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Prediction of retention indexes

III. Silylated derivatives of polar compounds

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ABSTRACT

Polar compounds containing hydroxyl, amino and carboxyl groups, singly or in combination, can be chromatographed after the polar functional groups are silylated. The silylated derivatives of acids, alcohols, amines, diols, amino alcohols, amino acids are shown to behave chromatographically as hydrocarbons, and their retention indexes can be readily predicted from their base values. The column difference, namely, the difference between the retention indexes of the analyte on polar and non-polar columns is minimal for the silylated derivatives in comparison to that observed for the underivatized analytes. This minimal column difference is attributed to the hydrocarbon-like chromatographic characteristics of the silylated derivatives. The retention indexes of the silyl derivatives appear to correlate with the atom number Z of the analyte.

INTRODUCTION

Compounds containing functional groups, such as hydroxyl, amino and carboxyl groups singly or in combination in the form of alcohols, phenols, aliphatic and aromatic amines, glycols, amino alcohols, carboxylic acids and amino acids, are polar and hydrophilic. These compounds are difficult to chromatograph and often yield skewed and asymmetrical elution peaks. Sometimes these compounds may not emerge from the column at all. This difficulty can be overcome by derivatizing the polar compounds to lessen the hydrophilicity of the substituent groups.

We have shown earlier [1] that the methyl esters of aliphatic acids have retention indexes (I) identical to those of *n*-alkanes with an equal number of atoms when chromatographed on non-polar columns (SE-30, DB-1). We have also shown [1] that methylation and alkylation alter the polarity and functionality of amino and hydroxyl groups, and the methylated and alkylated derivatives behave chromatographically as aliphatic hydrocarbons. In this report, we will show that silylated derivatives of compounds containing amino, hydroxyl and carboxyl groups, either singly or in combination, may also show chromatographic characteristics similar to that of *n*-alkanes. Silylation minimizes the intramolecular interaction between functional groups and facilitates the prediction of retention indexes.

EXPERIMENTAL

Trimethylchlorosilane (TMCS), N,O-bis(trimethylsilyl)acetamide (BSA), dimethyl-*tert.*-butylsilyltrifluoroacetamide (MTBSTFA) and dimethyl-

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formamide (DMF) were purchased from Pierce (Rockford, IL, USA). *tert.*-Butyldimethylchlorosilane (TBDMS-CL) was purchased from Fluka (Buchs, Switzerland). All other chemicals were obtained from Aldrich (Milwaukee, WI, USA). Solvents used were of analytical grade and dry.

Silylation method A

Approximately 1.0 mg of solid or 5.0 μl of liquid analyte is introduced into a 0.6-ml Reacti-Vial (Regis). To the reaction vials, is also added 0.1 ml of the silylating reagent consisting of a mixture of 3.0 ml MTBSTFA, 1.0 ml DMF and 80 mg TBDMS-CL. The vial is capped and heated at 75–80°C for 45 min [2,3]. After cooling the reaction mixture is injected directly into the gas chromatograph. This reaction will replace the active hydrogen in the analyte molecule with a TBDMS group.

Silylation method B

This method is similar to method A, except that a different silylating reagent consisting of 3.0 ml BSA, 1.0 ml DMF and 0.08 ml TMCS is used. The reaction mixture is heated at 75–80°C for 15 min or longer. This reagent will replace the reactive hydrogen atoms in the analyte molecule with TMS groups [2,3].

Chromatography

All chromatographic runs were performed on Hewlett-Packard Model 5890 and 5880A gas chromatographs, equipped with thermal conductivity detectors. Integration was performed by a HP Model 3393 integrator on Model 5890 and by Level 4 Integration on Model 5880A. The non-polar column used was a fused-silica capillary column DB-1 (15 m × 0.53 mm I.D., film thickness 1.5 μm) obtained from J&W Scientific, Folsom, CA, USA. The polar columns used were stainless-steel columns (3.05 m × 3.175 mm I.D.) packed with 10% Carbowax 20M (CW-20M) on 80–100 mesh Supelcoport and fused-silica capillary DB-Wax columns (15 m × 0.53 mm I.D., film thickness 1.0 μm) from J&W Scientific. The operating conditions were similar to those described [1,4]. Briefly, the injector was at 250°C and the detector at 300°C. The oven temperature was programmed to begin at 40°C isothermally for 4 min and then linearly increased at a rate of 8°C/min to 200°C, which was the final temperature for the DB-Wax column. The DB-1 column was maintained at 200°C for 1 min and then linearly increased again at a rate of 5°C/min to 280°C. The maximum oven temperature was maintained for additional 20 min if necessary. In some cases, the temperature program was initiated from 100°C. The early peaks overlapped in the chromatogram but their retention times were listed separately in the report by the electronic integrator.

The I values were calculated according to Van den Dool and Kratz [5]. The I values obtained with the initial temperature at 40°C differed only insignificantly from those obtained with the initial temperature at 100°C.

RESULTS AND DISCUSSION

Methods of prediction

We reported [1] that the retention index of an analyte on non-polar and polar columns can be predicted using the equation:

$$I_p = 100Z + \Sigma m_i - \Sigma n_i \quad (1)$$

where I_p is the predicted retention index; Z the total number of carbon atoms and carbon equivalent atoms such as nitrogen, oxygen, etc.; m_i and n_i represent group retention factors (*GRF*s) of substituents and functional groups the presence of which in the molecule either increases or decreases the retention index. Detailed steps for predicting the retention index from chemical structure are given in ref. 1.

When the I values of the series of homologues or their silylated derivatives are plotted against the total number of carbon, oxygen and nitrogen atoms (Z) in the molecule, a straight line is obtained and may be represented by linear regression equations as follows:

$$I = AZ + B \quad (2)$$

$$= AZ' + B' \quad (3)$$

and

$$B' = B - A(Z' - Z) \quad (4)$$

where the regression coefficient A represents the I increment for atom addition; Z and Z' are the numbers of atoms in the underivatized compound and its silylated derivative, respectively; the intercept B represents the total of the *GRF* value and the

I increment from the carbon atoms of the silylating reagent; B' differs from B in that it contains only the *GRF* value of the silylating group without the contribution from the carbon atoms. The relationship of B' with B is given by eqn. 4.

According to Kováts' convention, A is assigned a value of 100 for the series of normal alkanes and the m_i, n_i and B are zero. Both eqns. 1 and 2 yield a value of $100Z$ which is known as the base value. For monofunctional and monosubstituted compounds such as alcohols, amines, aldehydes, etc., the A values are close to 100 and the I values predicted from eqns. 1 and 2 are practically identical, within 3% error of the observed value. In highly polar compounds such as acids and amides the A values are significantly less than 100 [1,4], and the I values predicted by eqn. 1 may show large deviations from the observed values. It may be pointed out that the carboxyl and the amide groups are consisted of carbonyl, hydroxyl and amino groups in combination and may be considered bifunctional chromatographically.

The value of A is sensitive to multisubstitution and polyfunctionality. The presence of highly polar substituents in the molecule can depress A. The decrease in A may also affect the *GRF* value; as a result, the I values of polyfunctional and multisubstituted compounds cannot be accurately predicted by eqn. 1. Accurate A values can only be obtained using eqn. 2, but if the homologues of the analyte are not readily available, accurate prediction of the I values will become extremely difficult.

The strongly non-polar nature of the silylated derivatives allows their I values to be readily predicted directly from the base values [4]. If the analyte contains additional functional groups that are not affected by silylation, their group retention factors (*GRF*s) or functionality constants must be taken into consideration in predicting I.

Linear regression equations for different homologous series of silylated derivatives are listed in Table I. These equations are based on Z and B or on Z' and B' values, as defined above. The statistics of the regression coefficient (A) and the intercept (B or B') of various homologous series were calculated using the Statistical Analytical System (SAS) procedure on a 486 personal computer[a].

Polyfunctionality and derivatization

In polyfunctional compounds intramolecular interaction of the functional groups can affect the A and *GRF* values so that their I values cannot be readily predicted using eqn. 1. Derivatization modifies the polarity of the analyte to facilitate the prediction of I.

