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Characterization of polyphenols from plant materials through their silylation and ^{29}Si NMR spectroscopy – line assignment through ^{29}Si, ^{13}C spin–spin couplings

Jan Schraml,[1]* Vratislav Blechta,[1] Jan Sýkora,[1] Ludmila Soukupová,[1] Petra Cuřínová,[1] David Proněk[2] and Jaromír Lachman[2]

[1] Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Rozvojová 135, 16502, Prague 6, Czech Republic
[2] Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Agriculture, Kamýcká 129, 16521 Prague 6, Czech Republic

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The lines in ^{29}Si NMR spectra of silylated polyphenols and some other compounds are difficult to assign owing to the absence of couplings with protons outside the silyl group. The assignment can be derived through small $^{n}J(^{29}\mathrm{Si}, ^{13}\mathrm{C})$ couplings ($n > 1$). Using a previously described method for measurements of these couplings, the assignment procedure is demonstrated here on three examples of trimethylsilylated phenols: 7-hydroxyflavone, ferulic acid, and quercetin. In some cases the procedure can be used to identify carbon atoms to which the siloxy groups are attached. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: NMR ^{29}Si NMR; ^{13}C NMR; $^{n}J(^{29}\mathrm{Si}, ^{13}\mathrm{C})$; line assignment; long-range couplings; polyphenols; flavonoids

INTRODUCTION

Polyphenols are important constituents of plant materials.[1] They are important not only for plant biochemistry but also as nutrition components of herbivores and omnivores. Considerable research effort has been made in understanding their antibacterial action, their role as antioxidants, and in connection with occurrence of cancer or cardiovascular diseases in human beings.[2–4] Obviously, separation of polyphenols from plant materials, analysis of their mixtures, and identification of individual polyphenols are prerequisites for such research.

Several years ago, we have shown that trimethylsilylation followed by measurements of ^{29}Si NMR spectra ('^{29}Si NMR tagging') could be a useful tool for analysis of lignins, which are mixtures of compounds similar to the polyphenols encountered in nutritious plants (for a review see Ref. 5). Since then, advancement of NMR technology (especially higher magnetic fields, more sensitive probes, and development of LC-NMR) has lowered ^{29}Si NMR detection limits,[6] making application of ^{29}Si NMR tagging worthwhile to apply to polyphenols produced by plants. In a preparatory study, we have trimethylsilylated some model compounds containing several phenolic hydroxyl groups, yielding ^{29}Si NMR spectra consisting of several lines for each compound. This is of advantage in analyses of complicated mixtures as it increases the reliability of components identification. However, assignment of these lines to different silicon nuclei in the molecule is a difficult task.

The difficulty stems from the fact that the 'phenolic' trimethylsiloxy groups are attached to aromatic carbons and there are no silicon spin–spin couplings to protons outside the trimethylsilyl group ($^{n}J(^{29}\mathrm{Si},^{1}\mathrm{H})$, $n > 2$). A similar situation is encountered in trimethylsilyl esters of polycarboxylic acids. The only possibility left for exact experimental assignment in such cases is based on silicon coupling to carbon-13 nuclei ($^{n}J(^{29}\mathrm{Si},^{13}\mathrm{C})$, $n > 1$), provided the ^{13}C NMR spectra are assigned at least partially. Two methods were developed specifically for this purpose: one termed *heteronuclear INADEQUATE*[7] and the other utilizing selective ^{29}Si decoupling.[8] Recently, we have reported two sensitive methods for measurements of small $^{n}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings[9], which can be employed also for this purpose. Both methods utilize INEPT-type polarization transfer for signal enhancement and pulsed magnetic field gradients for coherence pathway selection; the first one, (Si,C)gCOSY, detects ^{13}C signals and the other, (Si,C,Si)gHMQC, detects ^{29}Si signals.

