SIMPLE METHOD FOR OBTAINING SATURATED COMPOUNDS FROM A MIXTURE USING A MICRO COLUMN OF PALLADIUM CHLORIDE ON SILICIC ACID

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SUMMARY

In a relatively simple procedure, a melting point capillary containing a small bed of palladium chloride on silicic acid is used to complex completely microgram amounts of unsaturated components from a mixture brought in contact with it. This permits the facile recovery of fully saturated compounds in the effluent from the column in near quantitative yield. It was not found possible (with methyl esters of fatty acids) to obtain unsaturated members from the complex in an unchanged condition and in satisfactory yield; consequently, the method is advocated only as a rapid means to recover saturates from a mixture. Besides methyl esters of fatty acids, hydrocarbons, wax esters, glyceryl-1-alkyl ethers, unesterified fatty acids, fatty alcohols, cholesteryl esters, triglycerides, fatty aldehydes, and sterols were studied.

INTRODUCTION

Separation of saturated from unsaturated compounds is an important initial step in the analysis of a complex lipid class. This was accomplished earlier by forming mercuric acetate addition compounds with double bonds followed by chromatographic separation of the mixture[1,2]. In the last fifteen years, however, this has been largely replaced by argentation chromatography[3–5]. For microscale separation of saturated from unsaturated components of a lipid class, argentation thin-layer chromatography (TLC) on silica gel has been by far the method of choice. Although this technique is extremely useful, it has some well known inconveniences including plate preparation, instability of silver ions in the layer, darkening of the plate, blackening of fingers, and corrosion of the metal of certain spreaders. There is also the possibility that clean-cut separation of saturated from unsaturated constituents may not be achieved because of a large disproportionality of one or the other classes or by the short-chain members in the saturated class tailing into the long-chain members of the unsaturated class immediately below it.

During the latter stages in the development of a micro column hydrogenation system employing palladium chloride on Celite[6], we observed that unsaturated mem-

bers of lipid classes when once applied to the column were difficult to elute prior to hydrogenation, whereas saturated members were easily removed. We recently investigated a modification of this system with the objective of developing a rapid micro method for obtaining the saturated and unsaturated components comprising a given class of lipid. Although rapid separation of the saturated from the unsaturated components was achieved with near-quantitative recovery of the former, removal of the unsaturates from the column in an unchanged condition was not realized. Nevertheless, we thought that, in view of its speed and simplicity, the procedure for obtaining saturated compounds from a mixture would in itself be of considerable analytical value. Consequently, we studied this aspect extensively and now report our results for the major classes that occur in fats and oils and for some of the minor classes as well.

EXPERIMENTAL

*Materials**

Palladium chloride ($PdCl_2$) was from Alfa Inorganics Division of Ventron Corp., Danvers, Mass., U.S.A.; silicic acid, 100 mesh, No. 2847, was from Mallinckrodt, St. Louis, Mo., U.S.A.; melting point capillaries open at both ends, Kimble No. 34500, 100 × 1.6–1.8 mm and Critoseal (a vinyl plastic putty) were from A. H. Thomas Co., Philadelphia, Pa., U.S.A.; hexane (Mallinckrodt Nanograde) was purified as described for pentane[7] and stored over CaH_2; benzene (Mallinckrodt Nanograde) was used as received; alcohols, fatty acids, fatty acid methyl esters, cholesteryl esters, wax esters, polyunsaturated hydrocarbons and *cis*-9-octadecene were from Nu Chek Prep, Elysian, Minn., U.S.A.; glyceryl-1-alkyl ethers were from Supelco, Bellefonte, Pa., U.S.A.; *n*-alkanes and olefins were from Chemical Samples, Columbus, Ohio, U.S.A.; fatty aldehydes were prepared from the corresponding alcohol by the method of Schwartz and Osman[8]; sterols were from Applied Science, State College, Pa., U.S.A.