Silylation is one of the preferred derivatizing reactions. It is fast, attains completion readily and affords a high yield. A large number of silylating reagents are available commercially [3]. Reactivity of these silylating reagents decreases from alcohols to amines in the following order: alcohols (primary > secondary > tertiary) > phenols > carboxylic acids > primary amines > acid amides > secondary amines [2].

Silylation replaces the active hydrogen atoms in the –OH, COOH and $-NH_2$ groups with trimethylsilyl (TMS) or *tert.*-butyldimethylsilyl (TBDMS) groups. The TMS group is less bulky than the TBDMS group and can replace both active hydrogen atoms in the primary amino ($-NH_2$) group, whereas the TBDMS can replace only one active hydrogen with ease. In general the TBDMS derivatives are more stable towards hydrolysis than the TMS derivatives.

The silicon atom in the silyl group does not make a contribution to I, as shown by the I of ethyl ester of 2-(trimethylsilyl)acetic acid (see Table X). The TMS group contains three methyl carbon atoms; its incorporation into a molecule will increase its I value by 300 units. The TBDMS group contains five methyl carbon atoms and one quaternary carbon atom and will contribute a net increment of 500 ($= 600 - 100$) to I. Silylation masks the functionality and eliminates the *GRF* value of the functional group. Unlike the underivatized compounds, the silylated derivatives of alcohols, amines and carboxylic acids exhibit comparable I values on nonpolar (SE-30, DB-1) and polar (CW-20M, DB-Wax) columns.

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[a] The regression analysis was performed with SAS/STAT statistical analysis procedures on a 486 personal computer. Data given for each homologous series include (i) the number of data points (n), (ii) standard errors of the regression coefficient and the intercept (S.E.), (iii) the coefficient of determination (R^2), (iv) the probability of getting a greater F statistic than that observed if the hypothesis is true; *i.e.*, the significance probability (p). The meaning of these terms is given in ref. 6.

TABLE 1

LINEAR REGRESSION EQUATIONS FOR COMPOUNDS OF MONOFUNCTIONALITY AND POLYFUNCTIONALITY AND THEIR SILYLATED DERIVATIVES ON NON-POLAR AND POLAR COLUMNS

I = retention index; Z = the number of C plus N and O atoms; Z' = Z + the number of C atoms in the silylating agent.

	I	
	DB-1	CW-20M, DB-Wax
(I) Homologous series with monofunctional groups		
(A) *n*-Alkanoic acids		
Free acids	(93.37 ± 0.90) Z + (257.26 ± 9.44)[a]	(105.60 ± 0.88) Z + (971.50 ± 9.59)
	(n = 13, R^2 = 0.9990, p = 0.0001)	(n = 8, R^2 = 0.9996, p = 0.0001)
O-(TMS) derivatives	(94.79 ± 0.53) Z + (321.17 ± 5.50)	
	(94.79 ± 0.53) Z' + (36.81 ± 6.96)	
	(n = 13, R^2 = 0.9997, p = 0.0001)	
O-(TBDMS) derivatives	(96.41 ± 0.82) Z + (521.31 ± 8.89)	(98.13 ± 1.34) Z + (598.10 ± 15.70)
	(96.41 ± 0.82) Z' − (57.15 ± 13.55)	(98.13 ± 1.34) Z' + (9.34 ± 23.47)
	(n = 12, R^2 = 0.9993, p = 0.0001)	(n = 10, R^2 = 0.9985, p = 0.0001)
(B) *n*-Alkanols		
Free alcohols	(99.71 ± 0.81) Z + (157.94 ± 9.86)	(101.29 ± 0.63) Z + (630.52 ± 7.75)
	(n = 11, R^2 = 0.9994, p = 0.0001)	(n = 11, R^2 = 0.9996, p = 0.0001)
O-(TMS) derivatives	(96.67 ± 0.15) Z + (319.26 ± 1.83)	
	(96.67 ± 0.15) Z' + (29.26 ± 2.24)	
	(n = 11, R^2 = 1.0000, p = 0.0001)	
O-(TBDMS) derivatives	(98.08 ± 0.44) Z + (519.25 ± 5.37)	(94.98 ± 0.50) Z + (534.30 ± 6.05)
	(98.08 ± 0.44) Z' − (69.21 ± 7.81)	(94.98 ± 0.50) Z' − (35.58 ± 8.79)
	(n = 11, R^2 = 0.9998, p = 0.0001)	(n = 11, R^2 = 0.9998, p = 0.0001)
(C) *n*-Aliphatic primary amines		
Free amines	(101.71 ± 0.61) Z + (117.56 ± 5.94)	
	(n = 9, R^2 = 0.9997, p = 0.0001)	
Acetone adducts	(99.37 ± 1.32) Z + (350.06 ± 11.65)	(98.17 ± 0.16) Z + (582.66 ± 1.32)
	(99.37 ± 1.32) Z' + (51.94 ± 15.47)	(98.17 ± 0.16) Z' + (188.14 ± 1.78)
	(n = 6, R^2 = 0.9993, p = 0.0001)	(n = 7, R^2 = 1.0000, p = 0.0001)
N-(TMS) derivatives	(98.46 ± 0.25) Z + (365.98 ± 2.39)	(91.12 ± 1.11) Z + (606.28 ± 9.24)
	(98.46 ± 0.25) Z' + (70.59 ± 3.05)	(91.12 ± 1.11) Z' + (332.93 ± 12.37)
	(n = 9, R^2 = 1.0000, p = 0.0001)	(n = 6, R^2 = 0.9994, p = 0.0001)
N,N-Bis(TMS) derivatives	(99.37 ± 1.32) Z + (350.06 ± 11.65)	
	(99.37 ± 1.32) Z' + (51.94 ± 15.47)	
	(n = 6, R^2 = 0.9993, p = 0.0001)	
N-(TBDMS) derivatives	(100.47 ± 1.22) Z + (569.84 ± 13.30)	
	(100.47 ± 1.22) Z' + (32.95 ± 20.20)	
	(n = 7, R^2 = 0.9993, p = 0.0001)	
(II) Homologous series with multiple functional groups		
(A) ω-Amino-1-alkanols		
Free amino alcohols	(106.70 ± 8.13) Z + (321.80 ± 50.09)	
	(n = 5, R^2 = 0.9829, p = 0.0010)	
O-(TMS) derivatives	(96.50 ± 1.70) Z + (458.60 ± 10.48)	
	(96.50 ± 1.70) Z' + (169.10 ± 15.49)	
	(n = 5, R^2 = 0.9991, p = 0.0001)	
O,N,N-Tris(TMS) derivatives	(96.30 ± 2.72) Z + (889.80 ± 16.78)	
	(96.30 ± 2.72) Z' + (23.10 ± 41.01)	
	(n = 5, R^2 = 0.9976, p = 0.0001)	
O,N-Bis(TBDMS) derivatives	(102.20 ± 2.02) Z + (1045.40 ± 12.47)	
	(102.20 ± 2.02) Z' − (181.00 ± 36.53)	
	(n = 4, R^2 = 0.9988, p = 0.0001)	

(Continued on p. 117)

TABLE I *(continued)*

	I	
	DB-1	CW-20M, DB-Wax
(B) α-Amino acids		
O,N-Bis(TMS) derivatives	(70.50 ± 2.48) Z + (689.50 ± 18.80)	
	(70.50 ± 2.48) Z' + (266.50 ± 33.59)	
	(n = 4, R^2 = 0.9975, p = 0.0012)	
O,N-Bis(TBDMS) derivatives	(71.80 ± 4.31) Z + (1097.50 ± 32.69)	
	(71.80 ± 4.31) Z' + (235.90 ± 94.19)	
	(n = 4, R^2 = 0.9928, p = 0.0036)	
(C) Terminally substituted amino acids		
O,N,N-Tris(TMS) derivatives	(110.78 ± 2.18) Z + (824.10 ± 17.25)	
	(110.78 ± 218) Z' − (82.88 ± 36.56)	
	(n = 5, R^2 = 0.9986, p = 0.0001)	
O,N-Bis(TBDMS) derivatives	(103.11 ± 2.18) Z + (1023.95 ± 17.21)	
	(103.11 ± 2.18) Z' − (213.40 ± 42.97)	
	(n = 5, R^2 = 0.9987, p = 0.0001)	
(D) Alkane diols		
O,O-Bis(TMS) derivatives	(93.50 ± 7.79) Z + (614.50 ± 39.49)	
	(93.50 ± 7.79) Z' + (53.50 ± 85.97)	
	(n = 3, R^2 = 0.0031, p = 0.0529)	
O,O-Bis(TBDMS) derivatives	(97.50 ± 4.91) Z + (1007.17 ± 24.86)	
	(97.50 ± 4.91) Z' − (162.83 ± 83.52)	
	(n = 3, R^2 = 0.9900, p = 0.0320)	
(E) Acid amides		
N-(TBDMS) derivatives	(44.00 ± 15.01) Z + (909.33 ± 76.05)	
	(44.00 ± 15.01) Z' + (689.33 ± 150.61)	
	(n = 3, R^2 = 0.8957, p = 0.2093)	
O,N-Bis(TBDMS) derivatives	(55.00 ± 6.35) Z + (1117.33 ± 32.17)	
	(55.00 ± 6.35) Z' + (512.33 ± 101.75)	
	(n = 3, R^2 = 0.9868, p = 0.0732)	