Feasibility of this approach to ^{29}Si line assignment is demonstrated here on three model compounds of increasing complexity. The models were derived by trimethylsilylation (R = $\mathrm{Si(CH_3)_3}$) of the parent compounds (R = H): 7-hydroxyflavone (**1-H**), ferulic acid (**2-H**), quercetin (**3-H**) (Scheme 1). Of the two mentioned methods of measurements, the latter has certain advantages (in general, narrow ^{29}Si lines

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*Correspondence to: Jan Schraml, Institute of Chemical Process Fundamentals, Academy of Sciences, Czech Republic, Rozvojova 135, Prague 6, 16502, Czech Republic. E-mail: schraml@icpf.cas.cz

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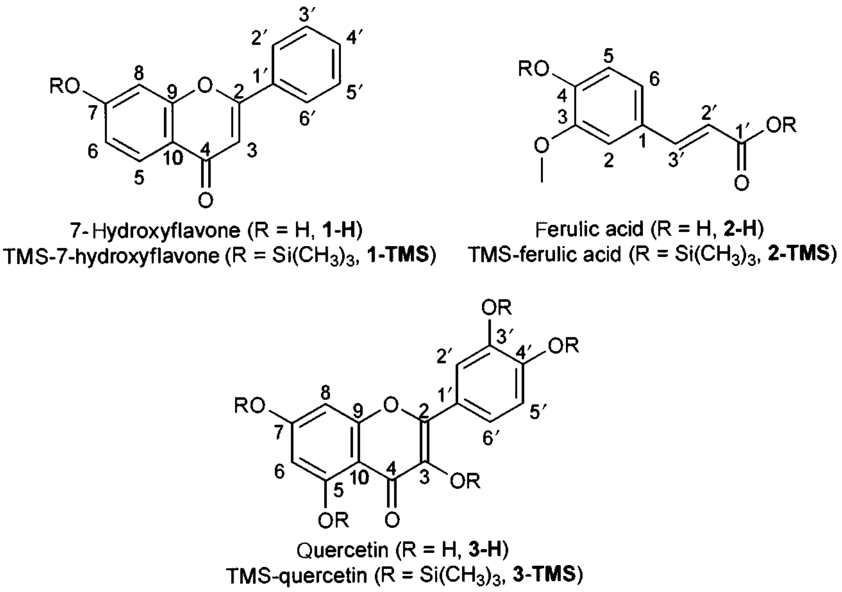

7-Hydroxyflavone (R = H, **1-H**)
TMS-7-hydroxyflavone (R = $Si(CH_3)_3$, **1-TMS**)

Ferulic acid (R = H, **2-H**)
TMS-ferulic acid (R = $Si(CH_3)_3$, **2-TMS**)

Quercetin (R = H, **3-H**)
TMS-quercetin (R = $Si(CH_3)_3$, **3-TMS**)

Scheme 1. Carbon numbering in the studied compounds.

provide better resolution along F_2 axis than ^{13}C lines do and, specifically, our probe has roughly 10 times higher sensitivity in ^{29}Si channel than in ^{13}C channel) and so it was employed here.

EXPERIMENTAL

Synthesis

The parent compounds were all of commercial origin: 7-hydroxyflavone (7-hydroxy-2-phenyl-4*H*-chromen-4-one, Aldrich 98%), ferulic acid ((*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid, Fluka), and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, Lachema, p.a.). They were all trimethylsilylated by the same procedure yielding 7-trimethylsiloxy-2-phenyl-4*H*-chromen-4-one (**1-TMS**), trimethylsilyl ester of (*E*)-3(4-trimethylsiloxy-3-methoxyphenyl)acrylic acid (**2-TMS**), and 2-(3,4-bis(trimethylsilyl)phenyl)-3,5,7-tris(trimethylsiloxy)-4*H*-chromen-4-one (**3-TMS**).

The phenols were trimethylsilylated by bis(trimethylsilyl)acetamide (BSA) in 100% stoichiometric excess in a small volume of acetonitrile as a solvent. The reaction mixture was stirred at 65 °C for 2 h. The solvent and the excess of silylation reagent were distilled off in vacuum 1 kPa at 65 °C.

