PROC. N.S. INST. SCI (1986)
Volume 37, pp. 13-19

DISILOXANES AND OTHER ARTIFACTS FROM A REVERSED-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY COLUMN IDENTIFIED BY MASS SPECTROMETRY†

DOUGLAS W. RUSSELL
Biochemistry Department
Dalhousie University
Halifax, N.S.
Canada B3H 4H7

HUGH A. GILLIS*
Atlantic Research Laboratory
National Research Council of Canada
Halifax, N.S.
Canada B3H 3Z1

When a mixture of hydrophobic compounds was separated by reversed-phase high-performance liquid chromatography, one product was heavily contaminated with a mixture of long-chain alkyl disiloxanes. These compounds, together with silanols, silyl ethers, and poly(dimethylsiloxanes), were formed by hydrolysis of the bonded phase.

Quand un mélange de composés hydrophobiques a été séparé par la chromatographie liquide à haute performance en phase inverse un des produits a été fortement contaminé par un mélange des disiloxanes alkyles à chaînes longues. Ces composés, ainsi que les silanols, les ethères silylés, et les poly(diméthylesiloxanes), ont été formés par l'hydrolyse de la phase stationnaire.

Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely used to solve many analytical and preparative isolation problems. We recently employed it to separate the components of a mixture of homologous and isomeric cyclodepsipeptides (Russell 1966) that had defied resolution by other techniques, including a 500-transfer counter-current distribution (Bertaud *et al* 1965). Four components, of M_r = 624, 638, 652, and 652 severally, were obtained almost pure. A fifth peak, examined by high-resolution mass spectrometry, gave ions at, *inter alia*, 624, 652, 666, which we at first thought were molecular ions of three new homologous cyclodepsipeptides. However, thermal fractionation of the material in the ion source of the mass spectrometer showed that a small amount of cyclodepsipeptide ($C_{35}H_{62}N_4O_8$, M_r = 666) was accompanied by a larger amount of less-volatile material with a spectrum quite unlike that of a cyclodepsipeptide. We here report identification of the major component of this material as dioctadecyltetramethyl disiloxane. Small amounts of a homologue were also detected, and we offer evidence for the additional presence of silyl ethers, silanols, and poly(dimethylsiloxanes) in the column effluent.

Methods

Preparative HPLC was performed on a 25 x 1.0 cm LC-18-DB Semiprep column (Supelco Canada Ltd., Oakville, Ontario L6K 2V1). The apparatus (Waters Associates, Milford, Mass 01757) consisted of a pump (501) controlled by a gradient controller (680), an injector (U6K), a column heater with controller (TCM), and a differential

* Present address: Chemistry Department, St. Francis Xavier University, Antigonish, N.S., Canada B2G 1C0
† NRCC No. 28089.

refractometer (R401) driving a reporting integrator (3390A; Hewlett Packard (Canada) Ltd., Kirkland, Québec H9J 2M5). The column was operated isocratically at 50° with methanol + water, 82 + 12 by volume, flowing at 3.5 mL min^{-1}. Cyclodepsipeptides were injected in chloroform + ethanol, 3 + 1 by volume. Chloroform (Fisher Spectranalysed) was purified by washing with H_2SO_4 and H_2O and drying with K_2CO_3 (Vogel, 1951). Commercial "absolute" ethanol was first refluxed over and then distilled from CaO. Cyclodepsipeptides (20 mg mL^{-1}) in the solvent mixture were repeatedly injected in 25-μL portions, and materials emerging in five peaks were collected separately. Material in the fifth peak (retention volume = 77-79 mL) was recovered by evaporating the methanol and extracting the aqueous residue with chloroform. The solid product obtained by evaporating the chloroform was examined by mass spectroscopy.

A ZAB-EQ mass spectrometer from VG Analytical (Manchester, England) equipped with a VG 11-250 data system was used for these studies. It was operated at a resolving power in the range 2000-5000. The magnet was scanned at 10 s $decade^{-1}$. Accurate molecular weights were determined by the manual peak-matching method, ions from perfluorokerosene being used as references. The precision of these molecular weights is estimated as $\pm$ 3 ppm for intense ions, but is somewhat less for weaker ions that may not be completely resolved from isobaric impurity ions. In this study samples were introduced by way of a temperature-controlled probe.