[a] The linear regression equations are given in the form of (A ± S.E.) Z + (GRF ± S.E.) where A is the regression coefficient, GRF the intercept, and S.E. the standard errors. The statistics given are the number of data points (n), standard errors for the regression coefficient and the intercept (S.E.), the coefficient of determination (R^2) and the significance probability (p), *i.e.*, the probability of getting a greater F statistic than that obtained if the hypothesis is true. It should be pointed out that all regression analysis can be seriously distorted by a single incorrect data value (see ref. 6).

Carboxylic acids

Upon silylation, the active hydrogen in the carboxylic acid group is replaced by the silyl group. The *I* values of the TMS esters on DB-1 column are approximated by their base values, and those on CW-20M column by their base values plus 150. The value of 150 represents the *GRF* for the residual polarity of the TMS ester group (–CO–O–TMS) on the polar column. The *I* values of the TBDMS esters on non-polar and polar columns can be calculated from their base values. Since the quaternary carbon atom in the TBDMS group has a *GRF* of −100 on non-polar and polar columns, this value must be subtracted from the base value. The *GRF* for the residual polarity of the TBDMS ester group (–CO–O–TBDMS) has a value of +100 on polar column, which must be added to obtain the predicted *I*. The TBDMS ester group appears to be less polar than the TMS ester group and has a smaller *GRF* value. Table II compares the observed and predicted *I* values of TMS and TBDMS derivatives of homologous aliphatic carboxylic acids on non-polar and polar columns.

Plotting the observed *I* values of the TMS and TBDMS esters and the free acids on DB-1 and CW-20M columns against the number of atoms (Z) in the parent molecule yields straight lines, as shown in Fig. 1. The column difference (ΔI), defined as the

TABLE II

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED ESTERS OF *n*-ALKANOIC ACIDS ON DB-1 AND CW-20M

Listed in the table are the following values: (1) for TMS esters: (i) I_p (on DB-1) = base value, (ii) I_p (on CW-20M) = base value + 100; (2) for TBDMS esters: (i) I_p (on DB-1) = base value − 100, (ii) I_p (on CW-20M) = base value; (3) the *GRF* for the silyl ester group on CW-20M = +100; (4) the *GRF* for the quaternary C atom in TBDMS group = −100.

Compound and silylated esters	Formula	On DB-1 column[a]			On CW-20M column[a]		
		I_{obs}	I_p	Difference (%)	I_{obs}	I_p	Difference (%)
Formic acid	CH_2O_2	512					
O-(TMS)-	$C_4H_{10}O_2$	612	600	3.5			
Acetic acid	$C_2H_4O_2$	642					
O-(TMS)-	$C_5H_{12}O_2Si$	705	700	0.71			
O-(TBDMS)-	$C_8H_{18}O_2Si$	930	900	3.33			
Propionic acid	$C_3H_6O_2$	743					
O-(TMS)-	$C_6H_{14}O_2Si$	800	800	0			
O-(TBDMS)-	$C_9H_{20}O_2Si$	1015	1000	1.5			
n-Butanoic acid	$C_4H_8O_2$	823			1600		
O-(TMS)-	$C_7H_{16}O_2Si$	891	900	1	1025	1000	2.5
O-(TBDMS)-	$C_{10}H_{22}O_2Si$	1098	1100	0.18	1200	1200	0
n-Pentanoic acid	$C_5H_{10}O_2$	925			1706		
O-(TMS)-	$C_8H_{18}O_2Si$	975	1000	2.5	1123	1100	2.3
O-(TBDMS)-	$C_{11}H_{24}O_2Si$	1184	1200	1.33	1294	1300	0.46
n-Hexanoic acid	$C_6H_{12}O_2$	992			1834		
O-(TMS)-	$C_9H_{20}O_2Si$	1071	1100	2.64	1200	1200	0
O-(TBDMS)-	$C_{12}H_{26}O_2Si$	1276	1300	1.85	1385	1400	1.07
n-Heptanoic acid	$C_7H_{14}O_2$	1103			1916		
O-(TMS)-	$C_{10}H_{22}O_2Si$	1166	1200	2.83			
O-(TBDMS)-	$C_{13}H_{28}O_2Si$	1378	1400	1.57	1474	1500	1.73
n-Octanoic acid	$C_8H_{16}O_2$	1187					
O-(TMS)-	$C_{11}H_{24}O_2Si$	1260	1300	3.08	1400	1400	0
O-(TBDMS)-	$C_{14}H_{30}O_2Si$	1482	1500	1.2	1582	1600	1.13
n-Nonanoic acid	$C_9H_{18}O_2$	1280			2132		
O-(TMS)-	$C_{12}H_{26}O_2Si$	1358	1400	3.01			
O-(TBDMS)-	$C_{15}H_{32}O_2Si$	1578	1600	1.38	1638	1700	1.01
n-Decanoic acid	$C_{10}H_{20}O_2$	1374			2238		
O-(TMS)-	$C_{13}H_{28}O_2Si$	1455	1500	3.01			
O-(TBDMS)-	$C_{16}H_{34}O_2Si$	1674	1700	1.53	1777	1800	1.28
Lauric acid	$C_{12}H_{24}O_2$	1545			2451		
O-(TMS)-	$C_{15}H_{32}O_2Si$	1651	1700	2.88			
O-(TBDMS)-	$C_{18}H_{38}O_2Si$	1872	1900	1.47	1977	2000	1.15
Myristic acid	$C_{14}H_{28}O_2$	1747			2660		
O-(TMS)-	$C_{17}H_{36}O_2Si$	1845	1900	2.89			
O-(TBDMS)-	$C_{20}H_{42}O_2Si$	2075	2100	1.19	2176	2200	1.09
Palmitic acid	$C_{16}H_{32}O_2$	1956					
O-(TMS)-	$C_{19}H_{40}O_2Si$	2036	2100	3.05			
O-(TBDMS)-	$C_{22}H_{46}O_2Si$	2263	2300	1.61	2370	2400	1.25

[a] Base value = 100 *Z*, where *Z* is the number of carbon and carbon equivalent atoms. I_{obs} = observed *I*; I_p = predicted *I*; Difference (%) = (difference between I_{obs} and I_p) × 100/(I_{obs} or I_p).

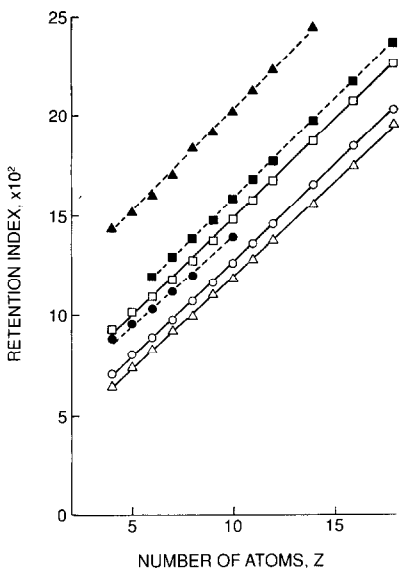

Fig. 1. Convergence of retention indexes of silylated alkanoic acid esters on non-polar and polar columns. The homologues of free acids and their TMS and TBDMS esters are given as △, ○ and □ on DB-1 column (solid lines) and as ▲, ● and ■ on DB-Wax column (broken lines), respectively.

difference between *I* on polar and non-polar columns [4], appears to be much smaller for the silylated acid esters than for the underivatized acids. This clearly demonstrates that silylation alters the polarity and polarizability of the analyte and that the silylated derivatives mimic the chromatographic behavior of *n*-alkanes.

