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^{29}Si and ^{13}C NMR Spectra of Trimethylsilylated Amino Acids

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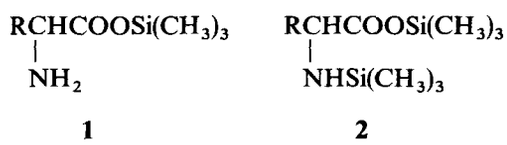
The ^{29}Si and ^{13}C NMR chemical shifts are reported for trimethylsilyl derivatives of 25 amino acids, the majority of which occur naturally as protein constituents of foodstuffs. The ^{29}Si chemical shifts in trimethylsilyl esters of amino acids roughly correlate linearly with the p*K* values of the parent amino acid. No such simple correlations were found for the shifts in the fully trimethylsilylated amino acids. The ^{29}Si chemical shifts of various functionalities encountered fall into distinct spectral regions, but the shifts of $(CH_3)_3SiN$ or $(CH_3)_3SiOOC$ groups alone are not sufficiently characteristic to identify the acid but their combination is in most cases.

KEY WORDS ^{29}Si NMR ^{13}C NMR Amino acids Trimethylsilyl derivatives Chemical shifts p*K* values

INTRODUCTION

Amino acid analysis is often required as a part of routine or research food and clinical analyses. Many different approaches to this analysis have been tried. One of the most common methods is based on trimethylsilylation of the amino acids followed by gas chromatographic determination of the volatile trimethylsilyl (tms) derivatives[1] (for a list of recent references, see Refs 2 and 3). One of the advantages of the method is that the derivatives are prepared in a single step. Although trimethylsilylated amino acids have been intensely studied, just two short papers[4,5] have been devoted to ^{29}Si NMR spectra of only five trimethylsilylated amino acids. While it was realized that ^{29}Si NMR tagging[6] could be useful in amino acid analysis, NMR sensitivity at that time was prohibitively low. Both investigations took place before the invention of polarization transfer techniques,[7] which have reduced dramatically the measuring time and the amount of the trimethylsilyl derivative required.[8] In this paper we aim to fill this gap and report the spectral parameters of trimethylsilyl derivatives of common amino acids encountered in foodstuffs.

Depending on the experimental conditions, trimethylsilation of simple α-amino acids yields two products, monotrimethylsilylated amino acids (**1**) and ditrimethylsilylated amino acids (**2**):



1 **2**

(of course, other functional groups present in some amino acids can also be silylated; a doubly silylated amino group has been reported only for $(tms)_3$glycine[1]). Although the *N*-silylated derivatives **2** are moisture sensitive, the requirement for a quantitative conversion of amino acids into their tms derivatives requires silylating conditions leading to products **2**. In fact, contrary to literature reports,[9] we experienced difficulties in our attempts to isolate the products of type **1**.

EXPERIMENTAL

Sample preparation

All the compounds studied were prepared by trimethylsilylation of carefully dried parent amino acids which were all of the L form. With the exceptions listed below, all amino acids were silylated with trimethylsilyldimethylamine following essentially the procedure of Smith and Shewbart.[10] The products were isolated by distillation *in vacuo*. Silylation of six amino acids (norleucine, cysteine, asparagine, glutamine, lysine and tryptophan) and preparation of fully silylated glycine required the use of *N,O*-bis(trimethylsilyl)-trifluoroacetamide[11] in acetonitrile. The solvent and reaction by-products were removed by stripping with a stream of dry nitrogen at 80 °C.

In addition to disilylated compounds of type **2**, we also attempted to prepare monosilylated compounds of type **1**. In contradiction with the literature,[9] all our attempts to prepare pure compounds **1** failed and we always obtained a mixture of products **1** and **2** which we could not separate by distillation. For this reason, only ^{29}Si chemical shifts determined in the product mixtures are reported for the monosilylated compounds.