NMR spectra

^{13}C and ^{29}Si NMR spectral measurements in solution were performed on a Varian UNITY-500 spectrometer (operating at 499.9 MHz for ^{1}H, at 125.7 MHz for ^{13}C, and at 99.3 MHz for ^{29}Si NMR measurements) using a 5-mm Si{C,H} Nalorac probe. The spectrometer is equipped with a X,Y,Z-Performa gradient module, has four r.f. channels, two of them fitted with waveform generators. The standard vnmr 6.1C software was used for all the experiments except for (Si,C,Si)gHMQC pulse sequence. All the spectra of the pertrimethylsilylated compounds were recorded at 25 °C in 0.3–0.7 M solutions in dry $CDCl_3$ (Aldrich, 99.8 atom % D, stored over activated molecular sieve 3A) containing 1% (v/v) of hexamethyldisilane (Aldrich, HMDSS, ^{29}Si secondary reference). The ^{13}C NMR spectra were measured using a spectral width of 30 000 Hz. WALTZ decoupling was applied both during acquisition (1 s) and relaxation delay (10 s). Zero filling to 128 K and a mild line-broadening were used in data processing. The spectra were referenced to the line of the solvent ($CDCl_3$, $\delta = 76.99$ ppm). The ^{29}Si NMR spectra were measured by an INEPT pulse sequence as modified for $Si(CH_3)_3$ groups[10] (75 ms polarization and 16 ms refocusing delays optimized for polarization transfer by $^{2}J(^{29}\text{Si–C–}^{1}\text{H}) = 6.7$ Hz from a nine-spin proton system) using relaxation delay 10 s, spectral width 8000 Hz, acquisition time 2 s, FID (free induction decay) data zero filled to 128K. The spectra were referenced to the line of HMDSS ($\delta = -19.79$ ppm).

The (Si,C,Si)gHMQC correlation experiment employed the pulse sequence described earlier[9]with the INEPT part as described above for the INEPT experiment; each sample was measured with two values of polarization transfer delay (Si > C) in the gHMQC part of the sequence, 160 and 500 ms. Intensity of the first gradient pulse was 9.93 gauss cm^{-1}, intensity of the second one alternated between 2.74 and -22.5 gauss cm^{-1} for the two FIDs needed for phase-sensitive 2D detection. The 90° pulses of ^{1}H, ^{13}C, and ^{29}Si were 18, 20, and 8 μs long, respectively. For the selected pulse (i.e. the first 90° carbon pulse) qSNEEZE[11] shape 540 or 404 μs was used to cover 9000 or 11 000 Hz excitation bandwidth. Other parameters were: acquisition time 2 s, spectral width in F_2 (^{29}Si) dimension 2000 Hz, with zero filling to 16 K data points and Gaussian broadening (5 s), relaxation delay 8 s, gradient duration 1 ms, spectrometer recovery time 1 ms. Spectral width in F_1 dimension was 8000 Hz or 10 000 Hz, $2 \times 64t_1$ increments, 24 scans per increment (total experiment time 8 h), zero filling to 256 and Gaussian broadening (0.005 s). The resolution (especially along F_2 axis) was chosen for the assignment purposes and for more precise determination of the coupling constants, and the resolution could be increased substantially. The reported coupling constants were read off from the separations of lines in antiphase doublets; when the separation within the doublet becomes comparable to the linewidth, the apparent couplings are larger than the true couplings. Simulations indicate that for Lorentzian lines, the smallest separation observed here (0.4 Hz) corresponds to true coupling 0.08 Hz smaller. No correction for the line overlap was used.

The published assignments of the lines in the ^{13}C NMR spectra of the parent compounds (**1-H** and **3-H**) were derived from dimethylsulfoxide solutions[12–15]. Owing to small differences between some carbon chemical shifts, change of solvent, and substituent effects of TMS group, the ^{13}C assignments in the trimethylsilylated products had to be verified by a combination of usual procedures including HETCOR, gHMBC, and INADEQUATE (in the case of **1-TMS** and **3-TMS**). The assigned ^{13}C chemical shifts are summarized in Table 1, the detected $^{n}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings in Table 2.

