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MECHANISM OF THE REACTION GAS CHROMATOGRAPHY OF PHENYL-MERCURY(II) COMPOUNDS

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SUMMARY

By combining radio-gas chromatography with liquid scintillation counting and with thin-layer chromatography–autoradiography, the conversion of symmetrical phenylmercury(II) compounds into phenylmercury(II) chloride and mercury(II) chloride in gas chromatography in the nanogram range has been demonstrated. The chlorine atoms necessary for this reaction are supplied by the Si–Cl– groups of chlorosilanized materials. The composition of the eluates depends on the concentration of the Si–Cl– groups.

INTRODUCTION

In the clinical–toxicological investigation of chronic cases of mercury poisoning, and particularly in environmental and food analysis, gas chromatography (GC) of organomercurials has played a decisive role since 1965[1–20]. Recently, GC procedures for the determination of trace amounts of inorganic mercury, utilizing methylmercury(II) chloride, phenylmercury(II) chloride, bis(pentafluorophenyl)mercury(II) or diphenylmercury as derivatives, have been developed[21–26].

However, difficulties concerning the elution of organomercury compounds have been repeatedly reported[3,27–30]: these compounds frequently appear in the chromatograms as broad tailing peaks. The peak areas increase with increasing number of injections, and at no time is the elution quantitative. In extreme instances no peak in the customary sense is observed. These effects have hitherto been interpreted in terms of the decomposition of mercury compounds during the separation process and by adsorption on the carrier material. Systematic investigations on these problems were first carried out in the microgram range by Dressman[31] and Baughman *et al.*[32]. They identified the eluates of different phenylmercury(II) salts by IR spectroscopy and mass spectrometry, and demonstrated the formation of diphenylmercury and primarily phenylmercury(II) chloride following injection into the gas chromatograph. Phenylmercury(II) salts other than the chloride were not eluted as such. In the nanogram range, our own experience with the GC of diphenylmercury and phenylmercury(II) chloride confirmed the observations of other workers[27,29,30].

In this paper, radiochemical techniques are described, that permit eluates to

be analysed and their components to be identified even in the subnanogram range in order to elucidate the conversions that occur in reaction GC of phenylmercury compounds. The methods involve a combination of radio GC with liquid scintillation counting and also with thin-layer chromatography (TLC)–autoradiography.

EXPERIMENTAL

Apparatus
   A Packard Series 7700 gas chromatograph equipped with a ^{3}H electron-capture detector (ECD), $U = 150$ V, was used with argon as carrier gas at a flow-rate of 45 ml/min.
   Three model columns were tested: 3% ethylene glycol succinate (EGS) on Gas-Chrom Q, column temperature (T_c) = 190°, 3% silicone oil OV-17 on Gas-Chrom Q, $T_c = 200°$, and 5% silicone oil OV-17 on Chromosorb P AW, $T_c = 200°$. In order to avoid the formation of Si–Cl– groups on the surface of the carrier, the last carrier was not silanized with the common substituted chlorosilanes but with hexamethyldisilazane (HMDS). However, to check the influence of high concentrations of Si–Cl– groups on the result, all three column types were also operated with a reaction zone of 14% diphenyldichlorosilane (DPCS) on Kieselguhr in the injection port.
   A Packard Tri-Carb 3375 liquid scintillation counter was used with 4.2% gain and channel width 12–240 keV.

Radiochemicals
   Diphenylmercury (DPM*) was prepared according to the reaction

$$2\ ^{203}HgCl_2 + NaB(C_6H_5)_4 + 3\ H_2O \rightarrow 2\ ^{203}Hg(C_6H_5)_2 + 3\ HCl + NaCl +$$
$$+ B(OH)_3 \qquad (1)$$
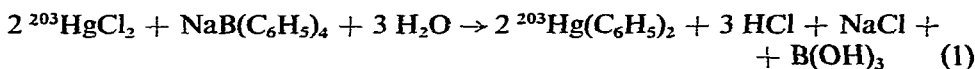
Initially the specific molar activity of this compound was $A_{spec} = 0.11 \cdot 10^{15}\ sec^{-1} \cdot mole^{-1}$ (= 2.9 kCi·mole^{-1}) and amounts of 2–250 ng were injected. The $^{203}HgCl_2$ was supplied by New England Nuclear (Boston, Mass., U.S.A.).
   Phenylmercury(II) chloride (PMC*) was prepared according to the reaction

$$^{203}HgCl_2 + {}^{203}Hg(C_6H_5)_2 \rightarrow 2\ C_6H_5{}^{203}HgCl \qquad (2)$$