Results and Discussion

Material present in peak 5 was examined for heterogeneity in the mass spectrometer by repetitive scanning while the probe temperature was raised in steps from 150°C to 190°C. As expected from the work of Bertaud *et al.* (1965), the mass spectra of scans obtained at a temperature > 160°C showed an ion of m/z = 666. This was accompanied by immonium peaks (Vetter 1972) at m/z = 72, 86, and 100, characteristic (Macdonald *et al.* 1964) of compounds containing certain amino acid residues. All four ions had identical temperature profiles and had disappeared completely by 190°C. The material remaining at this temperature gave ions, apparently at m/z = 624 and 652, which we thought at first were isomers of the known cyclodepsipeptides, sporidesmolides III (Russell *et al.* 1964) and II (Bertaud *et al.* 1965; Shemyakin *et al.* 1963) respectively. Precise mass measurements excluded this possibility. For example, $C_{32}H_{56}N_4O_8^{+\cdot}$ (sporidesmolide III) requires m/z = 624.410, whereas the isobaric ion being investigated had m/z = 623.598 $\pm$ 0.002 which, at lower resolution, had been "rounded off" to 624 by the data system. In what follows, m/z values for which such ambiguity exists are quoted to the nearest 0.1 mass unit to avoid confusion. The data system of the mass spectrometer provided three plausible compositions for this ion: $C_{40}H_{79}O_4$ (623.598), $C_{43}H_{79}Si$ (623.595), and $C_{41}H_{87}OSi_2$ (623.598). The rather high natural abundances of ^{29}Si (4.70%) and ^{30}Si (3.09%) permitted a clear choice between these possibilities by a comparison of predicted with observed isotope clusters. Similar measurements for all major ions of m/z $\geq$ 100 (relative abundance > 2%) showed conclusively that they possessed the general formula $C_nH_{2n+1}Si_2O$ (Table I). Two other ions (m/z = 651.6, 399) were too weak for useful isotope cluster measurements to be made, but are included in the Table on the basis of their precise masses and by analogy with the major ions. We conclude that the less-volatile material present in peak 5 is a mixture of disiloxanes, principally dioctadecyltetramethyldisiloxane. Thus, the ion of m/z = 651.6 is an even-electron ion and so cannot be a molecular ion. Furthermore, it is known that molecular ions of disiloxanes are not normally detected in EI mass spectra, since they lose an alkyl radical rapidly (Coutant and Robinson, 1974). The resulting even-electron ion fragments further by rearrangement reactions that lead to loss of (neutral) alkenes and produce a series of even-electron fragment ions. Examination of the mass spectrum leads to the conclu-

Table I Mass*, isotope cluster, and compositional data for ions of peak 5 material after evaporation of the depsipeptides.

Mass(A)	Relative Abundance,%†‡	Composition	m/z obsd.‡	m/z calcd.	Isotope Peak	% intensity obsd.‡	% intensity calcd.
652	0.4	$C_{41}H_{87}OSi_2$	651.630	651.630	—	—	—
624	8.7	$C_{39}H_{83}OSi_2$	623.598	623.598	A+1	53.7	55.6
					A+2	20.8	21.9
					A+3	5.6	6.1
413	2.0	$C_{24}H_{53}OSi_2$	413.368	413.364	A+1	39.7	38.2
					A+2	12.4	13.8
					A+3	3.1	3.1
399	0.3	$C_{23}H_{51}OSi_2$	399.352	399.348	—	—	—
385	85.9	$C_{22}H_{49}OSi_2$	385.333	385.332	A+1	34.7	35.8
					A+2	12.4	13.0
					A+3	2.6	2.8
371	6.9	$C_{21}H_{47}OSi_2$	371.316	371.317	A+1	32.4	34.6
					A+2	12.0	12.6
					A+3	2.2	2.6
133	100.0	$C_4H_{13}OSi_2$	133.051	133.051	A+1	15.2	14.9
					A+2	7.7	7.8
					A+3	0.7	0.7
119	15.2	$C_3H_{11}OSi_2$	119.035	119.035	A+1	12.7	13.8
					A+2	7.3	7.6

*For ions of $m/z \geq 100$.

†Except for $m/z = 652$ and 399, ions of relative abundance $< 2\%$ are omitted.

‡Values are means of 21 scans.

sion that two such series are present, arising from two homologous disiloxanes. These are formulated as I and II (Fig. 1). For reasons advanced below, alternative structures in which two long-chain alkyl groups are borne on the same carbon atom are considered less probable. The presence of about 4% of the mono-C_{20} homologue is consistent with the percentage of eicosanoic acid found along with stearic acid in the triacylglycerols in many natural oils (Deuel, 1951); natural stearic acid is a common source of C_{18} compounds. Formation of m/z = 623.6 by loss of C_2H_4 from m/z = 651.6 is inconsistent with the very low abundance of m/z = 399 which should be favoured over m/z = 623.6 because its formation involves loss of a larger alkene (Coutant and Robinson, 1974).