RESULTS AND DISCUSSION

The three studied compounds (**1-TMS**, **2-TMS**, **3-TMS**) were chosen to test the proposed approach on compounds with increasing complexity and, at the same time, to investigate derivatives of compounds found in natural material. Previous determination of $^{n}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings in the studied fragments (Si–O–C_6H_4 or Si–O–C(O)–C) indicated a possible obstacle to the straightforward application of the suggested simplistic approach – the $^{n}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings for $n = 2$ and $n = 3$ have comparable absolute values.[16] The recently proposed (Si,C,Si)gHMQC method holds promise to overcome this obstacle by providing a means to measure even smaller couplings expected for $n > 3$.

The simplest compound, **1-TMS**, containing only one line in the ^{29}Si NMR spectrum did not represent the ^{29}Si assignment problem but served as a test of this approach. As it is apparent from Fig. 1a, the coupling constants can be read from these experiments with a relatively high accuracy and precision. However, even the high precision (estimated at ±0.1 Hz) of the measured couplings does not allow the differentiation of two- and three-bond couplings based on their values. In the case of an unknown compound, such a result would not permit identification of the carbon atom to which the trimethylsiloxy group is attached. However, when it is a matter of ^{29}Si NMR line assignment on a known carbon skeleton, as is the case of **1-TMS**, almost the same couplings with three carbon nuclei in a row (C-6, 7, 8) are possible only if the coupling to the middle carbon (C-7) is through two bonds while the coupling with its neighbors is through three-bond. At the same time, this spectrum solves the assignment problem encountered in the ^{13}C NMR spectrum of the parent **1-H** compound. For this compound, contradictory assignments of the lines at $\delta = 161.91$ and 162.86 to C-2 and C-7 have been published.[14,15] In the trimethylsilyl derivative, **1-TMS**, the difference in chemical shifts between these two lines is larger ($\delta = 160.15$

Table 1. ^{29}Si chemical shifts and assigned ^{13}C chemical shifts of compounds **1–3**

	Compound				
Carbon	**1-H**[a]	**1-TMS**	**2-TMS**	**3-H**[b]	**3-TMS**
C-1	–	–	128.23	–	–
C-2	161.91[c]	162.89	122.02	146.9	147.75
C-3	106.62	107.21	150.82	135.9	137.36
C-4	176.37	177.62	146.76	176.0	173.26
C-5	126.69	126.88	120.80	160.9	156.29
C-6	115.13	118.69	110.59	98.3	109.67
C-7	162.86[c]	160.15	–	164.0	159.21
C-8	102.54	107.50	–	93.5	101.44
C-9	157.51	157.50	–	156.3	157.60
C-10	116.10	118.36	–	103.2	111.52
C- 1′	131.30	131.64	166.91	122.2	125.42
C- 2′	126.17	125.97	117.46	115.3	120.88
C- 3′	129.08	128.78	145.05	145.2	146.27
C- 4′	131.54	131.22	–	147.8	148.01
C- 5′	129.08	128.78	–	115.8	120.42
C- 6′	126.17	125.97	–	120.1	122.32
CH_3–Si	–	0.07	0.12, −0.38[d]	–	1.95, 0.36, 0.31, 0.21, 0.19
^{29}Si	–	22.29	23.48, 21.50	–	21.85, 21.62, 21.31, 21.02, 19.45

[a] Data from DMSO-d_6 solution, Ref. 14.

[b] Data from DMSO-d_6 solution, Ref. 12, except for small numerical differences the data agree with those of Ref. 13.

[c] Assignment of the two lines is interchanged in Refs 13 and 14.

[d] CH_3–O at 55.12 ppm.