Initially the specific molar activity of this compound was $A_{spec} = 12 \cdot 10^{12}\ sec^{-1} \cdot mole^{-1}$ (= 0.32 kCi·mole^{-1}) and amounts of *ca.* 200 ng were injected.
   Mercury(II) chloride was supplied with an initial specific molar activity of $A_{spec} = 6.25 \cdot 10^{12}\ sec^{-1} \cdot mole^{-1}$ (= 0.17 kCi·mole^{-1}) and *ca.* 100 ng were injected. The material was supplied by Amersham-Buchler (Braunschweig, G.F.R.).

Radio-gas chromatography
   As liquid scintillation counting (LSC) is the most sensitive method for measuring β-radiation, it was employed for the radio-GC of ^{203}Hg compounds in the nanogram

range. The effluents from the separation column were fed to the ECD or alternatively to a fraction collector by PTFE tubes via a T-piece. Heatable fraction collector tubing was used, discharging into small cold traps interchangeable within 3 sec. The whole fraction collector was cooled in a Dewar vessel by liquid nitrogen. The cold traps were also filled with liquid nitrogen. The carrier gas flow-rate was adjusted so that the argon condensed entirely in the cold traps, losses by aerosol formation thus being avoided.

To record the blank value, five fractions of 1 min each were cut at the beginning of each test series. Then the radioactive test solution was injected, and the eluates were separated by freezing in 10 fractions of 1 min each. The samples thus obtained were heated slowly until the argon had just evaporated. Next, the cold traps, each in a counting vial, were at once filled with 1.8 ml of PPO scintillator in dioxan so as to dissolve the condensed water and subsequently warmed to room temperature. The counting vials were then filled to 10 ml with PPO scintillator in toluene and thereby $97.2 \pm 1.6\%$ of the collected activity dissolved homogeneously in the LSC cocktail. For calibration purposes the same amount of test solution, having been injected into the gas chromatograph, was dissolved in a mixture of 1.8 ml of dioxan scintillator and 8.2 ml of toluene scintillator.

Thin-layer chromatography–autoradiography

To identify the eluted radioactive species, the eluates were collected as described above, dissolved in isobutyl methyl ketone and analysed by TLC. The thin-layer chromatograms were evaluated by autoradiography. This method has previously been reported elsewhere[33].

Neutron-activation analysis

As is to be shown later, DPM is converted into PMC in the gas chromatograph. The appropriate position for supplying the chlorine atoms for this reaction was localized by neutron-activation analysis. The material investigated was dimethyldichlorosilane-treated quartz-wool from the injection port of the GC column and the liquid phase OV-17. The material was irradiated for 20 min with a neutron flux of $1.8 \cdot 10^{12} \, \text{n} \cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ in a swimming pool reactor of the TRIGA I type*. After a decay time of 20 min the γ-spectra were recorded. A DIDAC 800 (Intertechnique) multi-channel analyser with a well-type NaI(T) detector served this purpose, calibration standards being 1.00, 10.0 and 100 μg of chlorine in the form of DDT.

Test-tube simulation of GC reactions

To check the correctness of the assumptions regarding the GC reactions of DPM and PMC, these reactions were test-tube simulated as follows. A few hundred micrograms of DPM dissolved in toluene were in each instance boiled with *ca.* 50 mg of DMCS-treated quartz-wool or with a few hundred milligrams of DPCS-impregnated Kieselguhr. The reaction products were detected by TLC as described elsewhere[33].

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* These experiments were performed in the Institute for Nuclear Medicine, Medical College, Hannover, G.F.R.

Model experiments on losses by adsorption on carrier material

As in all radio-GC experiments the elution yields were found to be considerably less than 100%, it was necessary to clarify where the losses of injected activity had occurred.

A separation column from which 96.8 ± 7.4% of the injected activity was eluted when it was empty was successively filled with various carriers: (a) commercial Gas-Chrom Q, (b) Gas-Chrom Q impregnated with 3% EGS and (c) Gas-Chrom Q dried in a stream of dry nitrogen at 100° and in addition exhaustively silanized with DPCS. To each packing was added 140 ng of DPM, and the adsorbed residues of each were measured by LSC 10 min after injection in a 500-mg aliquot suspended in a thixotropic gel.

