A NEW LIQUID PHASE FOR GAS CHROMATOGRAPHIC SEPARATIONS OF STEROIDS

Sir:

A new kind of thermostable polar liquid phase for gas chromatography has been found. It is silicone polymer QF-1, a fluorinated alkyl silicone of the Dow–Corning Corp.[1] This phase may be used in the same fashion as the non-polar silicone polymer SE-30. It has polar properties that suggest many applications in the identification, estimation and preparative separation of steroids and other natural products.

Polar phases useful at about 220° may be compared for degree of polarity by determining the separation factor for the $-CH_2CH_2-$ group in a homologous series of aliphatic compounds with about 20 to 30 carbon atoms. With 1% liquid phase columns, a series of wax esters (C_{24} to C_{36}) showed these separation factors for the $-CH_2CH_2-$ group at 190–210°:

Silicone polymer SE-30	1.87
Neopentyl glycol succinate (NGS)	1.74
Silicone polymer QF-1	1.66

The separation factor observed for QF-1 indicates a highly polar nature as far as methylene groups are concerned.[2]

The properties of QF-1 were investigated through use of a series of steroids to determine whether selective functional group retention effects were present. The results are summarized in the table. Non-polar retention effects are shown for comparison in the SE-30 data. The NGS values reflect polar properties leading to a considerable increase in retention times for hydroxy and keto compounds; the effect is comparable in magnitude for both functional groups, and a somewhat smaller effect is shown for ester groups. QF-1 showed a selective behavior. An increase in retention times was observed for compounds with oxygen-containing functional groups in the order ether < hydroxyl < ester < keto. Further, the retention times for hydroxy and keto steroids varied with structural variations to a far greater extent than has been observed for other phases. For example, QF-1 is the only phase found so far that is useful for the separation of cholesterol and cholestanol (separation factor, cholestanol/cholesterol, 1.09 for a 1% phase at 202°). Another example is the separation of 5α-pregnane-3β,20α-diol and 5α-pregnane-20β-ol-3-one; this pair has not been amenable to separation with other phases. A demonstration of this property is shown in Fig. 1, where a comparison is made of the behavior of five closely related steroids with SE-30 and QF-1 phases

Polar phases in use in gas chromatography at the present time all have oxygen- or nitrogen-containing functional groups. The use of fluoroalkyl silicones provides an alternate approach to the problem of obtaining low-bleed stereospecific polar phases for use in natural products work.

(1) We are indebted to the Dow–Corning Corp. for supplies of QF-1 polymers.

(2) Many more highly polar phases may be used at lower temperatures; the only polar phases currently used for steroid separations are a few thermostable polyesters, as for example NGS and EGIP (ethylene glycol isophthalate).

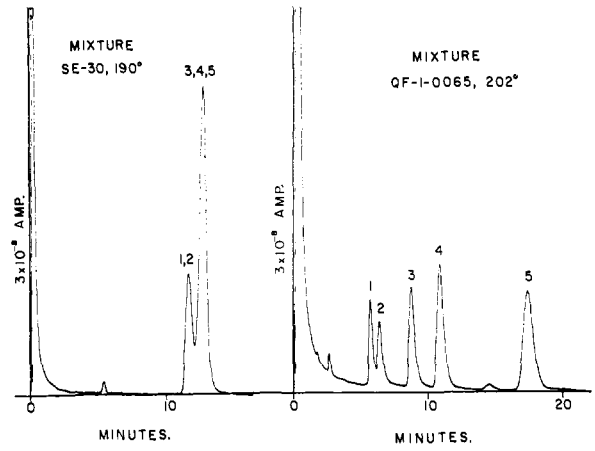

Fig. 1.—Comparison of separations for a mixture of five closely related steroids with SE-30 and QF-1 liquid phases. The columns were the same as those used to obtain the data in the table. The temperatures were 190° (SE-30) and 202° (QF-1). The compounds were (1) 5α-pregnane-3β, 20β-diol, (2) 5α-pregnane-3β,20α-diol, (3) 5α-pregnane-3β-ol-20-one, (4) 5α-pregnane-20β-ol-3-one, and (5) 5α-pregnane-3,20-dione. QF-1 liquid phase was obtained from the Dow–Corning Corp. as polymer QF-1-0065.