Table 2. Long-range $^{n}J(^{29}Si,^{13}C)$ coupling constants (absolute values) in Hz[a]

Compound	1-TMS	2-TMS		3-TMS				
Silicon δ	22.29	23.48	21.50	21.85	21.62	21.31	21.02	19.45
Carbon								
C-1			0.5					
C-2			0.5			0.5		2.9
C-3			1.3					2.6
C-4			2.4		0.2			0.5
C-5			1.6		2.1			
C-6	1.8		0.5	1.8	1.3			
C-7	2.0			2.0	0.6			
C-8	1.8			2.0	0.5			
C-9	0.4				0.5			
C-10	0.4				1.2			
C-1′		2.4				0.5	0.5	0.5
C-2′		2.2					1.3	
C-3′		0.4				1.8	2.3	
C-4′						2.2	1.5	
C-5′						1.3		
C-6′						0.5	0.5	
Si–O–C-[b]	7	1′	4	7	5	4′	3′	3

[a] No long-range ^{29}Si, ^{13}C couplings other than shown here were detected, estimated error ± 0.1 Hz. The values are line separations within the antiphase doublets, no correction for line overlap was used. The values $J < 0.6$ Hz reported here might overestimate the true couplings by as much as 0.1 Hz.

[b] Silicon line assignment indicated as the carbon number to which the trimethylsiloxy group is attached, see Scheme 1.