RESULTS AND DISCUSSION

To evaluate the radio-GC measurements the activity recovered during each minute fraction was referred to the total activity injected. These relative yields were plotted as points on the abscissa in the middle of the appertaining time sections, and all points were connected by a graph. Hence, the radio-gas chromatograms obtained in this way have a resolution of time no better than 1 min. Manually changing the cold traps causes an error of −5% on the ordinate.

Table I gives the total recoveries of activity (summed over 10 fractions), irrespective of their identities. Hence these results are not reproducible and are considerably less than 100% in all instances. Between 10 and 60 min after injection, small amounts of radioactivity were still found in the eluate. This bleeding decayed nearly exponentially with time, with no substance peaks being observable. Thus, from the EGS column 2.4 ± 1.1% and from the OV-17 column 23.5 ± 1.5% of the activity injected still appeared.

In Figs. 1–6 some of the radio-gas chromatograms are shown, together with the corresponding conventional gas chromatograms. In Figs. 1a, 2a and 3a it is noticeable that activity is eluted even before the intrinsic substance peak occurs.

Table II gives the results of the TLC–autoradiographic identification of the eluates. Some typical examples of these autoradiograms are shown in Figs. 1b, 2b and 3b.

TABLE I

TOTAL GC RECOVERIES (%) OF ACTIVITY INJECTED

Substance injected	Column		
	3% EGS*	3% OV-17*	5% OV-17**
DPM	48.4 ± 14.1	30.8 ± 11.9	24.5 ± 6.1
PMC	41.5 ± 12.0	79.1	6.9
HgCl₂	45.9 ± 5.9	27.1 ± 3.9	

* Carrier: Gas-Chrom Q.
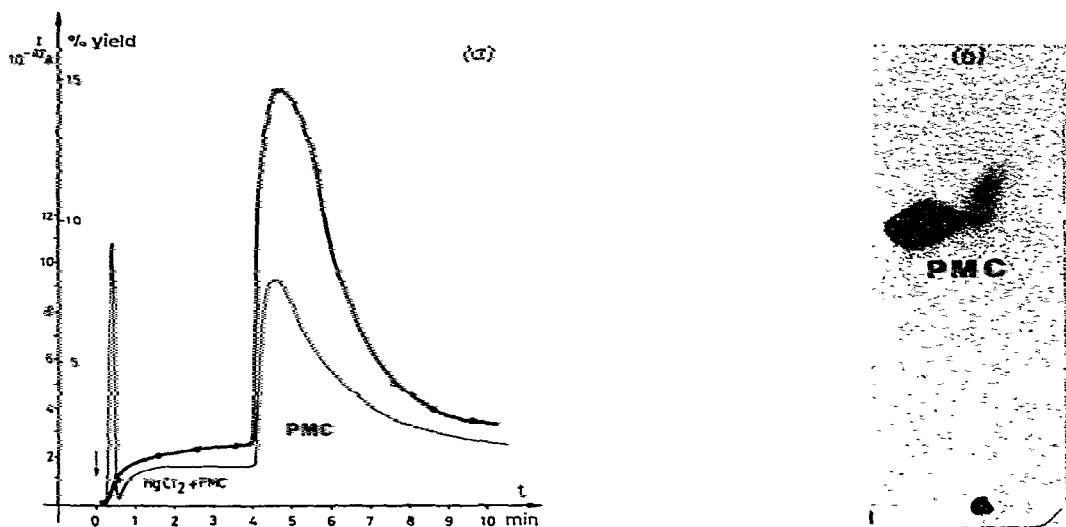** Carrier: Chromosorb P AW, silanized with HMDS.