To investigate the artifacts further, repeated injections were made on to the RP-HPLC column of the chloroform + ethanol solvent alone. The same operating conditions as before were used, and two fractions were collected: fraction A at 14-35 mL, representing a trailing shoulder to the main solvent peak, and fraction B at 49-70

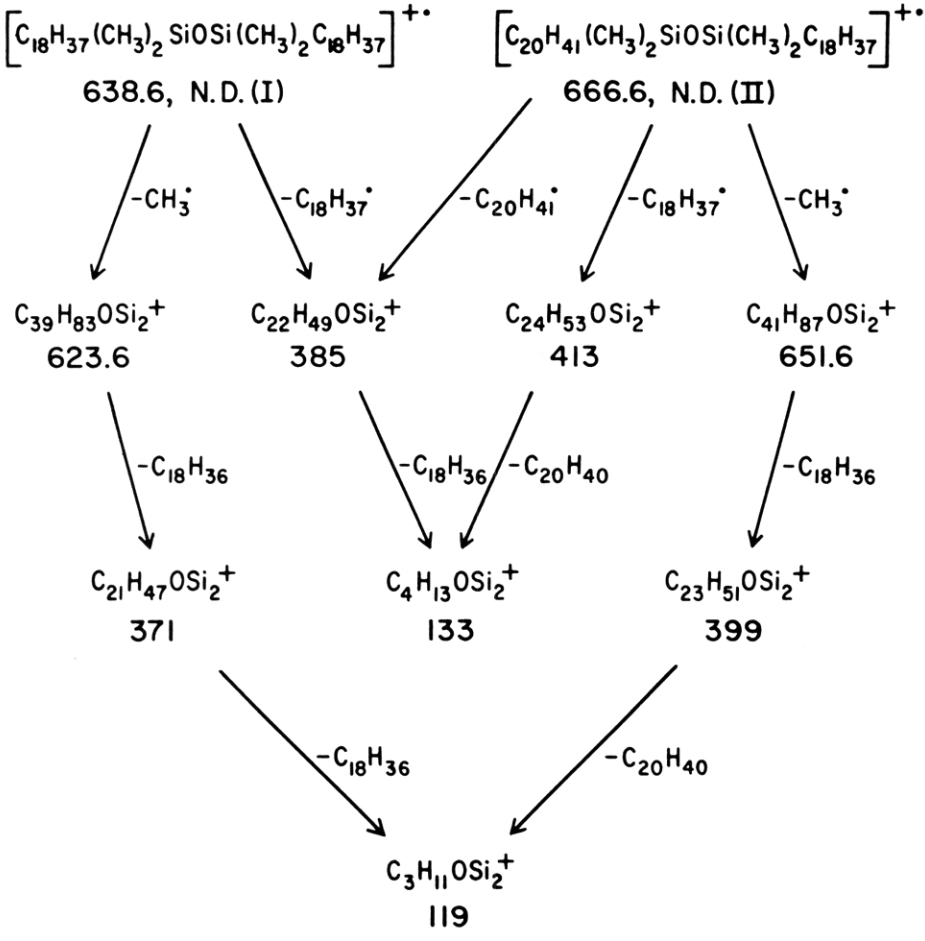

Fig 1. Interpretation of mass spectrum of less volatile material, retention volume 77-79 mL, eluted from the reversed-phase HPLC column. ND = not detected.

mL, representing the region in which cyclodepsipeptides had been eluted in the earlier experiment (retention volumes on the column decreased somewhat between the two experiments). Fraction B was examined first, and the initial probe temperature was lower than before. At 100°C a spectrum was observed that showed only six major ions. All could be accounted for as arising from dimethyloctadecylsilyloxy compounds III, IV, and V (Fig 2). No ions attributable to the structure $(C_{18}H_{37})_2SiOX$, where X=H, Me or Et, were detected. As the probe temperature was raised, ions of m/z = 89, 103, 341 and 327 disappeared. Ions of m/z = 75 and 313 persisted and were joined by the ions characteristic of I and II. Above 170°C, prominent peaks appeared at m/z = 231, 181, 169 and 131, which have not been identified.