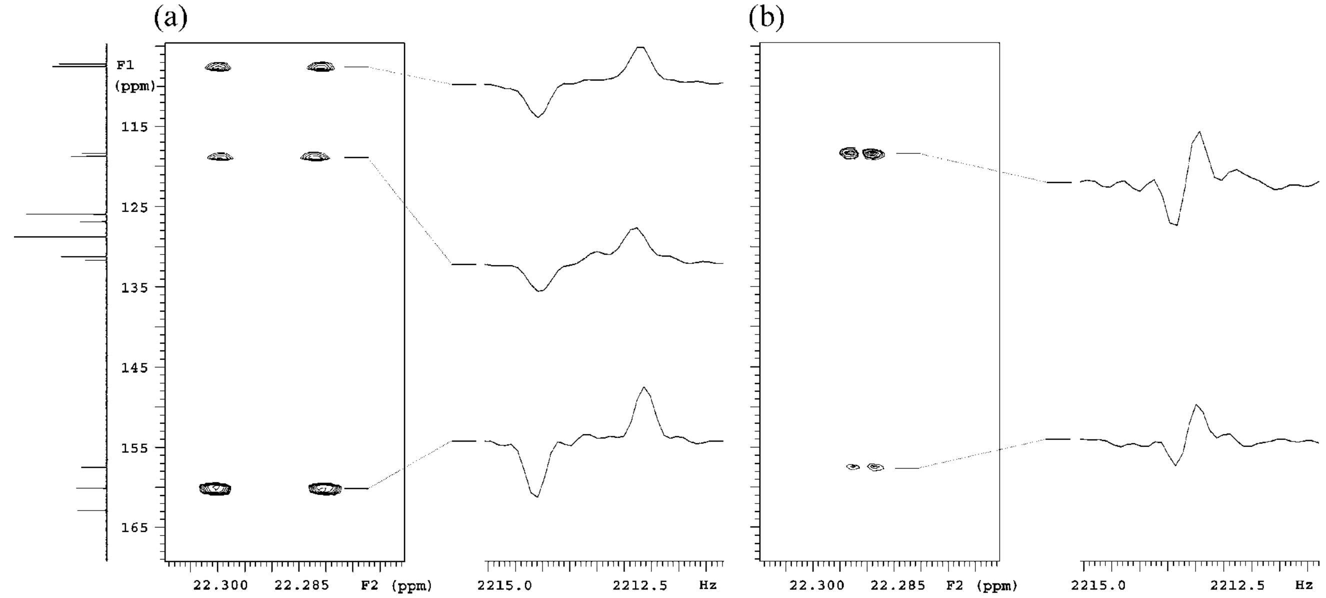

Figure 1. (a) A contour plot of the (Si,C,Si)gHMQC 2D spectrum of **1-TMS** measured with polarization transfer 160 ms (on the left – the relevant part of ^{13}C NMR spectrum; on the right – traces through the cross-peaks corresponding to the ^{13}C chemical shifts of C-8, C-6, and C-7. The separations of the lines in the antiphase doublets are absolute values of the coupling constants in Hz; down from the top these $^{2}J(^{29}Si,^{13}C)$ and $^{3}J(^{29}Si,^{13}C)$ couplings are 1.8, 1.8, and 2.0 Hz). (b) A contour plot of (Si,C,Si)gHMQC 2D spectrum of **1-TMS** measured with polarization transfer of 500 ms (on the right – traces through the cross-peaks corresponding to the ^{13}C chemical shifts of C-10 and C-9. The $^{5}J(^{29}Si,^{13}C)$ and $^{4}J(^{29}Si,^{13}C)$ couplings are 0.4 Hz each.

and 162.89) and, most significantly, the line at 162.89 ppm has no cross peak in the (Si,C,Si)gHMQC shown in Fig. 1, and so it cannot arise from C-7 but rather belongs to C-2 (in agreement with our assignment by other means). In an attempt to see smaller couplings, the experiment was repeated with a longer polarization delay of 500 ms (Fig. 1b) which makes couplings of 0.4 Hz to C-9 and C-10 visible ($^{4}J(^{29}Si,^{13}C)$ and $^{5}J(^{29}Si,^{13}C)$, respectively). With the current lack of understanding of these couplings, it is not clear why the $^{4}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ coupling to C-5 (δ = 126.88) is not detected.

The second model, **2-TMS**, is closer to real examples; it illustrates well what has been seen in the spectra of the first model. The ^{29}Si line at δ = 23.49 exhibits in the (Si,C,Si)gHMQC spectrum intense cross peaks with C-2′ and C-1′ and weak ones with C-3′ (Fig. 2). Without going to details of the coupling-constant values (Table 2), this silicon signal obviously originates from the trimethylsilyl group on C-1′. Similarly, as in the previous case, the couplings over two and three bonds are of similar magnitude while the coupling across four bonds is much smaller (about one-third). The horizontal separation of the cross peaks follows the trend one can expect: the largest separation is found in the cross peaks of C-4 ($^{2}J(^{29}\mathrm{Si},^{13}\mathrm{C})$) followed by C-5 and C-3 ($^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$) and finally the smallest, of C-6, C-2 and C-1 ($^{4}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ and $^{5}J(^{29}\mathrm{Si},^{13}\mathrm{C})$). Among weak couplings, the most intense cross peaks are due to $^{5}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ coupling of C-1. It is also noteworthy that in this particular case all these long-range couplings are revealed in one experiment employing a polarization transfer delay of 160 ms.

The third example, **3-TMS**, represents the real assignment problem. Five ^{29}Si NMR lines can be assigned on the basis of the experience gained on the previous two models as follows. The ^{29}Si line at δ = 21.85 is due to the silicon of the siloxy group on C-7 as there are three pairs of cross peaks in the (Si,C,Si)gHMQC contour plot (Fig. 3), with the separations within the pair corresponding to $^{2}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ or $^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings. They occur at the chemical shifts of C-6, C-7, and C-8 leaving no alternate assignment possible. This is entirely reminiscent of the above-described example **1-TMS**; also no smaller couplings are revealed for this silicon in the experiments with the short polarization delay. Analogously, the ^{29}Si line at δ = 21.62 is assigned to the silicon atom on C-5 as this silicon couples with C-5, C-6, and C-10 either through $^{2}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ or $^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings (the couplings to C-10 and C-6 are somewhat smaller). Cross peaks due to $^{4}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ or $^{5}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ are observed for C-7 and C-9. The ^{29}Si lines at δ = 21.31 and 21.02 can be assigned to the trimethylsiloxy groups on C-4′ and C-3′, respectively, in the same manner. Also, in these two cases the $^{2}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings are somewhat larger than the $^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings experienced by the *ortho* positioned carbons. Finally, the remaining ^{29}Si line at δ = 19.950, which must be due to siloxy group attached to C-3, manifests its position in line with other above-described observations by its $^{2}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ and $^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ couplings to C-2 and C-3. Surprisingly, the $^{3}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ coupling to C-4 has anomalous magnitude; it is as small as $^{4}J(^{29}\mathrm{Si},^{13}\mathrm{C})$ or $^{5}J(^{29}\mathrm{Si},^{13}\mathrm{C})$, comparable to the coupling with C-1′.