Preparation of $PdCl_2$ on silicic acid

$PdCl_2$ (170 ± 2 mg) was weighed into a 2-ml screw cap vial, and 0.4 ml conc. HCl was added. The vial was closed loosely with a foil-lined cap and heated gently on a hot plate until all of the $PdCl_2$ was dissolved (about 10 min), as indicated by the absence of particles settling to the bottom. The hot solution was transferred with a Pasteur pipette to a 7.5-cm glass mortar** containing 1.0 g ± 20 mg of silicic acid. The vial was rinsed with 0.4 ml of distilled water, the rinsings were transferred to the mortar and the contents were ground thoroughly with a glass pestle. Grinding was interrupted once or twice to scrape the sides and bottom of the mortar carefully with a flat spatula. Grinding was then continued until the mixture appeared to be homogeneously brown. The mortar and pestle were heated at 110° for 1.5–3.0 h, cooled in a desiccator, and the powder was reground briefly*** and transferred to a

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* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

** Glass is preferred to porcelain as the latter adsorbs $PdCl_2$ onto its surface.

*** As much pressure as physically possible should be applied to the pestle (see Discussion).

5-ml screw cap vial. The vial was closed by pulling the sleeve portion of a 7 × 11 mm septum-type rubber stopper (A. H. Thomas Co., Cat. No. 8753-D32) over the threads. The closure was punctured several times with a hypodermic needle so that a glass capillary could be inserted smoothly. The vial was stored in a desiccator over P_2O_5 when not in use.

Preparation of micro columns

A melting point capillary was sealed at one end by pushing it into Critoseal. It was weighed, the open end was inserted through the septum of the $PdCl_2$–silicic acid vial, and the capillary was dabbed several times into the powder. Approximately 4–6 mg was loaded into the capillary by this technique. If more was desired, the powder was pushed into the capillary with a tamper made from a wire (1.2–1.3 mm diameter) and the process was repeated. The powder was finally pushed into the capillary so that about 5 mm of space remained between the powder and the open end of the capillary, and a small plug of glass wool was pushed into that space. The capillary was cut just below the Critoseal plug, and the powder was tamped into a compact column with a wire from the top while another tamper was pushed against the glass wool plug.

General considerations

All solutions were introduced into the capillary with a hypodermic syringe. Air bubbles or spaces were avoided by expelling the solvent slowly from the syringe with the needle tip opening pressed against the wall of the capillary. If an occasional air bubble formed, it was removed by subjecting the capillary to a quick wrist-whipping motion. The capillary was inserted into a capillary holder (such as those supplied with Microcaps or one made from a septum) which was attached through a glass "Y" to a nitrogen tank and a pressure gauge graduated in quarter pounds (Fig. 1). With $PdCl_2$–silicic acid beds of approximately 4.5–7.0 mg, pressures of 2–4

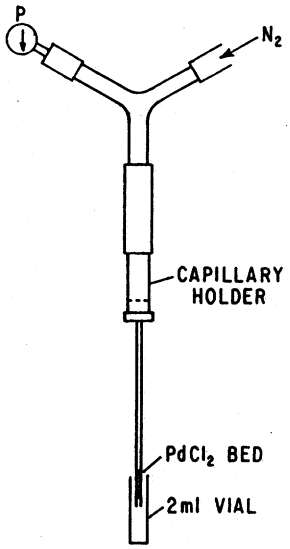

Fig. 1. Apparatus used for elution of micro $PdCl_2$–silicic acid columns.

p.s.i.g. were used. This gave flow-rates of between approximately 3.0 and 3.5 min/100 μl of hexane and slightly slower rates with mixtures of hexane and benzene. In instances where larger beds were used, flow-rates were slower and the nitrogen pressure was increased to obtain the desired flow-rate of 3.0–3.5 min/100 μl. The bed was washed with 100 μl of hexane* prior to introduction of the sample solution. The wash removed lipid contaminants transferred to the glass wool plug from the fingers and also lipids normally present on glass wool[7]. The flow-rate of the column was also established at this point with a pressure setting of about 3 p.s.i.g.; if outside of the 3.0–3.5 min/100 μl range, the pressure was adjusted accordingly with another 100-μl hexane wash.