The techniques used here were those developed earlier for separations of steroids by gas chromatography.[3] They are equally useful for separations

TABLE I

COMPARISON OF RELATIVE RETENTION TIMES FOR STEROIDS WITH NON-POLAR AND POLAR PHASES

Compound	Relative retention time SE-30,[a] 208°	NGS,[b] 210°	QF-1,[c] 195°
Cholestane	1.00[d]	1.00[e]	1.00[f]
Cholestanyl trifluoroacetate	1.65	2.22	3.23
Cholestanyl methyl ether	1.86	2.56	2.31
Cholestanol	2.04	6.34	3.48
Cholestan-3-one	2.20	6.78	6.77
Cholestanyl acetate	3.10	6.03	5.76
5α-Pregnane-3β,20β-diol	0.67	6.47	1.94
5α-Pregnane-3β,20α-diol	0.72	7.64	2.16
5α-Pregnane-3β-ol-20-one	0.67	6.52	2.98
5α-Pregnane-20β-ol-3-one	0.72	7.64	3.70
5α-Pregnane-3,20-dione	0.72	7.20	5.93
	215°[g]	215°[h]	202°[i]
Cholestane	1.00[j]	1.00[k]	1.00[l]
Cholesterol	1.94	6.48	3.08
Cholestanol	1.99	6.10	3.36

[a] Column, 1.8 m. × 3 mm. i.d., 0.75% SE-30 on 100–140 mesh Gas-Chrom P, 16 p.s.i. [b] Column, 1.8 m. × 4 mm. i.d., 0.75% neopentyl glycol succinate on 100–140 mesh Gas-Chrom P, 22 p.s.i. Retention times observed with this phase are dependent on both the temperature and age of the column. [c] Column, 1.8 m. × 5 mm. i.d., 1% QF-1 (10,000 cs.) on 100–140 mesh Gas-Chrom P, 14 p.s.i. [d] Time, 9.35 min. [e] Time, 3.65 min. [f] Time, 4.15 min. [g] Same column at 215°, 18 p.s.i. [h] Same column at 215°, 17 p.s.i [i] Same column at 202°, 14 p.s.i. [j] Time, 6.1 min. [k] Time, 3.1 min. [l] Time, 2.9 min.

(3) W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, *J. Am. Chem. Soc.*, **82**, 3481 (1960) (steroids); W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, *Biochem. Biophys. Res. Comm.*, **3**, 33 (1960) (sex hormones and bile acids); W. J. A. VandenHeuvel and

of many other classes of naturally occurring and synthetic compounds and they are particularly valuable in investigations of complex mixtures of natural origin.

E. C. Horning, *ibid.*, **3**, 356 (1960) (adrenal cortical steroid hormones); W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, "Separation of Steroids by Gas Chromatography," Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy, June 2–4, 1960 (sterols and sterol esters); C. C. Sweeley and E. C. Horning, *Nature*, **187**, 144 (1960) (steroids), H. Ziffer, W. J. A. VandenHeuvel, E. O. A. Haahti and E. C. Horning, *J. Am. Chem. Soc.*, **82**, 6411 (1960) (Vitamins D_2 and D_3); W. J. A. VandenHeuvel, E. C. Horning, Y. Sato and N. Ikekawa, *J. Org. Chem.*, in press (steroidal amines); E. O. A. Haahti, W. J. A. VandenHeuvel and E. C. Horning, *ibid.*, in press (polyester phases for steroids); W. J. A. VandenHeuvel and E. C. Horning, *ibid.*, in press (sapogenins); W. J. A. VandenHeuvel, J. Sjövall and E. C. Horning, *Biochim. Biophys. Acta*, in press (trifluoroacetyl derivatives of steroids); E. O. A. Haahti, W. J. A. VandenHeuvel and E. C. Horning, *Anal. Biochem.*, in press (urinary 17-ketosteroids).

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RECEIVED FEBRUARY 10, 1961

OBSERVATIONS ON THE COMPOUND REPORTED AS "DI-TRIMETHYL STANNYL ETHYLENE"

Sir:

Because of current interest in vinylmetallics[1] it was desired to obtain the compound reported[2] as $(CH_3)_3SnCH{=}CHSn(CH_3)_3$ in order further to study its chemical and spectroscopic properties.

Trimethyltin chloride (28.3 g., 0.142 mole) suspended in 250 ml. of anhydrous ammonia at $-70°$ was treated with sodium (6.53 g., 0.284 g. atom) in small portions, giving a yellow solution of trimethylstannylsodium. To this, chloroform (5.66 g., 0.0474 mole) was added with stirring over one hour, discharging the yellow color. After evaporation of ammonia, the reaction flask was washed with *n*-pentane, the solution filtered and solvent removed. The liquid remaining yielded 19.0 g. of distillate, boiling range 53–60° (6 mm.) The infrared spectrum for this gross reaction product in the region 4000–650 cm.$^{-1}$ showed absorptions from methyl groups on tin, similar to those given for tetramethyltin,[3] with the addition of a sharp strong peak at 955 cm.$^{-1}$.

The proton n.m.r. spectrum[4] for the gross product consisted of a complex series of peaks. Among other resonances discussed below, an intense peak at 9.81 indicated presence of hexamethylditin, a product reported in the earlier work.[2] Gross product was titrated with ethereal iodine and the trimethyltin iodide formed precipitated as the ammoniate. The solution was filtered and solvent removed. In this manner, 19.0 g. of reaction product yielded 20.0 g. (0.065 mole) of $(CH_3)_3SnNH_3{}^+I^-$, accounting for 10.6 g. (55%) of the gross product as hexamethylditin. There remained purified product, I, of which 8.0 g. distilled at 58–60° (6 mm.)