Besides assisting ^{29}Si line assignments, the couplings can also help in solving some ^{13}C assignment problems. For example, 2D (^{13}C–^{13}C) INADEQUATE of **3-TMS** would easily proceed from the characteristic chemical shift of C-4 to that of C-10. Further on there are, however, two coupling pathways from C-10 to C-7: (i) C-10 → C-5 → C-6 → C-7

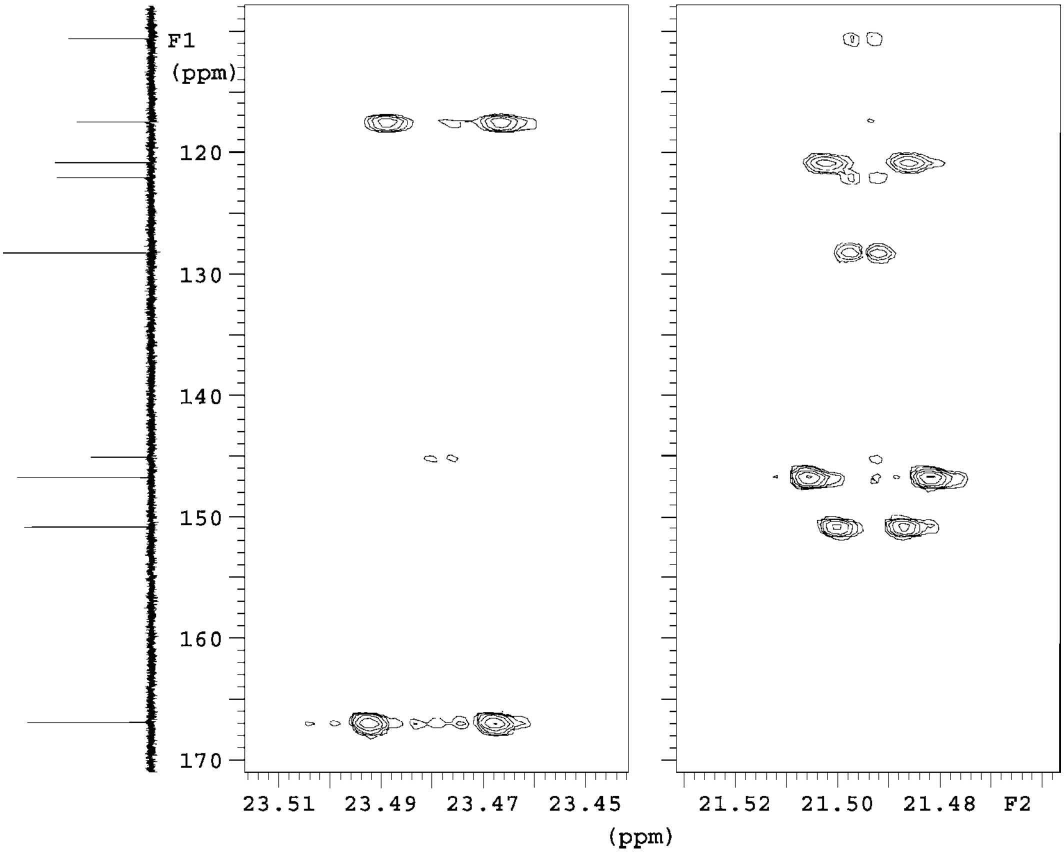

Figure 2. Relevant parts of the contour plot of the (Si,C,Si)gHMQC 2D spectrum of **2-TMS** measured with 160 ms polarization delay. (On the left – ^{13}C NMR spectrum).

