionates extremely rapidly at 110° in a helium atmosphere to nitrosobenzene and aniline, for injection of this substance gave excellent bands at 3:10 and 4:50 (observation of Dr. R. J. Fessenden). [g] With a second lot of packing cured in the same manner (12 days), retention times in the same column at 200° were respectively 4:42 and 3:57, and the peaks of the bands were barely resolved. Resolution was improved only slightly at lower temperature. [h] A quantity of 0.01 μl. of butyl acetate could be detected in presence of 1.0 μl. of butyl alcohol. With di-2-ethylhexyl phthalate as partitioning agent, the bands for these compounds were closer together and butyl acetate could be detected only when present in a ratio of at least 1:50.

to the point of ineffectiveness. Below 275°, contamination of chromatographed samples by "bleeding" of partitioning agent from the column is essentially *nil;* at 300° there is minor but observable contamination from bleeding until the column has been used for a thousand hours or longer.

The versatility of silicone grease as partitioning agent is illustrated in part by the data assembled[2] in Table II. Among other compounds successfully chromatographed are lactones, nitrogen heterocycles, saturated hydrocarbons up to C_{36}, oximes,[3] esters of fatty acids up to C_{32}, alkylphosphonic esters, and other organophosphorus compounds,[4] alkylnaphthalenes, and complex reaction mixtures containing hydroxy nitriles.[5] The iodides from a Zeisel analysis may be readily identified, both qualitatively and quantitatively as methyl, ethyl, isopropyl or *n*-propyl iodides. Among the very few types of compounds not successfully chromatographed are alkanediols. No signal could be observed after injection of decanediol. Esters of dibasic acids, including diethyl malonate and diethyl oxalate, are readily separated. Among compounds chromatographed satisfactorily but not separated are: 2- and 3-bromopentanes, methyl stearate and methyl oleate, 2- and 3-octadecanones, the isomeric half esters of methylsuccinic acid.

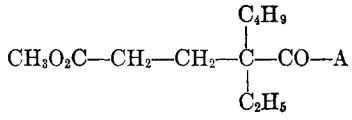
Perhaps one of the more interesting entries in Table II is the separation of the isomeric half esters, I and III, of α-butyl-α-ethylglutaric acid. In fractional distillation, there has been detected no difference in the boiling points of these compounds. The isomeric keto esters, II and IV, were not separable on silicone grease but were separable on Reoplex-400 (Geigy). Also of interest is the separation on silicone grease of the *cis*- and *trans*-isomers of *sym*-dimethylsuccinic anhydride. Although fatty acids may be chromatographed quite satisfactorily,

(2) These data have been excerpted from those accumulated in various investigations in these laboratories. Principal contributors have been R. E. Bozak, Joan S. Fessenden, R. J. Fessenden, E. R. Harris, R. B. Hutchison, K. W. Kraus, F. J. Schmitz, and P. Tavs.

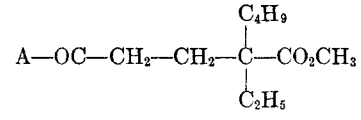
(3) Although di-2-ethylhexyl phthalate was used for separation of oximes in work recently published [J. Cason and E. R. Harris, *J. Org. Chem.*, **24**, 676 (1959)], silicone grease is equally effective.

(4) J. Cason and W. N. Baxter, *J. Org. Chem.*, **23**, 1302 (1958).

(5) J. Cason, K. W. Kraus, and W. D. MacLeod, Jr., *J. Org. Chem.*, **24**, 392 (1959).



I. A = OH
II. A = C_4H_9



III. A = OH
IV. A = C_4H_9

the corresponding esters give sharper bands and larger signals with a thermal detector. Strangely enough, for equal weights of half esters I and III, a significantly larger signal is received from ester III. This is a definite exception to the report[6] that signal is a function of square root of molecular weight. Lower molecular weight acids, including formic and acetic acids may be separated on the silicone grease; however, broad unsymmetrical bands of somewhat variable retention times are obtained. The separation of branched-chain and normal fatty acid esters has been reported.[7]

EXPERIMENTAL

Preparation of column packing. There were used 4 parts of silicone grease[1] to 10 parts by weight of 30–60-mesh Celite (Johns-Manville "Chromosorb" or Celite firebrick which had been pulverized and sieved). The grease was dispersed by warming and stirring in 7–8 parts by weight of chloroform. A few minutes of vigorous mixing and stirring by hand are required for complete dispersion. The Celite, moistened with chloroform, was added to the stirred dispersion of grease, and the resultant mixture was shaken vigorously for a few minutes. The chloroform was removed at reduced pressure and the packing material was dried at 100° in a vacuum. Such packing material is just short of becoming sticky and has an appearance similar to the Celite before impregnation.