The infrared spectrum for I was similar to that described for the gross product. Removal of hexamethylditin was not accompanied by loss of any bands in the region 4000–650 cm.$^{-1}$.

A Raman spectrum[5] for I recorded these shifts in cm.$^{-1}$ from the mercury exciting line of 4358 Å. (relative intensities in parentheses): 106 (4.4), 128 (6.7), 150 (7.6), δSn–C; 469 (3.7), 518 (10), 610 (0.4), νSn–C; 956 (0.7), δC–H; 1196 (2.7), 1352 (0.3), δCH_3; 2897 (2.3), 2913 (3.1), 2981 (1.7), νC–H. These lines correspond approximately to those of tetramethyltin[3] or hexamethylditin,[6] with the addition of the line at 956 cm.$^{-1}$. Absence of any lines in the region 1500–1800 cm.$^{-1}$ of the Raman spectrum, however, is a compelling argument against postulating any C=C bonds[7] in I.

Cleavage[8] of 1.05 g. of I by 137 cc. (6.1 mmole) of dry hydrogen chloride yielded a solid and a noncondensable gas identified as methane (infrared spectrum), and no other volatile products, in particular, *no ethylene*. The solid, identified as trimethyltin chloride by its infrared spectrum and melting point (38–39°), weighed 1.2 g. (6.05 mmole). A second cleavage, carried out on 2.31 g. of I with 150 cc. (6.7 mmole) of dry hydrogen chloride, yielded a liquid, a solid, and no volatile gases. The liquid was separated, and identified as tetramethyltin by infrared spectrum and vapor pressure at 0°; found, 33 mm., reported[9] 34 mm. The solid was identified as trimethyltin chloride; yield of each of these products was 6.5 ± 0.2 mmole.

The accumulated data give no support to the earlier formulation of I as $(CH_3)_3SnCH{=}CHSn(CH_3)_3$. Instead, all evidence leads to $(CH_3)_3SnCH_2Sn(CH_3)_3$ as the composition and structure of I. Analysis yielded: C, 24.5; H, 5.70; required for $C_7H_{20}Sn_2$: C, 24.6; H, 5.9; for $C_8H_{20}Sn_2$: C, 27.2; H, 5.70. Bis-trimethyl stannylmethane, independently synthesized from dichloromethane and trimethylstannylsodium according to Kraus and Neal,[10] gave infrared and proton n.m.r. spectra identical with those of I.

It is evident that trimethylstannylsodium and chloroform in liquid ammonia yield the products $(CH_3)_3SnCH_2Sn(CH_3)_3$ and hexamethylditin in equimolar quantities (over-all yields 70–80%) by the reactions

(1) H. D. Kaesz and F. G. A. Stone, Chapter 3, A.C.S. Monograph 147, "Organometallic Chemistry" (Editor, H. Zeiss), Reinhold Publishing Corp., New York, N. Y., 1960.

(2) C. A. Kraus and A. M. Neal, *J. Am. Chem. Soc.*, **52**, 4426 (1930).

(3) W. F. Edgell and C. H. Ward, *ibid.*, **77**, 6486 (1955).

(4) Proton resonances were obtained using a Varian V-4310A spectrometer, 40 Mcs. Resonances were observed for neat liquids and then measured for 10–20% solutions in carbon tetrachloride relative to the methyl-group protons of 1–2% internal toluene standard. Tetramethylsilane under these conditions appeared at 91.7 ± 0.5 c.p.s. All resonances are reported here as τ values, $\pm 0.01\tau$; *cf.* L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 47, and G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(5) Cary Raman Spectrophotometer, Model 81, Applied Physics Corporation—instrument settings: region 100–200 cm.$^{-1}$, single slit/2 cm.$^{-1}$, sensitivity 1 × 200; region 200–3000 cm.$^{-1}$, single slit/10 cm.$^{-1}$, sensitivity 1.35 × 10; sample volume, 0.25 ml.

(6) M. P. Brown, E. Cartmell and G. W. A. Fowles, *J. Chem. Soc.*, 506 (1960).

(7) R. N. Jones and C. Sandorfy, Chapter IV, "Chemical Applications of Spectroscopy" (Editor, W. West), Interscience Publishers, New York, N. Y., 1956, p. 368.

(8) Carried out in a high vacuum system; all cc. given at S.T.P.

(9) R. T. Sanderson, "Vacuum Manipulation of Volatile Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 148.

(10) C. A. Kraus and A. M. Neal, *J. Am. Chem. Soc.*, **52**, 695 (1930).