Fractionation of sample

The lipid classes studied and data pertinent to fractionation and subsequent steps are given in Table I. A hexane* solution of the lipid class was injected into the capillary, and sufficient hexane was injected to bring the volume in the capillary to 100 μl. The solution was stirred in the capillary with a fine wire (pipet probe). The capillary was inserted in the holder, and collection of effluent into a 2-ml screw cap vial was begun. After all the solution had passed over the bed, the solvent system listed in Table I was injected into the capillary in 100-μl aliquots. At the end of the sequence, the solvent in the receiver was removed at room temperature under a nitrogen stream. Standards contained in the same volume and type of solvents were evaporated simultaneously. Residues were dissolved in CS_2 for gas chromatographic analysis or in hexane for TLC.

Gas–liquid chromatography (GLC)

A Hewlett-Packard 5750A instrument with flame ionization detector was used for GLC. Two columns were employed: (A) 240 cm × 0.3 cm I.D. silanized stainless steel packed with 90–100 mesh Anakrom ABS containing 7.5% stabilized ethylene glycol adipate plus 2% phosphoric acid, and (B) 183 cm × 3 mm I.D. glass packed with 1% Se-30 on 100–120 mesh Gas-Chrom Q. Quantitation was by peak height measurements and/or instrumental peak area measurements. Recoveries of saturated components were based on these measurements before and after passage of the mixture over the $PdCl_2$ column. All classes were chromatographed underivatized except for the glyceryl-1-alkyl ethers. These were converted to isopropylidene derivatives[9] prior to GLC.

TLC on silica gel G

250-μl thick precoated 2.5 × 10 cm glass slides (Analtech, Newark, Del., U.S.A.) were dipped into a 5% solution of silver nitrate in acetonitrile and air dried for 1 h prior to being spotted. Developing solvents are given in Table I. Spots were revealed by spraying with 50% aq. sulfuric acid and charring.

TABLE I

COMPOSITION OF LIPID CLASSES AND PERTINENT DATA FOR OBTAINING SATURATED COMPONENTS FROM A MIXTURE

Class	*Amount put over column (μg)*	*Column size (mg)*	*Unsaturates per mg of bed (μg)*	*Eluting solvents sequence (μl)*	*Analytical conditions*
Hydrocarbons					GLC on column A 120–200° at 8°/min
n-Eicosane	34				
n-Tetracosane	25	9.0		500 Hexane	
1-Docosene	36		4.0		
cis,cis-9,12-Octadecadiene	21		2.3		
All-*cis*-9,12,15-octadecatriene	22		2.4		
Wax esters					GLC on column B 270°
Palmityl palmitate	95				
Palmityl stearate	130	6.2		200 Hexane, 300 20% benzene in hexane	
Oleyl stearate	93		15.0		
Stearyl oleate	79		12.7		
Methyl esters				200 Hexane, 300 20% benzene in hexane	GLC on column A 185°
Methyl myristate	3				
Methyl palmitate	40				
Methyl stearate	20	5.0			
Methyl palmitoleate	4		0.8		
Methyl oleate	60		12.0		
Methyl linoleate	10		2.0		
Methyl linolenate	4		0.8		
Glyceryl-1-alkyl ethers					GLC on column A 220° as isopropylidene derivatives
Glyceryl-1-tetradecyloxy	39				
Glyceryl-1-hexadecyloxy	39				
~~Glyceryl 1 (batyl alcohol)~~					
Glyceryl-1-octadecyloxy	63	5.6		500 1% methanol in CH_2Cl_2	
~~Glyceryl 1 (chimyl alcohol)~~					
Glyceryl-1-*cis*-octadec-9-enyloxy	98		17.5	~~CH_2Cl_2~~	
~~Glyceryl 1 (selachyl alcohol)~~					
Free fatty acids				200 Hexane, 300 50% benzene in hexane	GLC on column A 220°
Myristic	53				
Heptadecanoic	27				
Nonadecanoic	48	5.5			
Palmitoleic	45		8.2		
Elaidic	46		8.3		
Fatty alcohols				200 Hexane, 300 50% benzene in hexane	GLC on column A 185°
Myristyl	18				
Palmityl	17				
Stearyl	21	4.3			
Palmitoleyl	22		5.1		
Palmitelaidyl	11		2.6		
Vaccenyl	20		4.6		
Linoelaidyl	22		5.1		
Cholesteryl esters				200 Hexane, 300 20% benzene	Ag^+ TLC, 40% benzene
Cholesteryl butyrate	31				