The mass spectral behaviour of fraction A was somewhat different. Below 100°C the spectrum was dominated by ions of m/z = 73 and 89, which were most probably

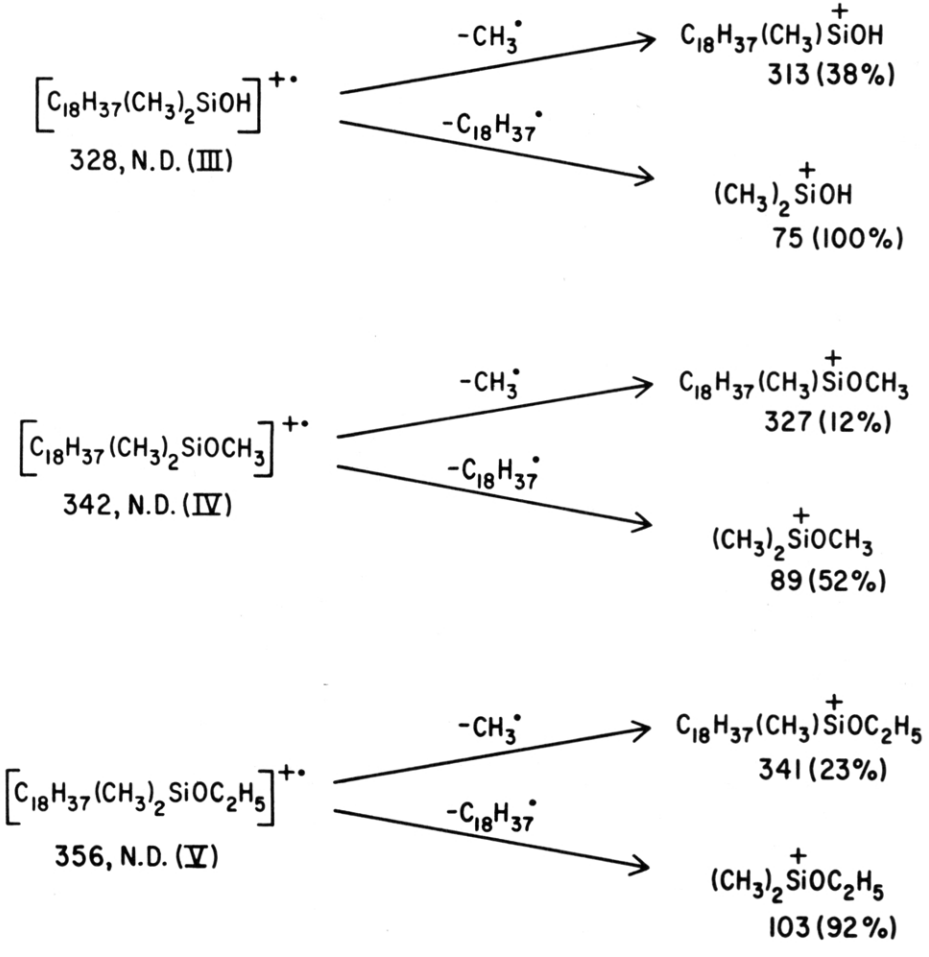

Fig 2. Mass numbers, relative abundance, and proposed structures of ions in the mass spectrum of more-volatile material, retention volume 49-70 mL, eluted from the reversed-phase HPLC column. N.D. = not detected.

Me_3Si^+ and Me_3SiO^+ respectively, arising from the relatively polar trimethylsilanol. At 100°C these were joined by the six characteristic ions of Fig 2, and also by prominent ions at m/z = 147 and 163. The first may be formulated as $Me_3SiOSiMe_2^+$, which could arise either from hexamethyldisiloxane or from an alkyl (presumably mainly octadecyl) pentamethyldisiloxane. The nature of the second was less obvious, but isotope cluster measurement supported the composition $C_5H_{15}Si_2O_2$ (Found: A+1, 16.0; A+2, 8.3; calcd.: A+1, 15.3; A+2, 7.2%). This ion, formulated as $Me_3SiOSi(Me)_2O^+$, is characteristic of poly(dimethylsiloxanes), the presence of which was confirmed by the appearance of low-abundance ions at m/z = 119, 133, 193, 207, 221, 237, 267, 295, 311, 355, 385 (Bertrand *et al.* 1987). No other ions of $m/z > 100$ were detected at this probe temperature, but further heating produced additional ions characteristic of the disiloxane I.

The bonded phases used in RP-HPLC are alkylated siloxanes, in which the Si-O bond is subject to hydrolysis. For example, Crowther *et al.* 1984 analysed the composition of such phases by vigorous acid hydrolytic removal of the bonded phase followed by gas chromatography of the products, which under their conditions were exclusively the disiloxanes formed by condensation of the first-formed trialkylsilanols. Thus, a support prepared by reacting silica gel with chlorodimethyloctylsilane gave on hydrolysis a quantitative yield of *sym*-dioctyltetramethyldisiloxane. Our results, in which dioctadecyltetramethyldisiloxane was a major component of the two well-separated fractions, A and B, show that hydrolysis of such bonded supports occurs continuously to some extent under much milder conditions. Under these conditions, too, some silanol escapes condensation and appears in the free state, while the presence of methanol and ethanol leads to some alcoholysis forming trialkylalkoxysilanes.