Fig. 1. (a) Radio-GC (————) and conventional GC (————) of 231 ng of labelled diphenylmercury in toluene. Column: 3% OV-17. (b) TLC–autoradiogram of the main peak substance. Left spot, eluate fraction between 4 and 10 min; right spot, phenylmercury(II) chloride as a reference substance. TLC plate, silica gel; solvent, benzene. Autoradiography: 39-h exposure with Kodirex X-ray film.
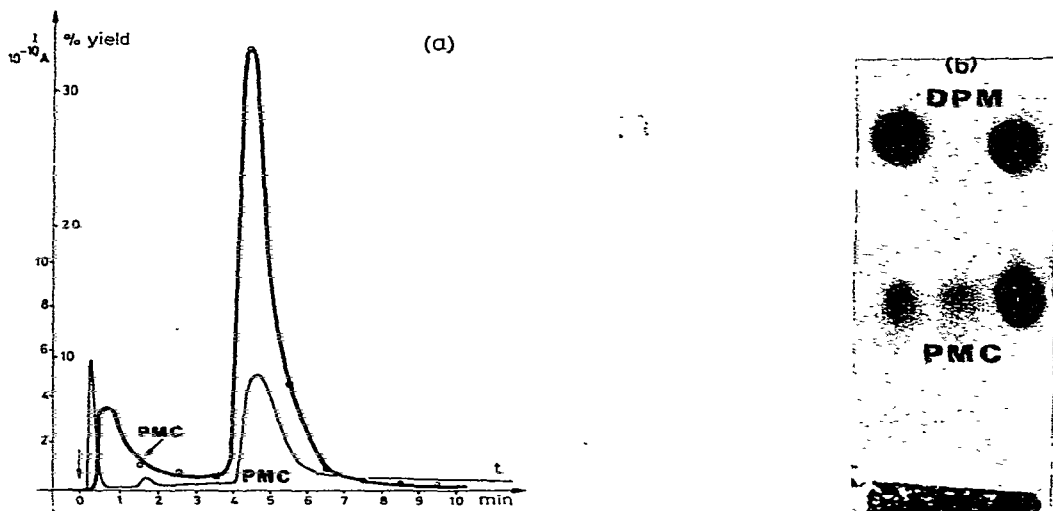

Fig. 2. (a) Radio-GC (————) and conventional GC (————) of 259 ng of labelled phenylmercury(II) chloride in toluene. Column: 3% EGS. (b) TLC–autoradiogram of the first peak. Left, eluate between 0 and 3 min with PMC and DPM as reference substances; middle, eluate only; right, PMC and DPM as reference substances. TLC plate, silica gel; solvent, benzene. Autoradiography: 95-h exposure with Agfa-Gevaert Safety E X-ray film.

The substance producing the main peak was still identified as PMC (Fig. 1b), irrespective of whether PMC or DPM was injected. DPM must consequently have reacted to give PMC on the column. The following substances may be considered as suppliers of the chlorine atoms necessary for this reaction: (1) the quartz-wool with
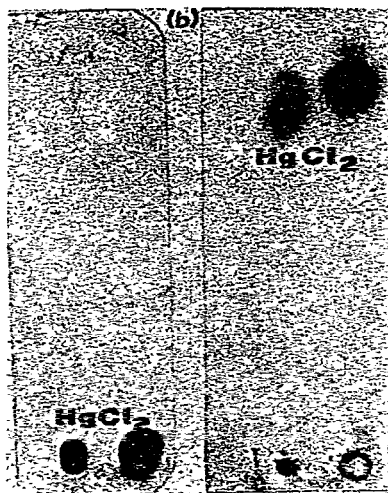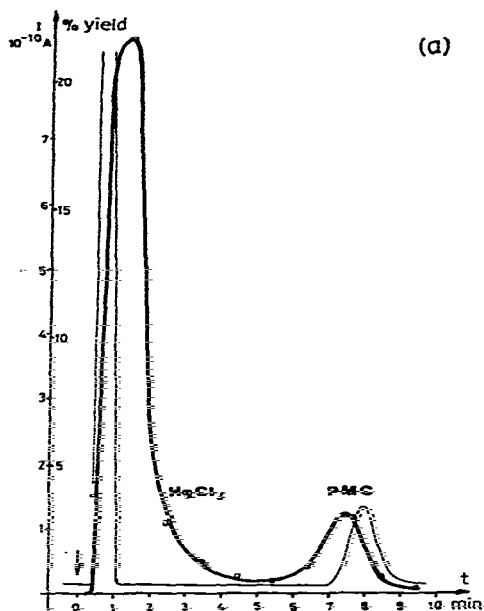
Fig. 3. (a) Radio-GC (———) and conventional GC (———) of 19.6 ng of labelled diphenylmercury in toluene. Column: 3% EGS with a reaction zone of 14% diphenyldichlorosilane on Kieselguhr. (b) TLC–autoradiogram of the first peak. Left: eluate fraction between 0 and 5 min (left) and $^{203}HgCl_2$ (right); solvent, benzene. Right: same TLC; solvent, isobutyl methyl ketone–benzene (3:1, v/v). Autoradiography: 20.5-h exposure with Kodirex X-ray film.
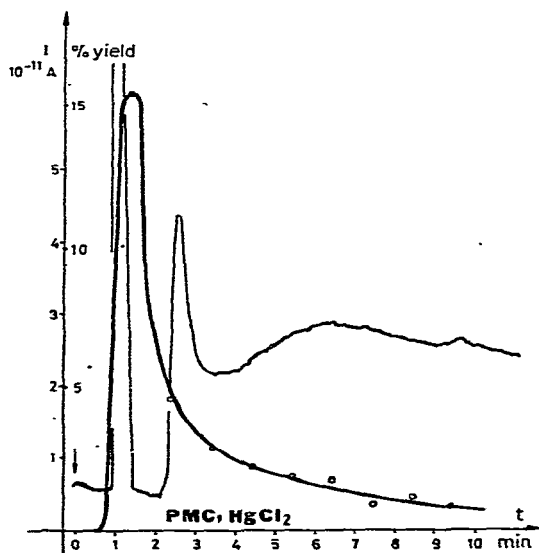