TABLE I (*continued*)

Class	*Amount put over column (μg)*	*Column size (mg)*	*Unsaturates per mg of bed (μg)*	*Eluting solvents sequence (μl)*	*Analytical conditions*
Cholesteryl stearate	32	7.1		in hexane	in hexane
Cholesteryl oleate	28		3.9		
Cholesteryl elaidate	32		4.5		
Cholesteryl linoleate	23		3.2		
Cholesteryl linolelaidate	28		3.9		
*Triglycerides**				200 Hexane, 300 65% benzene in hexane	Ag^+ TLC, benzene
MMM	33				
POP	28	6.6	4.2		
PPO	49		7.4		
POO	41		6.2		
Fatty aldehydes				200 Hexane, 300 20% benzene in hexane	GLC on column A 180°
Palmitaldehyde	26				
Stearaldehyde	12	5.0			
Oleyl aldehyde	22		4.4		
Linoleyl aldehyde	28		5.6		
Linolenyl aldehyde	20		4.0		
Sterols				200 Hexane, 500 35% benzene in hexane	GLC on column B 195° to 255° at 6°/min
Cholesterol	30		6.4		
Campesterol	27	4.7	5.7		
β-Sitosterol	64		13.6		
Desmosterol	33		7.0		
Stigmasterol	43		9.1		

* M = Myristyl; P = Palmityl; O = Oleyl.

RESULTS AND DISCUSSION

Under the conditions specified, the $PdCl_2$–silicic acid column quantitatively and selectively retained the unsaturated members from a mixture with saturated components of a given class of lipid. This is illustrated in Figs. 2 and 3 for the various classes studied. Only three exceptions were noted; these are discussed below under the free fatty acid and methyl ester classes. All fully saturated compounds were recovered in quantitative or near quantitative yield relative to standards.

Hydrocarbons

The GLC patterns of the hydrocarbon mixture before and after contact with the $PdCl_2$ are shown in Fig. 2A and a, respectively. Fig. 2a shows that only the alkanes have passed through the column. Recovery relative to the standards was 97%. The small peak with a retention time identical to 1-docosene in Fig. 2a is believed to be a saturated impurity in 1-docosene because Ag^+ TLC shows a small spot moving ahead of the major spot. Other olefins which were examined and found to be completely removed when passed over the $PdCl_2$ column in the indicated ratio were: *cis*-9-octadecene (4 μg/mg packing); *trans*-9-octadecene (2.9 μg/mg packing); and