The packing material is cured by heating in a slow stream of nitrogen at 325–335°; higher temperatures tend to impair resolution and give too rapid a curing for satisfactory control. In large batches, an exothermic reaction may be noticeable as the temperature approaches 300° and this may necessitate turning off the heater for a few minutes. Heating may be accomplished[8] in a Pyrex tube, on which an electric heating wire has been wound. The heated tube should be placed in a vertical position with a short (20 mm.) outlet tube at the bottom of not less than 10 mm. o.d. During the first 30–50 hr. of heating, both a volatile, low-melting solid and a mobile liquid are swept from the tube; care must be exercised that the outlet is not plugged by the solid.

After 50–70 hr. of heating, when evolution of solid material has nearly ceased, heating should be discontinued, and the packing material should be tested for retention times of suitable compounds.[8] For some lots of silicone grease, this initial heating period has been sufficient. A retention time of 2–4 min. for methyl decanoate at 180° in a 1.5-m. column, helium pressure of 15–20 cm. of mercury, is in the range that is satisfactory. A representative rate of decrease of retention time with heating may be noted in Table I. The grease described as Lot II in this table gave, at 305° in the 2.5-m. column, a retention time for ethyl 10-methyltetracosanoate of 34 min. before curing, 22 min. after curing. A curing period of 5–8 days is normal, and the longest period that has been used was 12 days. If very high molecular weight materials are to be chromatographed, retention times may be shortened by extending the heating period, but deterioration of resolution eventually occurs. It is usually better to reduce retention times by decreasing the content of grease on the packing.

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(6) R. H. Eastman, *J. Am. Chem. Soc.*, **79**, 4243 (1957).

(7) J. Cason and P. Tavs, *J. Biol. Chem.*, **234**, 1401 (1959).

(8) Commercial models of gas chromatography apparatus usually are not designed to permit heating above 300°; however, if one has apparatus which will withstand temperatures up to 340°, heating is conveniently done in the chromatography tube, where retention time may be checked by simply lowering the temperature to a suitable value. Heating may then be resumed as indicated.

Thermal Decomposition of Di-*n*-butyl Maleate

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During work on the addition of maleic acid derivatives to unsaturated fatty acids,[1] it was found that di-*n*-butyl maleate undergoes a decomposition reaction which has not been previously reported. The decomposition appears to be related to the well known pyrolysis of esters to form alkenes.[2] It may also be related to the decarboxylation of diaryl fumarates to form, first, aryl cinnamates and, then, stilbenes.[3] It differs from these reactions and is unusual in that decomposition is initiated at the relatively low temperature of 265°, the boiling point of dibutyl maleate. Another unusual feature of the decomposition is formation of 1-butanol.

When di-*n*-butyl maleate is heated to 260–265°, the liquid turns deep red, the refractive index rises, and there is loss in weight. Heating for 2.5 hr. transforms about 40% of the ester into higher boiling material. 1-Butanol is found in the cold trap when this material is distilled under vacuum. If the ester is swept with nitrogen while being heated, 1-butene can be trapped from the off-gas. Attempts to distill dibutyl maleate at atmospheric pressure result in a slow distillation of butanol and evolution of butene. Low weight recoveries in these experiments indicated the presence of another volatile product, shown to be carbon dioxide.

Beside volatile products, there is a considerable residue of dark, viscous tar. This residue could be

(1) W. R. Miller, E. W. Bell, H. M. Teeter, and J. C. Cowan. Presented before the 32nd Fall Meeting of American Oil Chemists' Society, October 1958, Chicago, Ill.

(2) W. J. Bailey and W. N. Turek, *J. Am. Oil Chemists' Soc.*, **33**, 317 (1956).

(3) L. B. Flett and W. H. Gardner, *Maleic Anhydride Derivatives*, John Wiley and Sons, New York, 1952, p. 248.