Fig. 4. Radio-GC (———) and conventional GC (———) of 23.5 ng of labelled diphenylmercury in toluene. Column: 5% OV-17 on Chromosorb P AW, silanized with hexamethyldisilazane.
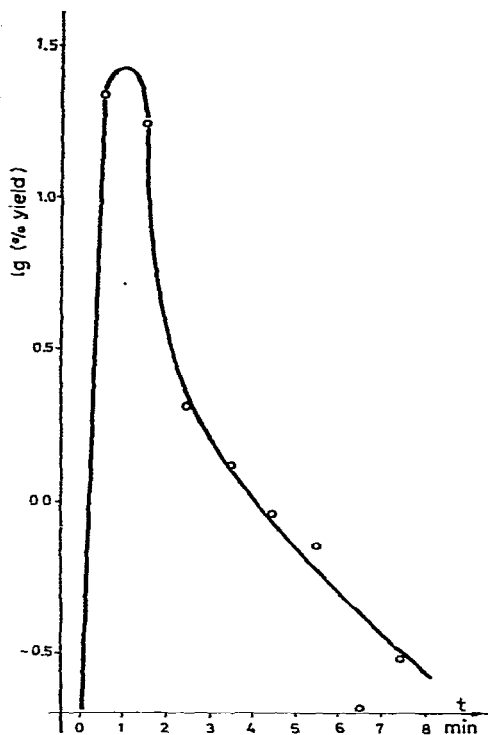
Fig. 5. Radio-GC of *ca.* 100 ng of ^{203}HgCl$_2$ in water. Column: 3% EGS, thoroughly cleaned by prolonged heating.
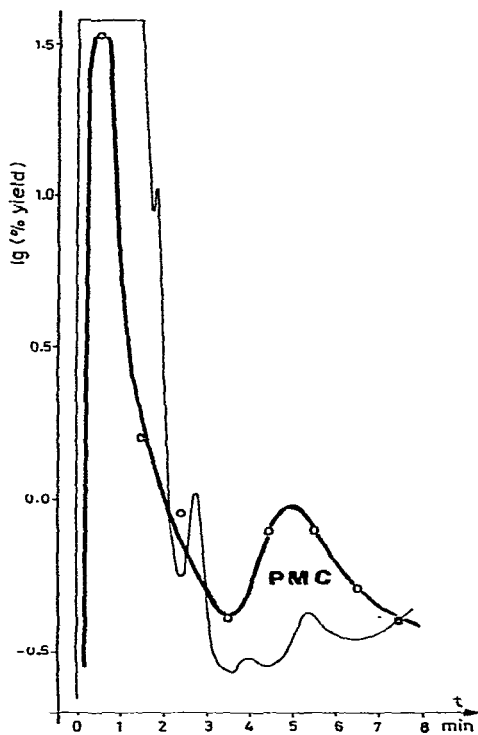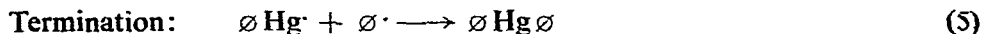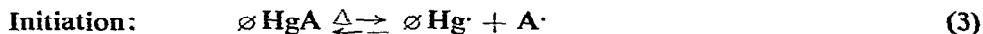
Fig. 6. Radio-GC (————) and conventional GC (————) of *ca.* 100 ng of ^{203}HgCl$_2$ in water, 10 min after injection of 2 μg of diphenylmercury. Column: 3% EGS.