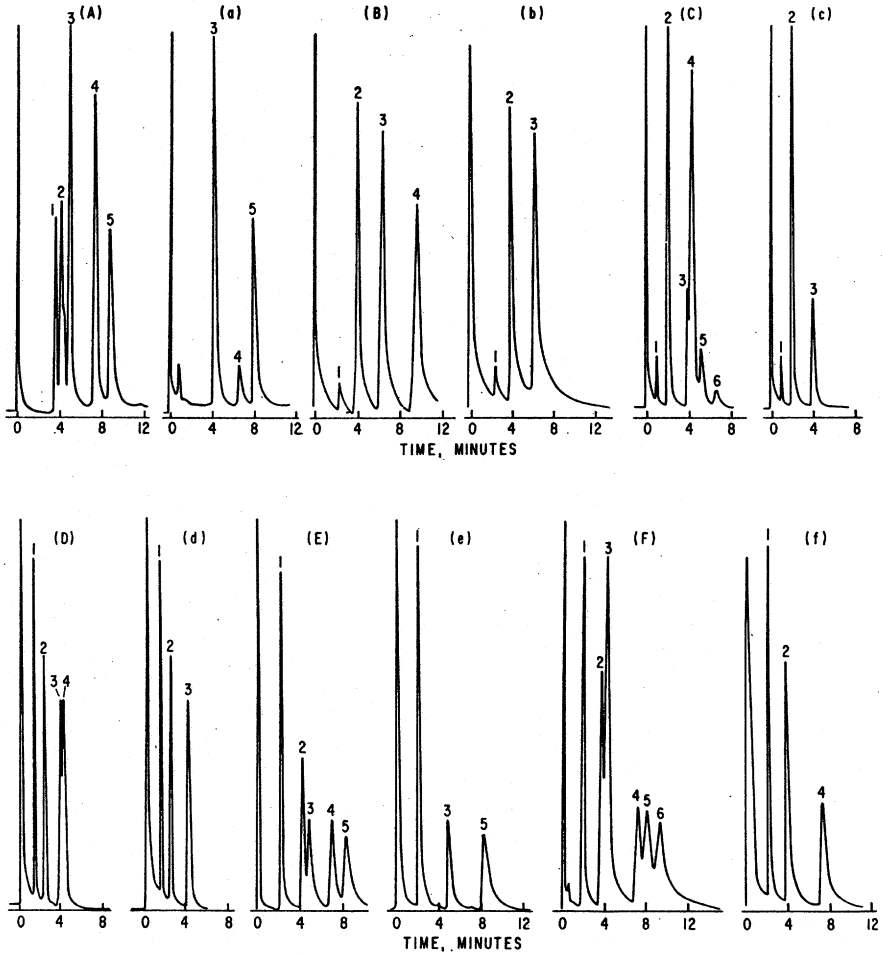

Fig. 2. Gas–liquid chromatograms of various lipid classes before (capital letter) and after (lower case letter) passage over $PdCl_2$–silicic acid micro column. (A) Hydrocarbons: peaks 1–5 are, respectively, *cis,cis*-9,12-octadecadiene; all-*cis*-9,12,15-octadecatriene; *n*-eicosane, 1-docosene; *n*-tetracosane; (a) peaks 3–5 are, respectively, *n*-eicosane; a saturated impurity in 1-docosene; *n*-tetracosane. (B) Wax esters: peaks 1–4 are, respectively, impurity in palmityl palmitate; palmityl palmitate; palmityl stearate; mixture of stearyl oleate and oleyl stearate; (b) peaks 1–3 are, respectively, impurity in palmityl palmitate; palmityl palmitate; palmityl stearate. (C) Methyl esters of fatty acids: peaks 1–6 are, respectively, myristate; palmitate; stearate; oleate; linoleate, linolenate; (c) peaks 1–3 are, respectively, myristate; palmitate; stearate. (D) Glyceryl-1-alkyl ethers (gas chromatographed as isopropylidene derivatives: peaks 1–4 are, respectively, glyceryl-1-tetradecyl ether; chimyl alcohol; batyl alcohol; selachyl alcohol; (d) peaks 1–3 are, respectively, glyceryl-1-tetradecyl ether; chimyl alcohol; batyl alcohol. (E) Free fatty acids: peaks 1–5 are, respectively, myristic; palmitoleic; heptadecanoic, elaidic; nonadecanoic; (e) peaks 1,3,5 are, respectively, myristic; heptadecanoic; nonadecanoic. (F) Fatty alcohols: peaks 1–6 are, respectively, myristyl; palmitelaidyl plus palmitoleyl; stearyl; vaccenyl; linoelaidyl plus linoleyl; (f) peaks 1,2,4 are, respectively, myristyl; palmityl; stearyl.

palmityl;

squalene (19.8 μg/~~ml~~ mg packing). The latter olefin, which is widely distributed in lipids, did not elute from the column even with 50% benzene in hexane.