The compounds (20 mg or less) were dissolved in 0.7 ml (5 mm NMR tubes) of dry deuteriochloroform

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(Institute of Nuclear Research, Swierk, Poland) containing 1% (v/v) of hexamethyldisilane (HMDSS) (PCR Research Chemicals). Before the ^{29}Si NMR measurements the samples and their dilution were checked by recording ^{13}C NMR spectra. The ^{13}C NMR chemical shifts were referenced to the central line of deuteriochloroform ($\delta = 76.99$). In cases when the ^{13}C chemical shift of HMDSS differed from $\delta = -2.48 \pm 0.02$, the sample was further diluted until this criterion was met.

NMR measurements

All the NMR measurements were performed on a Varian Unity-200 spectrometer (operating at 50.3 MHz for ^{13}C and at 39.7 MHz for ^{29}Si NMR measurements), using the standard software and APT and INEPT pulse sequences. The spectra were recorded in the temperature range 22–24 °C. The ^{29}Si NMR spectra were measured by the INEPT sequence (with the usual parameters[12]) with a spectral width of 4000 Hz. The acquisition delay (1.0 s) during which the WALTZ[13] decoupling of protons was employed was followed by a relaxation delay of 5 s. FID data (8K) were zero filled to 32K and a mild exponential broadening (1.0 Hz) was used in the data processing. The ^{29}Si $\pi/2$ pulses were at maximum 17 μs long whereas the ^{1}H $\tau/2$ pulses were 10 μs in a 5 mm switchable probe. The ^{29}Si chemical shifts were referenced to the line of HMDSS at $\delta = -19.79$. The ^{13}C NMR spectra were measured with one pulse sequence using a spectral width of 16000 Hz. WALTZ decoupling was applied both during relaxation delay (5 s) and acquisition time (1 s). Zero filling to 64K and a mild exponential broadening (1–3 Hz) were used in the data processing. Owing to dilution monitoring the chemical shifts are very reproducible. Repeated chemical shift measurement of independently synthesized sample always reproduced the shift to within ±0.02 ppm.

Line assignment

The lines in ^{13}C NMR spectra were assigned according to the published chemical shifts in the parent amino acids.[14] Since the ^{29}Si chemical shifts of $(CH_3)_3SiOOC$ and $(CH_3)_3SiN$ differ considerably,[5] it was no problem to differentiate these two. Since the exact assignment of ^{29}Si lines in compounds containing two $(CH_3)_3SiOOC$ groups requires either heteronuclear INADEQUATE[15] or selective $\{^{29}Si\}$–^{13}C decoupling[16] experiments, we assigned these lines only tentatively by comparison with other data. For assignment of ^{29}Si lines to two $(CH_3)_3SiN$ groups in compounds such as tms derivatives of asparagine, glutamine or lysine we employed the BINEPTR technique[17] (which is a modification of the SPINEPTR pulse sequence[18]). In this application one should be aware that the residual couplings seen in the BINEPTR spectra are not the two-bond couplings ^{29}Si–N–^{1}H but the three-bond couplings ^{29}Si–C–^{1}H. Thus, as shown in Fig. 1, in the BINEPTR spectrum of *N,N',O*-tris(trimethylsilyl)glutamine one of the $(CH_3)_3SiN$ lines is split to a doublet [$(CH_3)_3SiNHCH$ group] and the other line is a singlet [due to the $(CH_3)_3SiNHCO$ group], similarly as for the $(CH_3)_3SiOOC$ line.

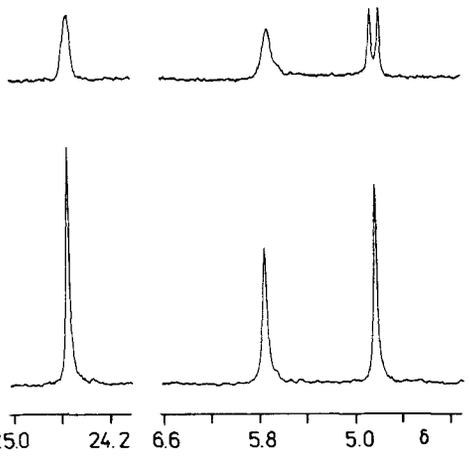

Figure 1. ^{29}Si NMR spectra of *N,N',O*-tris(trimethylsilyl)glutamine. Bottom, spectrum measured with INEPT pulse sequence; top, spectrum measured with BINEPTR pulse sequence. Only relevant regions are shown.