which the ends of the separation column were plugged (this had been deactivated by silanization with DMCS) and (2) the liquid phase OV-17 (which is prepared by hydrolysis of DMCS and DPCS). The neutron-activation determination of chlorine in these materials gave 120 ppm of Cl in the quartz-wool and 7.0 ppm of Cl in the silicone oil OV-17*. The chlorine therein is probably bonded to silicon. Thus the source supplying the chlorine atoms necessary for transforming Hg to HgCl compounds was detected.

Dressman[31] has previously considered on-column reactions like these. For the DPM formation which he observed he suggested a free-radical mechanism:

Initiation: $\varnothing\,\mathrm{HgA} \underset{\longleftarrow}{\overset{\triangle}{\longrightarrow}} \varnothing\,\mathrm{Hg\cdot} + \mathrm{A\cdot}$ (3)

Propagation: $\mathrm{A\cdot} + \varnothing\,\mathrm{HgA} \longrightarrow \mathrm{AHgA} + \varnothing\cdot$ (4)

Termination: $\varnothing\,\mathrm{Hg\cdot} + \varnothing\cdot \longrightarrow \varnothing\,\mathrm{Hg}\,\varnothing$ (5)

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* The carrier material is conceivable as a chlorine source also, as it was treated with DMCS. Cl could not be determined therein by neutron-activation analysis because of the large contents of other nuclei capable of being activated, such as ^{23}Na, ^{27}Al and ^{39}K.

TABLE II

IDENTIFICATION OF ELUATES

a. Substance injected: DPM.

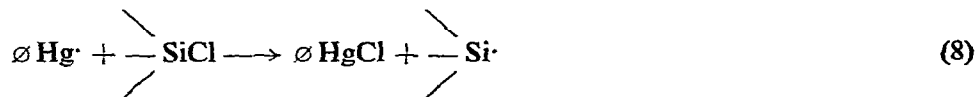| Substance injected	Column type	Eluates	
		Range before solute peak	Solute peak
A. DPM	3% EGS on Gas-Chrom Q	PMC, HgCl₂	PMC, no DPM
	3% OV-17 on Gas-Chrom Q	PMC, HgCl₂	PMC, no DPM
	5% OV-17 on Chromosorb P AW	PMC, HgCl₂	
B. DPM	3% EGS on Gas-Chrom Q*	HgCl₂	PMC
	3% OV-17 on Gas-Chrom Q*	HgCl₂, PMC	PMC
	5% OV-17 on Chromosorb P AW*	HgCl₂,	PMC
C. PMC	3% EGS on Gas-Chrom Q	PMC**	PMC
	3% OV-17 on Gas-Chrom Q	PMC**	PMC

* These columns contained a special reaction zone with DPCS as described under *Apparatus*.
** HgCl₂ below limit of detection.

Although he did not ascertain a chloride source, he explained the formation of PMC by a nucleophilic substitution:

$$\varnothing \, HgA + Cl^- \longrightarrow \varnothing \, HgCl + A^- \tag{6}$$

On the basis of the above, the following reaction mechanism for the on-column conversion of DPM to PMC is proposed:

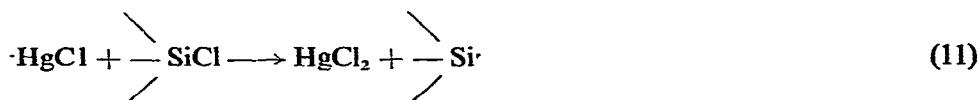$$\varnothing \, Hg \, \varnothing \; \overset{\Delta}{\underset{\longleftarrow}{\longrightarrow}} \; \varnothing \cdot + \varnothing \, Hg \cdot \tag{7}$$

$$\varnothing \, Hg \cdot + -\overset{|}{\underset{|}{Si}}Cl \longrightarrow \varnothing \, HgCl + -\overset{|}{\underset{|}{Si}} \cdot \tag{8}$$

$$-\overset{|}{\underset{|}{Si}} \cdot + \varnothing \cdot \longrightarrow -\overset{|}{\underset{|}{Si}} - \varnothing \tag{9}$$