The capacity of the $PdCl_2$–silicic acid powder for monoenoic olefins is lower than it is for members of monoenoic oxygen-containing classes, undoubtedly because of the greater tendency of the latter to adsorb more strongly onto the silicic acid surface. That adsorption might be a prerequisite for complexation of double bonds by the $PdCl_2$, is suggested by the fact that when a much weaker adsorbent (Celite) was substituted for silicic acid in an otherwise identical system, no removal of olefins was observed.

Wax esters

Fig. 2B and b are the GLC patterns obtained for the mixture of wax esters before and after passage over the $PdCl_2$ column, respectively. Both oleyl stearate and stearyl oleate, which were not resolved by GLC, were quantitatively subtracted from the mixture. The saturated esters were recovered in 98% yield.

Fatty acid methyl esters

The GLC patterns of the fatty ~~and~~ acid methyl esters before and after passage over the $PdCl_2$ column are shown in Fig. 2C and c, respectively. The saturated constituents of the mixture were recovered in 98% yield, and all unsaturated components were completely removed by the $PdCl_2$ column. In addition to the methyl esters shown, the methyl esters of the following fatty acids were completely removed by the column when they were passed over the column in a ratio of 7.5–10.0 μg/mg packing: *trans*-9-octadecenoic, *trans*-6-octadecenoic, *cis*-16-octadecenoic *cis*-3-octadecenoic, *trans*-3-octadecenoic and 17-octadecenoic. However, both methyl *trans*-2-octadecenoic and a mixture of methyl *cis*- and *trans*-2-octadecenoate passed through the column quantitatively with the saturated methyl esters. Morris *et al.*[10] showed that the *trans*-2-octadecenoate was the least retarded of all positional isomers in the methyl octadecenoates during argentation TLC, and they indicated that this isomer as well as the *cis*-2-octadecenoate isomer may not complex at all because of the steric and/or delocalization of the π electrons of the double bond by the adjacent carbomethoxy group.

The effect of having a large preponderance of saturated methyl esters relative to unsaturated methyl esters was also examined. When a mixture having a ratio of 125:1 methyl palmitate to methyl oleate (1760 μg 16:0 to 14 μg 18:1) was passed over a 4.7 mg $PdCl_2$ column in the solvent sequence and volumes given in Table I, the oleate was quantitatively removed by the column and the palmitate was recovered in the effluent in 96% yield.

Glyceryl-1-alkyl ethers

This class was put through the $PdCl_2$ column in dichloromethane, and the saturated members were selectively eluted with that solvent and/or with 1% methanol in dichloromethane. After removal of the solvent, the glyceryl ethers in the residue were quantitatively converted to the isopropylidene derivatives for GLC[9]. The patterns obtained for this class before and after passage over the $PdCl_2$ column are shown in Fig. 2D and d, respectively. Only one glyceryl ether containing an unsaturated alkoxy group (*cis*-9-octadecenyloxy) was studied. It was completely removed from

the mixture. The three glyceryl ethers containing saturated alkoxy groups were recovered in 95% yield. The isopropylidene derivatives were gas chromatographed on an ethylene glycol adipate–phosphoric acid stationary phase, yet these acid-labile derivatives were stable, presumably because of the anhydrous conditions which must exist in the system.

Free fatty acids

The chromatograms of the free fatty acid mixture before and after being passed over the $PdCl_2$ column are shown in Fig. 2E and e, respectively, All unsaturated members were removed, and all saturated members were recovered in the effluent in 99% yield. As with the methyl esters, when *trans*-2-octadecenoic acid was studied it was found not to complex and was quantitatively recovered in the saturated fraction.

Fatty alcohols

All unsaturated alcohols in the mixture shown in Fig. 2F and f were subtracted by the $PdCl_2$ column, while the saturated compounds were eluted from the column and recovered in 100% yield. Unlike the double bond in 2-position-C-2 in the methyl octadecenoates, 2-octadecenol-1, when studied in a ratio of 12.1 μg/mg column packing, was complexed completely. The electron-withdrawing ability of the hydroxyl group is less than that of the carboxyl and carbomethoxy groups; this may be at least partially responsible for the ability of the double bond to be complexed.