Del Re calculations

Net atomic charges were calculated by the simple MO–LCAO Del Re method,[19] which does not require a knowledge or optimization of the molecular structure. Empirical parameters for different atoms and bonds were taken from the literature.[19–24].

RESULTS AND DISCUSSION

The assigned ^{29}Si and ^{13}C chemical shifts are summarized in Tables 1 and 2. Because of the small substituent effects of the trimethylsilyl group, the ^{13}C chemical shifts in the trimethylsilylated amino acids do not differ substantially from those reported for the parent amino acids.[14] They are given here for the sake of completeness and future reference but will not be discussed any further.

The ^{29}Si chemical shifts reported here fall well into the three regions noted earlier[5] on the basis of very limited data. The boundaries have to be modified, however: (i) the lines of $(CH_3)_3SiOOC$ are to be found in the region $\delta = 23.2$–26.1; (ii) the lines of $(CH_3)_3Si{-}X{-}CH$ groups (with X = O or S) occur in the region $\delta = 16.1$–19.5; and (iii) the silicon lines of $(CH_3)_3SiNH$ groups lie in the region $\delta = 3.8$–6.6. Outside this scheme are the lines of the $[(CH_3)_3Si]_2N$ group ($\delta = 8.19$ in $(tms)_3$glycine) and $(CH_3)_3SiN{<}^{C}_{C}$ group ($\delta = 8.37$ in $(tms)_2$sarcosine, $\delta = 12.73$ in $(tms)_3$histidine and $\delta = 10.27$ in $(tms)_3$tryptophan). The chemical shift values reported earlier[5] were almost all smaller [e.g. the shifts of $(CH_3)_3SiNH$ groups by 0.7 and $(CH_3)_3SiOOC$ groups by 1.0 ppm]. A cursory investigation of concentration dependence showed that high concentration, dictated by low sensitivity of the earlier measurement, is the cause of these differences. The present data were obtained under standard conditions

Table 1. ^{13}C chemical shifts in fully trimethylsilylated amino acids[a]

Parent amino acid	C_α	C_β	C_γ	C_δ	C_{other}	C=O	$(CH_3)_3Si$
Glycine[b]	45.12					174.55	−0.43
							−0.28
Glycine[c]	48.31					175.03	−0.30
							1.50
Sarcosine[b]	54.00				35.66[d]	173.80	−0.81
							−0.21
α-Alanine[b]	51.42	23.23				177.28	−0.34
							0.10
β-Alanine[b]	37.78	40.81				173.44	−0.19
							−0.08
Valine[b]	61.71	32.88	19.36			176.59	−0.30
			17.47				0.01
Norvaline[b]	55.73	38.74	18.61	13.88		177.05	−0.32
							0.04
Leucine[b]	54.43	45.90	24.45	22.03		177.49	−0.32
				23.01			0.04
Norleucine[b]	55.91	36.26	27.52	22.49	13.98[e]	177.05	−0.29
							0.07
Isoleucine[b]	60.76	40.12	24.64[f]	11.59		176.52	−0.30
			15.64[d]				0.02
Phenylalanine[b]	57.71	43.12			137.90[g]	175.88	−0.39
					129.49[h]		−0.09
					128.04[h]		
					126.33[i]		
Tyrosine[c]	57.88	42.36			153.66[j]	176.08	−0.36
					130.96[g]		−0.08
					130.57[h]		0.15
					119.73[h]		
Serine[c]	57.80	66.78				175.11	−0.59
							−0.27
							0.09
Threonine[c]	61.82	70.04	20.66			175.52	−0.20
							−0.02
							0.27
Cysteine[c]	57.55	32.60				174.61	−0.27
							0.09
							0.91
Cystine[k]	55.73	45.65				174.61	−0.31
	55.66						0.11
Methionine[b]	54.91	35.61	30.24	15.30[d]		176.41	−0.33
							0.03
Aspartic acid[c]	53.04	43.02				175.18	−0.37
						171.51	−0.24
							0.04
Glutamic acid[c]	55.15	31.13	31.88			176.37	−0.35
						173.90	−0.26
							−0.06
Asparagine[c]	53.50	43.56				175.94[l]	−0.74
						175.39[m]	−0.41
							0.15
Glutamine[c]	55.39	31.55	33.93			178.06[l]	−0.75
						176.44[m]	−0.33
							−0.03
Lysine[c]	55.92	34.53	22.67	36.41	41.72[n]	176.95	−0.30
							−0.06
							0.06
Histidine[c]	56.35	35.56			140.53[g]	176.42	−0.43
					139.30[o]		−0.35
					117.28[p]		−0.03
Tryptophan[c]	56.81	32.73			136.94[q]	176.48	−0.39
					131.56[q]		−0.27
					128.07[r]		−0.07
					121.56[r]		
					119.35[r]		
					119.14[r]		
					113.92[q]		
					112.80[r]		