Aliphatic fatty acids are monofunctional, while the alicyclic and aromatic acids, because of the ring function, are bifunctional. Silylation may also diminish the *GRF* of the ring function. The decrease is more pronounced with silylated alicyclic acid esters than with silylated benzoic acid ester. In bromo- and iodobenzoic acids, silylation may also decrease the *GRF*s of the halo atoms.

Aliphatic alcohols

Silylated derivatives of alcohols are ethers. The ether oxygen has a *GRF* of zero on non-polar column and a value of +70 on polar column [4]. Silylation masks completely the polarity of the ether oxygen of the silylated ethers on polar column and reduces its *GRF* to practically zero. As a result, silylated derivatives of alcohols exhibit approximately the same *I* values on CW-20M and DB-1 columns, as shown in Table III.

Table IV shows the *I* values of silylated ethers of alicyclic alcohols and phenols. The *GRF* values of the 6-membered alicyclic rings and the phenyl ring in the silylated ethers are not affected, but the functionality of the –OH group will be modified by silylation. In alicyclic silylated ethers, chain branching, due to the presence of iso-carbon, has a *GRF* value of −40 on DB-1 column which should be considered in obtaining the predicted *I*.

Primary aliphatic amines

The *I* values of primary aliphatic amines may be affected by the solvent used in diluting the analyte. The *I* values of the amines are higher by about 210 units in acetone than in ethanol. According to the structure–retention-index relationship the higher *I* observed in acetone should belong to a molecule with at least two more atoms than the amine molecule, suggesting the formation of Schiff's base or anil. The high temperature in the injection port accelerates the coupling of the amine and the solvent acetone. According to this scheme, the reaction product of *n*-hexylamine in acetone is shown as follows:

$$C_6H_{13}NH_2 + O{=}C(CH_3)_2 \rightarrow C_6H_{13}N{=}C(CH_3)_2$$

The identity of the anil was confirmed by analysis by gas chromatography–mass spectrometry.

The *I* values of the anils formed from amino alkanes and acetone can be predicted from the base values plus 52 which is the *GRF* for the anil $-N{=}(CH_3)_2$ group. For higher amines the anil reaction may be incomplete for the brief duration in the injection port. *n*-Decylamine and *n*-dodecylamine in acetone were found to show two chromatographic peaks each, one corresponding to the amine and the other to the acetone adduct or the anil. A comparison of the observed and predicted *I* values of *n*-alkylamines in acetone and in ethanol is given in Table V.

The primary amino $-NH_2$ group possesses two active hydrogen atoms. Depending on the condition of silylation and the molecular connectivity of the carbon atom to which the amino group is attached, one or two hydrogen may be replaced. Mono-substitution converts the primary amine to a secondary amine and disubstitution converts the primary

TABLE III

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED ETHERS FROM *n*-ALKANOLS

The predicted *I* values for TMS ethers are: I_p (DB-1) = I_p (CW-20M) = base value. The predicted *I* values for TBDMS ethers are: I_p (DB-1) = I_p (CW-20M) = base value −100, where −100 is the *GRF* for the quaternary C atom in TBDMS group.

Compound and silylated ethers	Formula	On DB-1 column[a]			On CW-20M column[a]		
		I_{obs}	I_p	Difference (%)	I_{obs}	I_p	Difference (%)
Ethanol	C_2H_6O	446			944		
O-(TMS)-	$C_5H_{14}OSi$	614	600	2.28	652	600	7.98
O-(TBDMS)-	$C_8H_{20}OSi$	827	800	3.38	834	800	4.08
1-Propanol	C_3H_8O	552			1025		
O-(TMS)-	$C_6H_{16}OSi$	705	700	0.71	730	700	4.11
O-(TBDMS)-	$C_9H_{22}OSi$	907	900	0.78	919	900	2.07
1-Butanol	$C_4H_{10}O$	659			1141		
O-(TMS)-	$C_7H_{18}O$	800	800	0	836	800	4.3
O-(TBDMS)-	$C_{10}H_{24}OSi$	1012	1000	1.2	1000	1000	0
1-Pentanol	$C_5H_{12}O$	767			1236		
O-(TMS)-	$C_8H_{20}OSi$	900	900	0	921	900	2.28
O-(TBDMS)-	$C_{11}H_{26}OSi$	1107	1100	0.36	1106	1100	0.55
1-Heptanol	$C_7H_{16}O$	974			1440		
O-(TMS)-	$C_{10}H_{24}OSi$	1092	1100	0.72	1108	1100	0.72
O-(TBDMS)-	$C_{13}H_{30}OSi$	1293	1300	0.54	1282	1300	1.38
1-Decanol	$C_{10}H_{22}O$	1267			1735		
O-(TMS)-	$C_{13}H_{30}OSi$	1379	1400	1.5	1385	1400	1.07
O-(TBDMS)-	$C_{16}H_{36}OSi$	1590	1600	0.63	1569	1600	1.94
1-Dodecanol	$C_{12}H_{26}O$	1420			1950		
O-(TMS)-	$C_{15}H_{34}OSi$	1575	1600	1.56	1573	1600	1.69
O-(TBDMS)-	$C_{18}H_{40}OSi$	1800	1800	0	1762	1800	2.11
1-Tetradecanol	$C_{14}H_{30}O$	1647			2157		
O-(TMS)-	$C_{17}H_{38}OSi$	1770	1800	1.67	1759	1800	2.28
O-(TBDMS)-	$C_{20}H_{44}OSi$	1987	2000	0.65	1969	2000	1.55
1-Hexadecanol	$C_{16}H_{34}O$	1852			2352		
O-(TMS)-	$C_{19}H_{42}OSi$	1965	2000	1.75	1959	2000	2.05
O-(TBDMS)-	$C_{22}H_{48}OSi$	2185	2200	0.68	2156	2200	2.01
1-Heptadecanol	$C_{17}H_{36}O$	1960			2475		
O-(TMS)-	$C_{20}H_{44}OSi$	2056	2100	2.1	2046	2100	2.57
O-(TBDMS)-	$C_{23}H_{50}OSi$	2280	2300	0.87	2244	2300	2.43
1-Octadecanol	$C_{18}H_{38}O$	2059			2534		
O-(TMS)-	$C_{21}H_{46}OSi$	2159	2200	1.86	2144	2200	2.54
O-(TBDMS)-	$C_{24}H_{52}OSi$	2395	2400	0.21	2339	2400	2.54

[a] See footnote in Table II.

amine to a tertiary amine. The *GRF* for residual amino functionality of an amino group connected to a secondary carbon atom is +50. Disubstitution with two TMS silyl groups occurs only in an amino group connected to a primary carbon atom with a *GRF* value of zero or below zero in some cases [4]. In contrast, the TBDMS group is bulkier than the TMS group and yields only mono-silylated derivatives. Structural identities of the mono- and di-TMS derivatives of *n*-hexylamine have been verified by gas chromatography–mass spectrometry analysis. Table VI compares the observed and predicted

TABLE IV

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SOME SILYLATED ETHERS (ON DB-1)[a]

The *GRF*s (m_i and n_i) used for *I* prediction have the following values: (1) alicyclic hydroxyl group = +119, (2) cyclopentane ring = +64, (3) quaternary carbon atom in TBDMS group = −100, (4) chain branching = −40, (5) cyclohexane ring = +62, (6) cyclohexene ring = +67, (7) phenyl ring = +58, (8) phenolic hydroxyl group = +211.