Whilst the ions listed in Table I could in principle be derived from isomeric disiloxanes such as $(C_{18}H_{37})_2MeSiOSiMe_3$, or even from homologues such as $(C_{18}H_{37})_2MeSiOMe_2(C_{18}H_{37})$, the failure to detect any $(C_{18}H_{37})_2SiOX^+$ ions argues strongly in favour of I as the correct structure for the major disiloxane.

Less-accessible silica-gel ≡Si-OH groups that escape derivatization by RMe_2SiCl are frequently "end-capped" with Me_2SiCl_2 which, in the presence of water, leads to the introduction of poly(dimethylsiloxane) groupings (Majors and Hopper 1974). The presence of trimethylsilyl groups (ions at m/z = 73, 89, 147) implies additional end-capping with chlorotrimethylsilane (Cooke and Olsen 1980). It might be expected that trimethylsilanol and poly(dimethylsiloxane) would be formed from these end-caps in the same way as the long-chain compounds I-V, and our failure to detect them in fraction B is puzzling. It is possible that the relative inaccessibility of these groups in the bonded phase largely protects them from solvolysis except when the reaction is catalysed by traces of HCl in the chloroform-containing solvent, so that they are eluted after each injection rather than continuously.

In summary, effluent from a reversed-phase HPLC column, even when operated under quite mild conditions, contained detectable quantities of all possible solvolysis products from the bonded phase. Workers using the mass spectrometer as an HPLC detector, when eluted solutes cannot be thermally fractionated, could be seriously misled unless the possible presence of these artifacts is borne in mind.

Acknowledgements

We thank Dr. A. G. McInnes for use of and advice on HPLC equipment, M. G. Flack and D. Embree for technical assistance.

References

Bertaud, W.S., Probine, M.C., Shannon, J.S. and **Taylor, A.** 1965. Isolation of a new depsipeptide from *Pithomyces chartarum*. *Tetrahedron* 21: 677-680.

Bertrand, M.J., Maltais, L. and **Evans, M.J.** 1987. Poly(dimethylsiloxane) as a reference standard for exact measurement in chemical ionization mass spectrometry. *Analyt. Chem.* 59: 194-197.

Cooke, N.H.C. and **Olsen, K.** 1980. Some modern concepts in reversed-phase liquid chromatography in chemically-bonded alkyl stationary phases. *J. Chromatog. Sci.* 18: 512-524.

Coutant, J.E. and **Robinson, R.J.** 1974. Mass Spectrometry, pp. 325-348, in Analysis of Silicones, Smith, A.L., Ed. John Wiley & Sons, N.Y.

Crowther, J.B., Fazio, S.D., Schiksnis, R., Marcus, S. and **Hartwick, R.A.** 1984. Analysis of the acid hydrolysis products of monofunctional chemically bonded stationary phases for high-performance liquid chromatography using capillary gas chromatography. *J. Chromatography* 289: 367-375.

Deuel, H.J. 1951. Lipids Vol. I. Chemistry, pp. 204-206, Interscience Publishers, N.Y.

Macdonald, C.G., Shannon, J.S. and **Taylor, A.** 1964. Mass spectra of cyclodepsipeptides: sporidesmolides. *Tetrahedron Letters*. 2087-2092.

Majors, R.E. and **Hopper, M.J.** 1974. Studies of siloxane phases bonded to silica gel for use in HPLC. *J. Chromatog. Sci.* 12: 767-778.

Russell, D.W. 1966. Cyclodepsipeptides. *Quart. Rev. Chem. Soc.* 20: 559-576.

Russell, D.W., Macdonald, C.G. and **Shannon, J.S.** 1964. The structure of the cyclohexadepsipeptide, sporidesmolide III. *Tetrahedron Letters* 2759-2764.

Shemyakin, M.M., Ovchinnikov, Y.A., Ivanov, V.T. and **Kiryushkin, A.A.** 1963. The structure and total synthesis of sporidesmolide II. *Tetrahedron Letters* 1927-1932.

Vetter, W. 1972. Amino acids, pp. 390-400, in Biochemical Applications of Mass Spectroscopy, Walter, G. R., Ed. John Wiley, N.Y.

Vogel, A.I. 1951. Practical Organic Chemistry 2nd edition, p. 174. Longmans, Green, London.

(Received 27 August 1987).