Table 1—*Continued*

Parent amino acid	C_α	C_β	C_γ	C_δ	C_{other}	C=O	$(CH_3)_3Si$
Proline[b]	61.64	31.73	25.86	46.99		177.45	−0.85
							−0.30
4-Hydroxyproline[c]	60.52	40.46	72.23	55.35		177.01	−0.96
							−0.01
							0.33

[a] All the chemical shift values are on the δ scale (ppm downfield from tetramethylsilane); estimated error ±0.02 ppm.
[b] Fully trimethylsilylated derivative is a bis(trimethylsilyl) derivative.
[c] Fully trimethylsilylated derivative is a tris(trimethylsilyl) derivative.
[d] CH_3.
[e] C_ω.
[f] CH_2.
[g] C_{ipso}.
[h] CH_{ortho}, CH_{meta}.
[i] C_{para}.
[j] O—C.
[k] Fully trimethylsilylated derivative is a tetrakis(trimethylsilyl) derivative.
[l] COO. [m] CON. [n] C_ε. [o] N=C—N. [p] N—C. [q] CH. [r] C.

Table 2. ^{29}Si chemical shifts in fully and monotrimethylsilylated amino acids[a]

Parent amino acid (abbreviation)	Si—N	Fully trimethylsilylated Si—OC(=O)	Si_{other}	Monotrimethylsilylated Si—OC(=O)
Glycine[b] (V)	5.75	24.36		24.74
Glycine[c]	8.19	23.70		—
Sarcosine[b] (Sar)	8.37	23.68		24.78
α-Alanine[b] (A)	3.89	24.17		24.77
β-Alanine[b] (BA)	4.00	23.35		23.75
Valine[b] (V)	4.51	23.63		24.46
Norvaline[b] (NV)	4.03	23.87		24.61
Leucine[b] (L)	4.11	23.78		24.61
Norleucine[b] (NL)	4.03	23.88		24.62
Isoleucine[b] (I)	4.37	23.57		24.36
Phenylalanine[b] (F)	4.31	24.15		24.23
Tyrosine[c] (Y)	4.24	24.07	19.38[d]	24.94
Serine[c] (S)	4.71	24.08	18.56[d]	24.92
Threonine[c] (T)	4.65	23.57	16.18[d]	24.65
Cysteine[c] (C)	4.78	24.83	16.89[e]	25.71
Cystine[f] (CC)	5.33	25.19		26.05
	5.40			
Methionine[b] (M)	4.78	24.52		25.23
Aspartic acid[c] (D)	4.92	24.13		24.67[g]
		24.77		25.56[g]
Glutamic acid[c] (E)	4.78	23.36		23.59[g]
		24.49		25.19[g]
Asparagine[c] (N)	5.41[h]	25.31		25.75
	6.56[i]			
Glutamine[c] (Q)	4.85[h]	24.56		25.24
	5.77[i]			
Lysine[c] (K)	3.47[j]	23.96		24.76
	4.12			
Histidine[c] (H)	4.25	23.62		25.54
	12.73[k]			
Tryptophan[c] (W)	4.14	23.68		
	10.27[k]			
Proline[b] (P)	3.91	23.25		24.80
4-Hydroxyproline[c] (HP)	5.49	23.66	16.47[d]	24.95