Compound and silylated ethers	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Cyclopentanol	$C_5H_{10}O$	774	600 + 119 + 64	783	1.15
O-(TBDMS)-	$C_{11}H_{24}OSi$	1119	1200 + 64 − 100 − 40	1124	0.44
Cyclohexanol	$C_6H_{12}O$	881	700 + 62 + 119	881	0
O-(TMS)-	$C_9H_{20}OSi$	1000	1000 + 62 − 40	1022	2.15
O-(TBDMS)-	$C_{12}H_{26}OSi$	1214	1300 + 62 − 100 − 40	1222	0.64
2-Cyclohexen-1-ol	$C_6H_{10}O$	887	700 + 67 + 119	886	0.11
O-(TMS)-	$C_9H_{18}OSi$	1027	1000 + 67 − 40	1027	0
2-Methylcyclohexanol	$C_7H_{14}O$	941	800 + 62 + 119 − 40	941	0
O-(TMS)-	$C_{10}H_{22}OSi$	1067	1100 + 62 − 2 × 40	1082	1.39
O-(TBDMS)-	$C_{13}H_{28}OSi$	1285	1400 + 62 − 100 − 2 × 40	1282	0.62
4-Methylcyclohexanol	$C_7H_{14}O$	941	800 + 62 + 119 − 40	941	0
O-(TMS)-	$C_{10}H_{22}OSi$	1071	1100 + 62 − 2 × 40	1082	1.02
O-(TBDMS)-	$C_{13}H_{28}OSi$	1279	1400 + 62 − 100 − 2 × 40	1282	0.23
Phenol	C_6H_6O	962	700 + 58 + 211	969	0.72
O-(TMS)-	$C_9H_{14}OSi$	1043	1000 + 58	1058	1.42
O-(TBDMS)-	$C_{12}H_{20}OSi$	1260	1300 + 58 − 100	1258	0.16
m-Cresol	C_7H_8O	1064	800 + 58 + 211	1069	0.47
O-(TMS)-	$C_{10}H_{22}OSi$	1134	1100 + 58	1158	2.07
O-(TBDMS)-	$C_{13}H_{22}OSi$	1348	1400 + 58 − 100	1358	0.74

[a] See footnote in Table II.

I values of some silylated alicyclic and aromatic amines. The aromatic amino group in aniline takes only one TMS group upon silylation, and the amino group in benzylamine is also mono-substituted. Silylation modifies the amine function in cyclohexylamine to make it resemble a hydrocarbon which introduces chain branching. Chain branching in alicyclic hydrocarbons chain branching has a *GRF* value of −40 [1].

Polyfunctional compounds

The *I* values of polyfunctional compounds are difficult to predict. Their *I* values cannot be estimated from the *GRF*s used for monofunctional compounds by the rule of additivity. The functional groups can interact intramolecularly among themselves to affect both *A* and *GRF* values so that the observed *I* and the predicted *I* based on the group additivity rule may be at variance. Alkylamines and aliphatic carboxylic acids can be chromatographed alone without derivatization, but underivatized amino acids, containing both free amino and carboxyl groups in the same molecule, have infinitely large *I* values and do not emerge from the column.

Derivatization by silylation greatly reduces the polarity of polyfunctional molecules and allows these molecules to be chromatographed as silylated derivatives. Their *I* values are readily estimated from the base values. A limited number of polyfunctional compounds in the following categories have been studied.

n-Alkanediols. Underivatized alkanediols yield asymmetrical peaks. Once derivatized, the TMS and TBDMS derivatives of the diols give sharp, symmetrical peaks. The *I* values of the TMS derivatives can be predicted from the base values, as shown in Table VII. The *I* values of TBDMS derivatives are predicted from the base values minus 200 which

TABLE V

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED DERIVATIVES OF *n*-ALKAMINES (ON DB-1)[a]

The *GRF*s (m_i and n_i) have the following values: (1) primary amine, $-CH_2NH_2$ = +133, (2) $-NHC_2H_5$ group = −30, (3) –NH–(TMS) group = +50, (4) $-N=(TMS)_2$ group = 0, (5) anil group = +52, (6) quaternary C atom in TBDMS group = −100, (7) chain branching = −40.

Compound and silylated derivatives	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Ethylamine	C_2H_7N	413	300 + 133 − 30	403	2.42
N-(TMS)-	$C_5H_{15}NSi$	660	600 + 50	650	1.52
n-Propylamine	C_3H_9N	515	400 + 133	533	3.38
N-(TMS)-	$C_6H_{17}NSi$	755	700 + 50	750	0.66
N,N-Bis(TMS)-	$C_9H_{25}NSi_2$	1031	1000	1000	3.01
n-Butylamine	$C_4H_{11}N$	626	500 + 133	633	1.1
Acetone adduct	$C_7H_{15}N$	835	800 + 52	852	1.99
N-(TMS)-	$C_7H_{19}NSi$	862	800 + 50	850	1.39
N,N-Bis(TMS)-	$C_{10}H_{27}NSi_2$	1115	1100	1100	1.35
N-(TBDMS)-	$C_{10}H_{25}NSi$	1085	1100 + 50 − 100	1050	3.23
n-Pentylamine	$C_5H_{13}N$	744	600 + 133	733	1.48
Acetone adduct	$C_8H_{17}N$	960	900 + 52	951	0.94
N-(TMS)-	$C_8H_{21}NSi$	963	800 + 50	850	1.39
N,N-Bis(TMS)-	$C_{11}H_{29}NSi_2$	1200	1200	1200	0
N-(TBDMS)-	$C_{11}H_{27}NSi$	1177	1200 + 50 − 100	1150	2.29
Isopentylamine	$C_5H_{13}N$	705	600 + 133 − 40	693	1.7
N-(TMS)-	$C_8H_{21}NSi$	920	900 + 50 − 40	910	1.09
n-Hexylamine	$C_6H_{15}N$	829	700 + 133	833	0.48
Acetone adduct	$C_9H_{19}N$	1046	1000 + 52	1052	0.57
N-(TMS)-	$C_9H_{23}NSi$	1055	1000 + 50	1050	0.47
N,N-Bis(TMS)-	$C_{12}H_{31}NSi_2$	1301	1300	1300	0.08
N-(TBDMS)-	$C_{12}H_{29}NSi$	1271	1300 + 50 − 100	1250	1.65
n-Heptylamine	$C_7H_{17}N$	937	800 + 133	933	0.43
Acetone adduct	$C_{10}H_{21}N$	1145	1100 + 52	1152	0.61
N-(TMS)-	$C_{10}H_{25}NSi$	1153	1100 + 50	1150	0.26
N,N-Bis(TMS)-	$C_{13}H_{33}NSi_2$	1390	1400	1400	0.71
N-(TBDMS)-	$C_{13}H_{31}NSi_2$	1373	1400 + 50 − 100	1350	1.68
n-Decylamine	$C_{10}H_{23}N$	1237	1100 + 133	1233	0.32
Acetone adduct	$C_{13}H_{27}N$	1442	1400 + 52	1452	0.69
N-(TMS)-	$C_{13}H_{31}NSi$	1447	1400 + 50	1450	0.21
N,N-Bis(TMS)-	$C_{16}H_{39}NSi_2$	1692	1700	1700	0.49
N-(TBDMS)-	$C_{16}H_{37}NSi$	1647	1700 + 50 − 100	1650	0.18
n-Dodecylamine	$C_{12}H_{27}N$	1444	1300 + 133	1433	0.76
Acetone adduct	$C_{15}H_{31}N$	1641	1600 + 52	1652	0.67
N-(TMS)-	$C_{15}H_{35}NSi$	1644	1600 + 50	1650	0.36
N,N-Bis(TMS)-	$C_{18}H_{43}NSi_{23}$	1893	1900	1900	0.37
N-(TBDMS)-	$C_{18}H_{41}NSi$	1879	1900 + 50 − 100	1850	1.54
n-Octadecylamine	$C_{18}H_{39}N$	2043	1900 + 133	2033	0.49
N-(TMS)-	$C_{21}H_{47}NSi$	2233	2200 + 50	2250	0.53
N,N-Bis(TMS)-	$C_{24}H_{55}NSi_2$	2486	2500	2500	0.56
N-(TBDMS)-	$C_{24}H_{53}NSi$	2489	2500 + 50 − 100	2450	1.57

[a] See footnote in Table II.

TABLE VI

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED DERIVATIVES OF SOME AMINES (ON DB-1)[a]

The *GRF*s (m_i and n_i) have the following values: (1) cyclohexene ring = +62, (2) –NH–(TMS) group attached to secondary C atom = +25, (3) chain branching = −40, (4) –NH–(TMS) group = +50, (5) phenyl ring = +58, (6) aromatic secondary amine, Ar–NH–(TMS) group = +130, (7) aromatic tertiary amine, Ar–N(TMS)R group = +26.