Cholesteryl esters

Esters of cholesterol containing an unsaturated acid were completely retained by the $PdCl_2$ column, whereas cholesteryl esters with saturated acid moieties were recovered in the effluent (Fig. 3). Although no attempt was made to check recoveries of the saturated cholesteryl esters, we did find that cholesteryl propionate (53 μg) when passed over a 4.6 mg $PdCl_2$ bed (11.5 μg/mg packing) could be recovered in 102% yield as determined by GLC*. This data suggests that the double bond at C-5 of the cholesterol moiety of the ester is not complexed or is complexed only weakly by the $PdCl_2$ bed in contrast to unesterified sterols as discussed below.

Triglycerides

Triglycerides containing one or more unsaturated fatty acids were retained by the $PdCl_2$ bed. Fully saturated triglycerides were found in the effluent (Fig. 3). Based on a recovery of 93% for tricaprin* by GLC, good recovery of saturated triglycerides is to be expected.

Fatty aldehydes

Because of space limitations, chromatograms of the fractionation of the fatty aldehyde mixture in Table I are not shown. As with the other classes discussed thus far, the saturated members were recovered in nearly quantitative (96%) yield and the unsaturated aldehydes were totally removed by the $PdCl_2$ column.

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* A 45 cm × 0.3 cm I.D. 3% JXR on 100–120 mesh Gas-Chrom Q stainless-steel column was used at 220° (cholesteryl propionate) or at 250° for tricaprin.

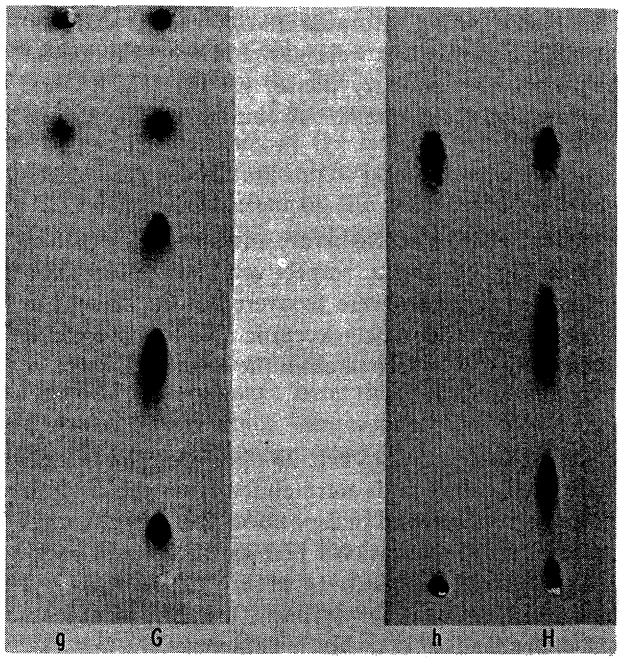

Fig. 3. Thin-layer chromatograms of some lipid classes before (capital letter) and after (lower case letter) passage over $PdCl_2$–silicic acid micro column. Left, cholesteryl ester;: (G) spots from top to bottom are, respectively, stearate; butyrate; elaidate; oleate plus linoleate; (g) stearate; butyrate. Right, triglycerides: (H) spots from top to bottom are MMM; POP + PPO; POO; (h) MMM. See text and Table I for details.

linoelaidate

Sterols

The unesterified sterols in the mixture studied (Table I) which contained unsaturation in the side chain, *i.e.*, stigmasterol and desmosterol (24-dehydrocholesterol) were completely retained by the $PdCl_2$ bed. However, sterols with saturated side chains were recovered in approximately 60% yield. When a fully saturated sterol (3β-cholestanol) was examined under identical conditions, it was recovered quantitatively. Thus it appears that the double bond at C-5 in unesterified sterols is complexed to some extent and to a much greater degree than is the double bond in cholesteryl esters.