[a] All the chemical shift values are on the δ scale (ppm downfield from tetramethylsilane); estimated error ±0.02 ppm.
[b] Fully trimethylsilylated derivative is a bis(trimethylsilyl) derivative.
[c] Fully trimethylsilylated derivative is a tris(trimethylsilyl) derivative.
[d] Si—O.
[e] Si—S.
[f] Fully trimethylsilylated derivative is a tetrakis(trimethylsilyl) derivative.
[g] Bis(trimethylsilyl) derivative.
[h] Si—α—NH. [i] Si—NHCO. [j] Si—ε—NH. [k] $Si—N_{heterocycl.}$.

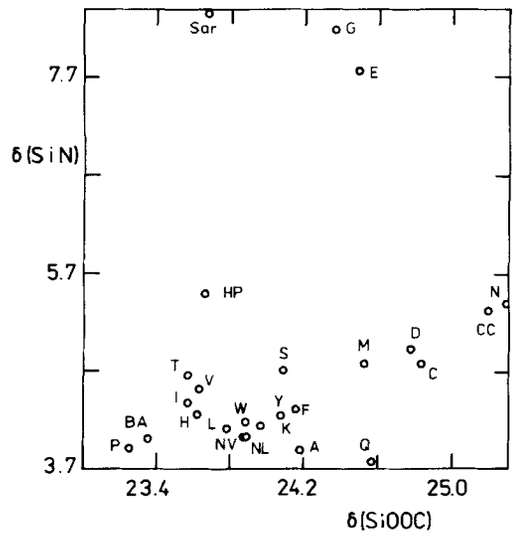

Figure 2. ^{29}Si chemical shifts in $(CH_3)_3SiN$ group *vs.* those in $(CH_3)_3SiOOC$ groups in fully silylated amino acids. For abbreviations, see Table 2; only the data from the first line in Table 2 are shown for compounds with more than two trimethylsilyl groups.

(solvent and concentration) for ^{29}Si NMR tagging employing the trimethylsilyl group[6] and hence are directly comparable with the chemical shifts found in other classes of compounds.

Apparently because of structural diversity of the important amino acids, no trends in the ^{29}Si chemical shifts are apparent from the data in Table 1. The calculated (Del Re method[19]) net atomic charges on the silicon atom do not vary with the molecular structure of the amino acid and hence cannot explain the observed changes.

There is a rough linear correlation between the ^{29}Si chemical shifts in the monosilylated amino acids and the pK values of the parent acids. However, the correlation [$\delta = 26.72\ (\pm 0.31) - 0.81\ (\pm 0.13)pK$, 21 data points, correlation coefficient = -0.813, for pK values from Ref. 25 and data in Table 2, excluding derivatives of phenylalanine and aspartic acid] is significantly different from the correlation published for simple carboxylic acids.[26–28] In the fully trimethylsilylated amino acids the analogous correlation is poor as the pK values of the parent amino acid do not adequately describe the acidity of *N*-trimethylsilylated acid.

As Fig. 2 illustrates, there is also a rough linear correlation between the ^{29}Si chemical shifts of $(CH_3)_3SiN$ and $(CH_3)_3SiOOC$ groups in fully silylated amino acids (excluding data points for sarcosine, glycine and glutamic acid derivatives). Since the ^{29}Si chemical shifts of $(CH_3)_3SiOOC$ or $(CH_3)_3SiN$ alone are not sufficiently characteristic to identify the fully trimethylsilylated amino acid, the plot of this type can be analytically utilized as each point with its coordinates [δ(SiO), δ(SiN)] is unique and well separated from all other points [with the exception of bis(trimethylsilyl) derivatives of norvaline and norleucine].