Compound and silylated derivatives	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Cyclohexylamine	$C_6H_{13}N$	851			
N-(TMS)-	$C_9H_{21}NSi$	1061	1000 + 62 + 25 − 40	1047	1.32
3-Aminoheptane	$C_7H_{17}N$	868		877	1.03
N-(TMS)-	$C_{10}H_{25}NSi$	1073	1100 + 25 − 40	1085	1.11
2-Aminopentane	$C_7H_{17}N$	868		877	1.03
N-(TMS)-	$C_{10}H_{25}NSi$	1081	1100 + 25 − 40	1085	0.37
3-Methylcyclohexylamine	$C_7H_{15}N$				
N-(TMS)-	$C_{10}H_{23}NSi$	1110	1100 + 62 + 50 − 2 × 40	1132	1.94
Aniline	C_6H_7N	955			
N-(TMS)-	$C_9H_{15}NSi$	1186	1000 + 58 + 130	1188	0.17
Benzylamine	C_7H_9N				
N-(TMS)-	$C_{10}H_{17}NSi$	1212	1100 + 58 + 50	1208	0.33
Phenethylamine	$C_8H_{11}N$	1115		1091	2.15
N-(TMS)-	$C_{11}H_{19}NSi$	1280	1200 + 58 + 50	1308	2.14
o-Toluidine	C_7H_9N	1068			
N-(TMS)-	$C_{10}H_{17}NSi$	1244	1100 + 58 + 130	1288	3.41
m-Toluidine	C_7H_9N	1064			
N-(TMS)-	$C_{10}H_{17}NSi$	1269	1100 + 58 + 130	1288	1.48
N-Methylaniline	C_7H_9N	1056			
N-(TMS)-	$C_{10}H_{17}NSi$	1195	1100 + 58 + 26	1184	0.92
N-Ethylaniline	$C_8H_{11}N$				
N-(TMS)-	$C_{11}H_{19}NSi$	1303	1200 + 58 + 26	1284	1.46

[a] See footnote in Table II.

represents the *GRF* values for the two quaternary carbon atoms in the TBDMS groups. The *I* values of the TMS and TBDMS derivatives of propylene glycol (*i.e.*, 1,2-propanediol) are lower than those of 1,3-propanediol, because the former contains a secondary alcoholic hydroxyl group.

ω-Amino-l-alkanols. ω-Amino-l-alkanols contain both terminal amino ($-NH_2$) and hydroxyl (–OH) groups. The amino groups of the amino alkanols can form anils with acetone. The hydroxyl group is more easily silylated than the primary amino group, and the TMS reagent will first react with the hydroxyl group under mild conditions to form the TMS ether with only one TMS group per molecule. A longer reaction time silylates both hydroxyl and amino groups, with the terminal amino group disubstituted. The fully silylated amino alcohol will contain three TMS groups per molecule. The TBDMS group is bulkier than the TMS group and will replace only one hydrogen from the amino group. As a result, the fully silylated amino alcohols will contain two TBDMS groups per molecule. Table VIII lists the observed and predicted *I* values of the amino alcohols.

Amino groups connected to secondary and tertiary carbon atoms are not readily silylated.

Amino acids. Underivatized amino acids are nonvolatile and cannot be analyzed by gas chromato-

TABLE VII

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF O,O-BIS(TMS)- AND O,O-BIS(*tert.*-BUTYLDIMETHYLSILYL)ALKANEDIOLS (ON DB-1)[a]

The *GRF*s (m_i and n_i) have the following values: (1) quaternary C atom in TBDMS group = −100, (2) the difference between primary and secondary alcohol function = −70.

Compound and silylated ether	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Ethylene glycol	$C_2H_6O_2$				
O,O-Bis(TMS)-	$C_8H_{22}O_2Si_2$	993	1000,	1000	0.7
O,O-Bis(TBDMS)-	$C_{14}H_{34}O_2Si_2$	1400	1600 − 2 × 100	1400	0
Propylene-1,2-diol	$C_3H_8O_2$				
O,O-Bis(TMS)-	$C_9H_{24}O_2Si_2$	1013	1100 − 70	1013	1.65
O,O-Bis(TBDMS)-	$C_{15}H_{36}O_2Si_2$	1424	1700 − 200 − 70	1430	0.42
1,3-Propanediol	$C_3H_8O_2$				
O,O-Bis(TMS)-	$C_9H_{24}O_2Si_2$	1073	1100,	1100	2.45
O,O-Bis(TBDMS)-	$C_{15}H_{36}O_2Si_2$	1489	1700 − 200	1500	0.73
1,4-Butanediol	$C_4H_{10}O_2$				
O,O-Bis(TMS)-	$C_{10}H_{26}O_2Si_2$	1180	1200,	1200	1.67
O,O-Bis(TBDMS)-	$C_{16}H_{38}O_2Si_2$	1595	1800 − 200	1600	0.31

[a] See footnote in Table II.

graphy. Amino acids can be chromatographed as TMS or TBDMS derivatives. The *I* values of silylated amino acids are determined by the number of silyl groups in the molecule and the proximity of the amino and carboxyl groups. The amino group at the α or β position to the carboxyl group is only monosubstituted, but the amino group connected to a methylene carbon at the terminal carbon atom is unhindered and will be disubstituted. The amino group in glycine is known to be disilylated by the TMS reagent [7].

Table IX lists the observed and predicted *I* values of TMS and TBDMS derivatives of some amino acid homologues containing ω, α or β amino groups. The terminal ω amino groups are disubstituted with TMS groups. The molecule that contains a silylated ester group and a terminal amino group with two TMS groups may further decrease its *I* by −60. Amino groups attached to secondary carbon atoms can only be monosubstituted, and the residual polarity of the resulting secondary amino group will have a *GRF* value of +50.

In α-amino acids, the close proximity of the silylated amino and carboxyl groups will reduce the *I* by −80 and decrease the value of *A* to about 71. In comparison, the isomeric ω-amino acids with the amino and carboxyl groups at each end of an alkane chain have normal *A* values near 100. Linear plots of *I vs. Z* of the TMS and TBDMS derivatives of isomeric α- and ω-amino acids are shown in Fig. 2. The α-amino acids have a smaller *A* value than the ω-amino acids. A small *A* value is associated with high polarity and the steric factor that reduces *I*. The statistical data for the regression coefficients and the intercepts are given in Table I.

Acid amides. Acid amides can accept one or two TBDMS groups. The first TBDMS group will be attached to the amide N atom and the second TBDMS group to the amide O atom. This result agrees with those of others [2].

$$\mathrm{R{-}CO{-}NH_2 + 2\ TBDMS \rightarrow R{-}\overset{\displaystyle O\text{–}TBDMS}{\overset{|}{C}}{=}N\text{–}TBDMS}$$

The acid amide group is highly polar and will affect the *A* values of mono- and di-TBDMS-substituted derivatives. The *A* for the monosubstituted derivatives is 44 and that for the disubstituted derivatives 55. Because the true value of *A* is so different from the assigned value of 100, the base value can no longer be used to predict *I* of these silylated derivatives. Accurate prediction can be

TABLE VIII

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED DERIVATIVES OF ω-AMINO-*n*-ALKANOLS (ON DB-1)[a]

The *GRF*s (m_i and n_i) have the following values: (1) primary alcohol group in a molecule with terminal $-NH_2$ group = +210, (2) primary alcohol $-CH_2OH$ = +156, (3) primary amine $-CH_2NH_2$ = +133, (4) quaternary C atom in TBDMS group = −100, (5) anil group = +52, (6) $-N=(TMS)_2$ group = 0, (7) –NH (TBDMS) group = +50.