An analogous reaction (SiCl₄ + ∅ Hg∅ → ∅ SiCl₃ + ∅ HgCl) was described by Ladenburg[34] as early as 1874. The approximately 10 μg amount of chlorine contained in the quartz-wool in the injection port of the column would suffice for the quantita-

tive conversion of $100 \mu g$ of DPM into PMC; this amount will not even approximately be injected during the service life of a column.

On simulating this reaction at the microgram level, PMC is detected by TLC in the reaction products, and the assumptions about the reaction mechanism have so far been confirmed by this procedure. .By making DPM react in this way with an excess of a chlorosilane, $HgCl_2$ is identified in the reaction product. The reaction was also capable of being repeated in the gas chromatograph: if the quartz-wool is replaced with a zone of DPCS-impregnated Kieselguhr 2 cm in length, mainly $HgCl_2$ is eluted from the column if DPM is injected [Table II (B) and Figs. 3a and 3b].

The mechanism of formation is analogous to eqns. 7–9:

$$\varnothing\, HgCl \overset{\Delta}{\underset{\rightleftharpoons}{\longrightarrow}} \varnothing \cdot + \cdot HgCl \tag{10}$$

$$\cdot HgCl + - \overset{\diagdown}{\underset{\diagup}{SiCl}} \longrightarrow HgCl_2 + - \overset{\diagdown}{\underset{\diagup}{Si}} \cdot \tag{11}$$

$$- \overset{\diagdown}{\underset{\diagup}{Si}} \cdot + \varnothing \cdot \longrightarrow - \overset{\diagdown}{\underset{\diagup}{Si}} - \varnothing \tag{9}$$

It is more difficult to explain why the PMC is eluted with no unequivocal retention time, irrespective of whether DPM or PMC was injected. In Fig. 1a the main peak of PMC appears after 5 min. Between 0 and 4 min a mixture of $HgCl_2$ and PMC emerges with almost uniform elution. This PMC cannot have been formed at the beginning of the GC separation process, and must have been produced from a substance with a shorter retention time. For this reaction, the mercury(II) chloride formed according to reactions 3–5 and/or 9–11 can be considered. After formation it is eluted considerably faster than the substances from which it was formed, and it is thereby re-phenylated. A phenylating reagent for this reaction may be DPM adsorbed on the carrier. In order to prove this assumption, *ca.* 100 ng of [203]$HgCl_2$ was first chromatographed on a column that had been cleaned by prolonged heating (*cf.*, Fig. 5). $HgCl_2$ was eluted as a single tailing peak. If to this column $2 \mu g$ of DPM was added, any $HgCl_2$ injected was partially converted into PMC (Fig. 6). If more DPM was adsorbed on the column, complete conversion of intermediately formed $HgCl_2$ into PMC would be expected,and this result is demonstrated in Fig. 2.

On the basis of the above, it was necessary to clarify whether the injected activity is retained by adsorption on the carrier and, if so, how much. The results showed that a commercially available DMCS-deactivated carrier (Gas-Chrom Q) adsorbs 33% of the injected activity in an air-dry state. After impregnation with 3% EGS, 31% is still retained. If, however, the material is dried and exhaustively silanized with DPCS, the losses by adsorption decrease to 8%. The identity of the adsorbed species could not be clarified, but the above-mentioned conversion of $HgCl_2$ into PMC suggested that it is DPM.

These results are of only a semi-quantitative nature. However, they give information on the column losses observed. The losses according to Table I amount to $51.6 \pm 15.5\%$ and $69.2 \pm 11.9\%$ for DPM and $58.5 \pm 12.0\%$ and 20.9% for PMC, respectively, which correspond with the above values of *ca.* 30%, at least in their order of magnitude.

CONCLUSION

⁣⁣⁣Based on these results, the preparation of separation columns was possible, allowing interference-free chromatography of phenylmercury(II) compounds. In such a column, symmetrical arylmercury compounds must react to yield arylmercury(II) chloride, and the column must be optimally permeable to arylmercury(II) chloride. Either condition can be fulfilled by very carefully deactivating and conditioning the surface-active materials with substituted chlorosilanes. Utilizing an ^3H ECD a limit of determination of 80 pg of DPM (absolute) was obtained on columns of this type.