CONCLUSION

The chemical shifts in fully silylated amino acids are difficult to interpret but they can be utilized for identification of the parent amino acid. Especially advantageous for such identification is a plot of ^{29}Si chemical shifts in $(CH_3)_3SiN$ groups *vs.* that in $(CH_3)_3SiOOC$ groups.

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REFERENCES

1. A. E. Pierce, *Silylation of Organic Compounds (a Technique for Gas-Phase Analysis)*. Pierce Chemical, Rockford, IL (1968).
2. G. van Look, *Silylating Agents*. Fluka Chemie, Buchs (1988).
3. M. H. Gordon, in *Principles and Applications of Gas Chromatography in Food Analysis*, edited by M. H. Gordon, p. 176. Ellis Horwood, Chichester (1990).
4. E. Bayer, G. Jung, E. Breitmaier, P. Hunziker, W. König and W. Voelter, in *Peptides 1971, Proceedings of the 11th European Peptide Symposium, Vienna, 1971*, edited by H. Nesvadba, p. 265. North-Holland, Amsterdam (1973).
5. J. Schraml, J. Pola, V. Chvalovský, H. C. Marsmann and K. Bláha, *Collect. Czech. Chem. Commun.* **42**, 1165 (1977).
6. J. Schraml, *Prog. Nucl. Magn. Reson. Spectrosc.* **22**, 289 (1990).
7. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.* **101**, 760 (1979).
8. J. Schraml, *Collect. Czech. Chem. Commun.* **48**, 1402 (1983).
9. K. Rühlmann and J. Hils, *Liebigs Ann. Chem.* **683**, 211 (1965).
10. E. D. Smith and K. L. Shewbart, *J. Chromatogr. Sci.* **7**, 704 (1969).
11. C. W. Gehrke, H. Nakamoto and R. W. Zumwald, *J. Chromatogr.* **45**, 24 (1969).
12. J. Schraml, *Collect. Czech. Chem. Commun.* **48**, 3402 (1983).
13. A. J. Shaka, J. Keeler and R. Freeman, *J. Magn. Reson.* **53**, 313 (1983).
14. E. Breitmaier and W. Voelter, *^{13}C NMR Spectroscopy*. Verlag Chemie, Weinheim (1974).
15. J. Past, J. Puskar, M. Alla, E. Lippmaa and J. Schraml, *Magn. Reson. Chem.* **23**, 1076 (1985).
16. J. Past, J. Puskar, J. Schraml and E. Lippmaa, *Collect. Czech. Chem. Commun.* **50**, 2060 (1985).
17. V. Blechta and J. Schraml, *J. Magn. Reson. A* **101**, 47 (1993).
18. J. Schraml, *J. Magn. Reson.* **59**, 515 (1984).
19. G. Del Re, *J. Chem. Soc.* 4031 (1958).
20. G. Del Re, B. Pullman and T. Yonezawa, *Biochim. Biophys. Acta* **75**, 153 (1963).
21. G. Del Re, in *Electronic Aspects of Biochemistry*, edited by B. Pullman, p. 221. Academic Press, New York (1964).
22. J. Nagy, P. Hencsei and J. Réffy, *Acta Chim. Acad. Sci. Hung.* **65**, 51 (1970).
23. J. Nagy and L. Párkányi, *Acta Chim. Acad. Sci. Hung.* **71**, 159 (1972).
24. P. Lazaretti and F. Taddei, *Org. Magn. Reson.* **3**, 113 (1971).
25. J. A. Dean (Ed.), *Lange's Handbook of Chemistry* 12th ed., p. 5–17 McGraw-Hill, New York (1978).
26. H. C. Marsmann and H.-G. Horn, *Z. Naturforsch., Teil B* **27**, 1448 (1972).
27. W. McFarlane and J. M. Seaby, *J. Chem. Soc., Perkin Trans. 2* 1561 (1972).
28. V. A. Pestunovich, M. F. Larin, E. I. Dubinskaya and M. G. Voronkov, *Dokl. Akad. Nauk SSSR* **233**, 378 (1977).