Compound and silylated derivatives	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
2-Amino-ethanol	C_2H_7NO	739	400 + 210 + 133	743	0.54
O-(TMS)-	$C_5H_{15}NOSi$	842	700 + 133	833	0.12
O,N,N-Tris(TMS)-	$C_{11}H_{31}NOSi_3$	1281	1300	1300	1.46
O,N-Bis(TBDMS)-	$C_{14}H_{35}NOSi_2$	1448	1600 − 200 + 50	1450	0.14
3-Amino-1-propanol	C_3N_9NO	842	500 + 210 + 133	843	0.12
O-(TMS)-	$C_6H_{17}NOSi$	948	800 + 133	933	1.58
O,N,N-Tris(TMS)-	$C_{12}H_{33}NOSi_3$	1371	1400	1400	2.07
O,N-Bis(TBDMS)-	$C_{15}H_{37}NOSi_2$	1560	1700 − 200 + 50	1550	0.64
4-Amino-1-butanol	$C_4H_{11}NO$	998	600 + 210 + 133	943	5.51
Acetone adduct	$C_7H_{15}NO$	1115	900 + 156 + 52	1108	0.63
O-(TMS)-	$C_7H_{19}NOSi$	1032	900 + 133	1033	0.1
O,N,N-Tris(TMS)-	$C_{13}H_{35}NOSi_3$	1459	1500	1500	2.73
O,N-Bis(TBDMS)-	$C_{16}H_{39}NOSi_2$	1666	1800 − 200 + 50	1650	0.96
5-Amino-1-pentanol	$C_5H_{13}NO$	1075	700 + 210 + 133	1043	2.97
Acetone adduct	$C_8H_{17}NO$	1219	1000 + 156 + 52	1208	0.9
O-(TMS)-	$C_8H_{21}NOSi$	1135	1000 + 133	1133	0.18
O,N,N-Tris(TMS)-	$C_{14}H_{37}NOSi_3$	1558	1600	1600	2.63
O,N-Bis(TBDMS)-	$C_{17}H_{41}NOSi_2$	1760	1900 − 200 + 50	1750	0.57
6-Amino-1-hexanol	$C_6H_{15}NO$	1156	800 + 210 + 133	1143	1.12
Acetone adduct	$C_9H_{17}NO$	1307	1100 + 156 + 52	1308	0.08
O-(TMS)-	$C_9H_{23}NOSi$	1231	1100 + 133	1233	0.16
O,N,N-Tris(TMS)-	$C_{15}H_{39}NOSi_3$	1669	1700	1700	1.82
O,N-Bis(TBDMS)-	$C_{18}H_{43}NOSi_2$	1859	2000 − 200 + 50	1850	0.48

[a] See footnote on Table II.

made, based on the true values of *A* and the regression equations. The *A* and *GRF* values for these derivatives are given in Table I under acid amides. Comparison of the predicted and observed *I* values is given in Table X.

Aliphatic dicarboxylic acids. Dicarboxylic acids such as oxalic acid, succinic acid, etc., can be silylated to yield disilylated acid esters. Their *I* values on non-polar column can be predicted from the base values minus 60. A *GRF* value of −60 is assigned to account for the presence of two terminal silyl groups in the di-acid molecule. The validity of this assumption will be further investigated. A comparison of the observed and predicted *I* values of these silyl derivatives is given in Table X.

Miscellaneous. The *I* values of miscellaneous silylated compounds may also be predicted from the base values according to the rules outlined above. Table X gives the comparison of the observed and predicted *I* values of some silylated derivatives. The presence of a silyl group in the molecule may diminish the *GRF*s of other substituents and functional groups. The vanishingly small *GRF*s for the alicyclic rings in the alicyclic hydrocarbon carboxylic acids and the reduced *GRF*s of the bromo (from +276 to +240) and iodo (from +380 to +325) substituents in halobenzoic acids are such examples.

Chain branching refers to branching from the main alkyl chain. At the point of branching a

TABLE IX

COMPARISON OF OBSERVED AND PREDICTED *I* VALUES OF SILYLATED DERIVATIVES OF α- AND ω-AMINO ACIDS (ON DB-1)[a]

The *GRF*s (m_i and n_i) have the following values: (1) $-CH_2-N(TMS)_2$ group = −60, (2) =CH–NH(TBDMS) group = +50, (3) proximity of =CH–NH(TBDMS) and =CH–COO(TBDMS) groups = −80, (4) quaternary C atom in TBDMS reagent = −100.

Compound and silylated derivatives	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
2-Amino acetic acid (glycine)	$C_2H_5NO_2$				
O,N,N-Tris(TMS)-	$C_{11}H_{29}NO_2Si_3$	1317	1400 − 60	1340	1.72
O,N-Bis(TBDMS)-	$C_{14}H_{33}NO_2Si_2$	1551	1700 + 50 − 200	1550	0.06
3-Amino-1-propionic acid (β-alanine)	$C_3H_7NO_2$				
O,N,N-Tris(TMS)-	$C_{12}H_{31}NO_2Si_3$	1434	1500 − 60	1440	0.42
O,N-Bis(TBDMS)-	$C_{15}H_{35}NO_2Si_2$	1629	1800 + 50 − 200	1650	1.27
4-Amino-1-butanoic acid	$C_4H_9NO_2$				
O,N,N-Tris(TMS)-	$C_{13}H_{33}NO_2Si_3$	1542	1600 − 60	1540	1.3
O,N-Bis(TBDMS)-	$C_{16}H_{37}NO_2Si_2$	1744	1900 + 50 − 200	1750	0.34
6-Amino-1-hexanoic acid	$C_6H_{13}NO_2$				
O,N,N-Tris(TMS)-	$C_{15}H_{37}NO_2Si_3$	1726	1800 − 60	1740	0.8
O,N-Bis(TBDMS)-	$C_{18}H_{41}NO_2Si_2$	1955	2100 + 50 − 200	1950	0.26
8-Amino-1-octanoic acid	$C_8H_{17}NO_2$				
O,N,N-Tris(TMS)-	$C_{17}H_{41}NO_2Si_3$	1931	2000 − 60	1940	0.46
O,N-Bis(TBDMS)-	$C_{20}H_{45}NO_2Si_2$	2159	2300 + 50 − 200	2150	0.42
2-Amino-1-propionic acid (alanine)	$C_3H_7NO_2$				
O,N-Bis(TMS)-	$C_9H_{23}NO_2Si_2$	1114	1200 − 80	1120	0.54
O,N-Bis(TBDMS)-	$C_{15}H_{35}NO_2Si_2$	1532	1800 − 200 − 80	1520	0.79
2-Amino-1-butanoic acid	$C_4H_9NO_2$				
O,N-Bis(TMS)-	$C_{10}H_{25}NO_2Si_2$	1184	1300 − 80	1220	2.95
O,N-Bis(TBDMS)-	$C_{16}H_{37}NO_2Si_2$	1600	1900 − 200 − 80	1620	1.64
3-Amino-1-butanoic acid	$C_4H_9NO_2$				
O,N-Bis(TMS)-	$C_{10}H_{25}NO_2Si_2$	1218	1300 − 80	1220	0.16
O,N-Bis(TBDMS)-	$C_{16}H_{37}NO_2Si_2$	1647	1900 − 200 − 80	1620	1.64
2-Amino-1-pentanoic acid (DL-norvaline)	$C_5H_{11}NO_2$				
O,N-Bis(TMS)-	$C_{11}H_{27}NO_2Si_2$	1247	1400 − 80	1320	5.53
O,N-Bis(TBDMS)-	$C_{17}H_{39}NO_2Si_2$	1661	2000 − 200 − 80	1720	3.88
2-Amino-1-hexanoic acid (DL-norleucine)	$C_6H_{13}NO_2$				
O,N-Bis(TMS)-	$C_{12}H_{29}NO_2Si_2$	1328	1500 − 80	1420	6.48
O,N-Bis(TBDMS)-	$C_{18}H_{41}NO_2Si_2$	1751	2100 − 200 − 80	1820	3.79

[a] See footnote in Table II.

methylene carbon atom is converted into a tertiary carbon atom; this change alters the molecular connectivity of the carbon atom and decreases the *GRF* by 40. In cyclohexanol, silylation not only eliminates the alcohol functionality but also reduces *I* due to effective chain branching, resulting from the added silyl group.

In a homologous series the *A* value may be strongly affected by large *GRF* values. Derivatization will generally reduce the latter but increase the former. There are indications that the *GRF*s of other functional groups in the molecule may be affected by the presence of highly polar and polarizable substituent groups. This may be one of the reasons that the *I* values of compounds of polyfunctionality are difficult to predict based on the *A* and *GRF* values alone.