The maximum capacity of the $PdCl_2$–silicic acid powder for unsaturated compounds of a given lipid class was not established. This capacity would ostensibly depend on factors such as number and position of double bonds, configuration of the bond, chain length, the presence of alkyl groups near the bond, and the activity of the silicic acid. Therefore it is recommended that the ratio of unsaturated components to unit weight of powder given in Table I not be exceeded. As the concentration of unsaturates in an unknown sample is not likely to be known without additional analysis or prior experience, one should assume that the total weight of the sample is represented by unsaturated components and proceed accordingly. Alternatively, if sufficient sample is available, one can carry out the fractionation using one ratio of sample to powder and repeat it using a narrower ratio. If the pattern and the ratio

of the peaks obtained by GLC of the effluent from the latter trial is the same as that obtained for the former, then only saturated components have been obtained from the sample.

The maximum capacity of the $PdCl_2$–silicic acid powder for unsaturated compounds also depends on how well the powder is ground after it is dried in the oven. During drying, apparently some $PdCl_2$ crystallizes out onto the silicic acid surface and becomes unavailable unless dispersed finely onto the surface; therefore, one must apply as much pressure as possible to the pestle during this final grinding step to impart greater capacity for unsaturates to the powder.

The $PdCl_2$–silicic acid powder retained its ability to subtract unsaturates from a mixture even after 3 months of use. In this time, the septum on the vial of powder was punctured over 100 times by Critoseal plugged capillaries. Whether any loss of activity occurred after 3 months was not ascertained, but repetition of the original conditions (Table I) gave identical results with all of the classes studied.

Preparation of the $PdCl_2$–silicic acid powder was reproducible, although slight variations in flow-rate and in capacity for unsaturates were observed. The flow-rate specified (3.0–3.5 min/100 μl) was arbitrarily chosen as this was the prevalent flow-rate throughout the early stages of the study. Slower flow-rates gave identical results and slightly faster flow-rates when encountered occasionally did also, but no attempt was made to study faster flow-rates comprehensively.

Although we weighed the $PdCl_2$–silicic acid powder in the capillary for each analysis, in practice, especially when a large number of fractionations are to be performed, it may be preferable to estimate the weight of powder, as each millimeter of the compacted bed is approximately equal to 1 mg.

The choice of solvents used to elute selectively the saturated components from a mixture on the micro $PdCl_2$ column was made on the basis of the relative mobility of the classes on silica gel G plates. The volume of solvent needed for complete elution of the saturates without eluting any unsaturated constituents was subsequently established. At the same time it was also ascertained that continued elution with at least 300 μl more of the last eluting solvent listed in Table I for a given class still does not elute any unsaturated component.

It is important, however, to understand that the volume of solvent used to elute completely the saturated members of a given class was established for that amount of $PdCl_2$–silicic acid specified in Table I. Variations in the amount of $PdCl_2$–silicic acid powder taken necessitate proportional adjustment of the solvents if quantitative recovery of the saturated members is to be obtained.

Small amounts of color eluted from the column with solvents containing more than 20% benzene or with dichloromethane. This color had no effect on the GLC characteristics of any of the recovered saturated compounds. The color remained at the origin of TLC plates developed with the solvents given in Table I.

A number of attempts were made (with the methyl esters) to recover unsaturated compounds from the column in an unchanged condition and in a respectable yield. These attempts included elution of the column with 1-hexene, cyclohexene, acetic acid solutions, solutions of strong organic bases, swamping the column with linolenic acid, and gassing the column with ammonia or hydrogen cyanide followed by washing with organic solvents. Although some monounsaturated fatty acid methyl esters could be eluted in 60–70% yield, analysis of double bond position[11] showed

that some double bond migration had occurred. A number of artifact peaks also were seen on GLC analysis of the eluted monounsaturated compound. Polyunsaturated members (in methyl esters) could not be eluted at all. No efforts were made to recover unsaturates from the $PdCl_2$–silicic acid column with the other classes studied.

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