REFERENCES

1 L. Friberg, B, Holmstedt, E. Johnsson, C. Ramel, S. Skerfving, A. Swensson and S. Tejning, *Nord. Hyg. Tidskr.*, 52 (1971) 37.
2 M. Fujiki, *Bunseki Kagaku (Jap. Anal.)*, 19 (1970) 1507.
3 S. Jensen, *Nord. Hyg. Tidskr.*, 50 (1969) 85.
4 L. R. Kamps and B. McMahon, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 590.
5 S. Kitamura, T. Tsukamoto, K. Hayakawa, K. Sumino and T. Shibata, *Igaku To Seibutsugaku (Med. Biol.)*, 72 (1965) 274.
6 S. Kitamura, T. Tsukamoto K. Hayakawa, K. Sumino and T. Shibata, *Igaku To Seibutsugaku (Med. Biol.)*, 73 (1966) 276.
7 T. Kosaka and Y. Takizawa, *Niigata Igakkai Zasshi (Niigata Med. J.)*, 80 (1966) 204.
8 T. Kosaka and Y. Takizawa, *Niigata Igakkai Zasshi (Niigata Med. J.)*, 80 (1966) 519.
9 K. Sumino, *Kobe J. Med. Sci.*, 14 (1968) 115.
10 K. Sumino, *Kobe J. Med. Sci.*, 14 (1968) 131.
11 Y. Takizawa and T. Kosaka, *Acta Med. Biol.*, 14 (1966) 153.
12 J. W. Huckabee, C. Feldman and Y. Talmi, *Anal. Chim. Acta*, 70 (1974) 41.
13 J. F. Uthe, J. Solomon and B. Grift, *J. Ass. Offic. Anal. Chem.*, 50 (1972) 583.
14 G. Westöö, *Acta Chem. Scand.*, 20 (1966) 2131.
15 G. Westöö, *Acta Chem. Scand.*, 21 (1967) 1970.
16 G. Westöö, *Chem. Fallout*, (1969) 75.
17 G. Westöö, B. Johansson and R. Ryhage, *Acta Chem. Scand.*, 24 (1970) 2349.
18 G. Westöö, *Acta Chem. Scand.*, 22 (1968) 1790.
19 G. Westöö, *Acta Chem. Scand.*, 22 (1968) 2277.
20 V. Zelenko and L. Kosta, *Talanta*, 20 (1973) 115.
21 P. Jones and G. Nickless, *Proc. Soc. Anal. Chem.*, 10 (1973) 269.
22 P. Jones and G. Nickless, *J. Chromatogr.*, 76 (1973) 285.
23 P. Jones and G. Nickless, *J. Chromatogr.*, 89 (1974) 201.
24 V. Luckow, *Dissertation*, Hannover, 1976.
25 P. Mushak, F. E. Tibbetts, P. Zarnegar and G. B. Fisher, *J. Chromatogr.*, 87 (1973) 215.
26 P. Zarnegar and P. Mushak, *Anal. Chim. Acta*, 69 (1974) 389.
27 R.Belcher, J. R. Majer, J. A. Rodriguez-Vazquez, W. I. Stephen and P. C. Uden, *Anal. Chim. Acta*, 57 (1971) 73.
28 S. Nishi and Y. Horimoto, *Bunseki Kagaku (Jap. Anal.)*, 17 (1968) 75.
29 S. Nishi, Y. Horimoto and R. Kobayashi, *Int. Symp. on Identification and Measurement of Environmental Pollutants*, National Research Council of Canada, Ottawa, 1971, p. 207.
30 G. Schwedt and H. A. Rüssel, *Z. Anal. Chem.*, 264 (1973) 301.
31 R. C. Dressman, *J. Chromatogr. Sci.*, 10 (1972) 468.
32 G. L. Baughman, M. H. Carter, N. L. Wolf and R. G. Zepp, *J. Chromatogr.*, 76 (1973) 471.
33 V. Luckow and H. A. Rüssel, *Chromatographia*, 9 (1976) 578.
34 A. Ladenburg, *Justus Liebigs Ann. Chem.*, 173 (1874) 143.