TABLE X

MISCELLANEOUS COMPOUNDS ON NON-POLAR AND POLAR COLUMNS[a]

On DB-Wax column

The *GRF*s (m_i and n_i) have the following values: (1) $-COOCH_3$ group = +295, (2) phenyl ring = +350, (3) the N in pyridine ring = +240, (4) C–NH–(TBDMS) group = +50, (5) quaternary C atom in TBDMS group = −100.

Compound	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Methyl (trimethylsilyl)acetate	$C_6H_{14}O_2Si$	1093	800 + 295	1095	0.18
Nicotinamide, O,N-Bis(TBDMS)-	$C_{18}H_{34}N_2OSi_2$	2513	2100 + 350 + 240 + 50 − 2 × 100	2540	1.06
Nicotinic acid, O-(TBDMS)-	$C_{12}H_{19}NO_2Si$	2002	1500 + 350 + 240 − 100	1990	0.59

On DB-1 column

The *GRF*s (m_i and n_i) have the following values: (1) phenyl ring = +58, (2) chain branching = −40, (3) two terminal silyl groups in a molecule = −60, (4) double bond in alkyl chain = +27, (5) alicyclic ring connected to silylated carboxyl group = +0, (6) aryl Br = +240, (7) aryl I = +325 (see text under *Miscellaneous*).

Compound	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Hexamethyldisiloxane	$C_6H_{18}OSi$	667		700	3.29
Methyl (trimethylsilyl)acetate	$C_6H_{14}O_2Si$	852		800	6.1
Oxalic acid, O,O-Bis(TBDMS)-	$C_{14}H_{30}O_4Si_2$	1531	1800 − 2 × 100 − 60	1540	0.58
Succinic acid, O,O-Bis(TBDMS)-	$C_{16}H_{34}O_4Si_2$	1739	2000 − 2 × 100 − 60	1740	0.06
2-Butene-1,4-dicarboxylic acid, O,O-Bis(TBDMS)-	$C_{18}H_{36}O_4Si_2$	1944	2200 + 27 − 2 × 100 − 60	1967	1.17
1,5-Dimethylhexylamine, N-(TBDMS)-	$C_{14}H_{33}NSi$	1354	1500 − 2 × 40 + 25 − 100	1345	0.66
Cyclobutane carboxylic acid, O-(TMS)-	$C_8H_{16}O_2Si$	1009	1000	1000	0.89
Cyclopentane carboxylic acid, O-(TMS)-	$C_9H_{18}O_2Si$	1096	1100	1100	0.36
Cyclohexane carboxylic acid, O-(TMS)-	$C_{10}H_{20}O_2Si$	1188	1200	1200	1.01
Cyclohexane carboxylic acid, O-(TBDMS)-	$C_{13}H_{26}O_2Si$	1410	1500 − 100	1400	0.71
Benzoic acid, O-(TMS)-	$C_{10}H_{14}O_2Si$	1232	1200 + 58	1258	2.07
Benzoic acid, O-(TBDMS)-	$C_{13}H_{20}O_2Si$	1458	1500 + 58 − 100	1458	0
o-Bromobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2BrSi$	1466	1200 + 240 + 58	1498	2.14
m-Bromobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2BrSi$	1472	1200 + 240 + 58	1498	1.74
p-Bromobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2BrSi$	1479	1200 + 240 + 58	1498	1.27
o-Iodobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2ISi$	1579	1200 + 325 + 58	1583	0.25
m-Iodobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2ISi$	1582	1200 + 325 + 58	1583	0.06
p-Iodobenzoic acid, O-(TMS)-	$C_{10}H_{13}O_2ISi$	1591	1200 + 325 + 58	1583	0.05
3,4,5-Triiodobenzoic acid, O-(TMS)-	$C_{10}H_{11}O_2I_3Si$	2381	1200 + 3 × 325 + 58 + 2 × 70	2273	0.29

(Continued on p. 128)

TABLE X *(continued)*

Compound	Formula	I_{obs}	$100Z + \Sigma m_i - \Sigma n_i$	I_p	Difference (%)
Formamide,					
O,N-Bis(TBDMS)-	$C_{13}H_{31}NOSi_2$	1341	(calc. from eqns. in Table I)	1337	0.82
N-(TBDMS)-	$C_7H_{16}NOSi$	1094	(calc. from eqns. in Table I)	1085	0.82
Acetamide,					
O,N-Bis(TBDMS)-	$C_{14}H_{33}NOSi_2$	1385	(calc. from eqns. in Table I)	1392	0.5
N-(TBDMS)-	$C_8H_{18}NOSi$	1112	(calc. from eqns. in Table I)	1129	1.51
Propionamide,					
O,N-Bis(TBDMS)-	$C_{15}H_{35}NOSi_2$	1451	(calc. from eqns. in Table I)	1447	0.28
N-(TBDMS)-	$C_9H_{20}NOSi$	1182	(calc. from eqns. in Table I)	1173	0.76
Hydroxylamine, N,O-Bis(TBDMS)-	$C_{12}H_{31}NOSi_2$	1266	1400 + 50 − 2 × 100	1250	1.26
Hydroxylamine, N,N,O-Tris(TMS)-	$C_9H_{27}NOSi_3$	1123	1100	1100	2.05

[a] See footnote in Table II.

CONCLUSIONS

Substituents that yield large column differences are carboxyl, phenolic and alcoholic hydroxyls, amino groups, etc. These highly polar groups in the form of acids, alcohols and amines, can be chromatographed underivatized, but when present in polyfunctional molecules, such as amino acids, these groups may interact intramolecularly to increase I, rendering the chromatography of the polyfunctional molecules extremely difficult without derivatization.

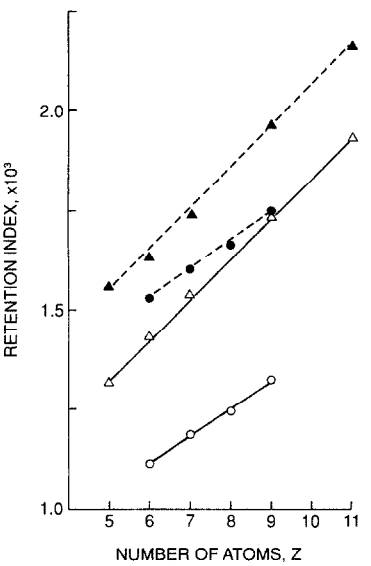

Fig. 2. Linear plots of retention indexes of TMS and TBDMS derivatives of α-amino acids (○, ●) and ω-amino acids (△, ▲) *vs.* the number of atoms (Z) on non-polar and polar columns. The I values of the homologues on non-polar column are connected by a solid line (———) and those on polar column by a broken line (– – –). The plots of TMS and TBDMS derivatives of the α-amino acids on DB-1 and DB-Wax columns have almost identical slopes; so have those of the ω-amino acids. The slopes of the silylated α-amino acids are less steep than those of the silylated ω-amino acids.

Derivatization, especially silylation, masks the functionality of substituent groups, minimizes their intramolecular interaction and allows the polyfunctional compounds to revert to a virtual hydrocarbon state. In this "reduced" state the silylated molecules exhibit chromatographic characteristics similar to that of aliphatic hydrocarbons, thus facilitating the prediction of their I values from base values. According to convention, the *n*-alkanes are assigned identical I values on polar and non-polar columns. Substituent groups retaining residual polarity and polarizability after silylation will show higher I values on polar than on non-polar columns. The convergence of the I values of silylated derivatives on these columns is a manifestation of their hydrocarbon-like chromatographic characteristics. From the I values of the silylated derivatives one can deduce the molecular size in terms of the number of atoms in the skeleton of the analyte molecule.

The relationship between retention index and molecular size of non-polar molecules and the relationship between structure and retention index will be useful for estimating the number of silylated groups in an analyte molecule. The size of the molecular skeleton and possibly the kind and number of functional groups in an analyte molecule can be derived from the I values and the column

differences before and after derivatization. Silylation, methylation and acetylation give different derivatives but may yield a core of useful shared information. In this manner, one can gain structural information from chromatographic data. Application of the method is straightforward and may be useful for routine separation, analysis, and tentative identification of unknown components in mixtures prior to detailed structural analysis by mass spectrometry.

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