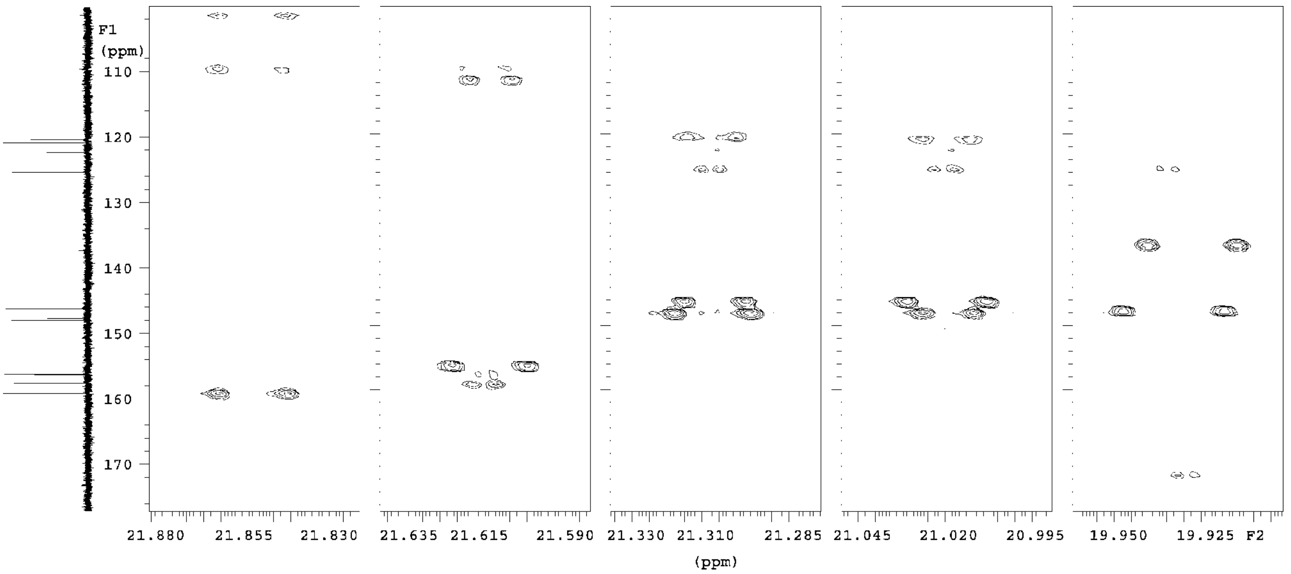

Figure 3. Relevant parts of the contour plot of the (Si,C,Si)gHMQC 2D spectrum of **3-TMS** measured with 160 ms polarization delay. (On the left is the ^{13}C NMR spectrum).

and (ii) C-10 → C-9 → C-8 → C-7 with only 5 ppm chemical-shift differences between the corresponding carbons. The two coupling pathways are indistinguishable according to INADEQUATE experiment. The $^2J(^{29}\text{Si},^{13}\text{C})$ and $^3J(^{29}\text{Si},^{13}\text{C})$ couplings unambiguously identify the C-5 carbon line and thus identify the carbon chemical shifts on each of the two coupling pathways.

CONCLUSION

The measurements of long-range $^nJ(^{29}\text{Si},^{13}\text{C})$ coupling constants in compounds containing several trimethylsilyl groups yield full and unambiguous assignments of all ^{29}Si lines provided the ^{13}C NMR signals are assigned. The method is especially valuable if siloxy groups are attached to quaternary carbons (especially aromatic or carboxylic) where other methods of assignment fail. In some cases, the couplings can be also used to identify the ^{13}C NMR line of the carbon to which the siloxy group is attached. When the trends in these coupling constants are better known (including their dependences on the number of intervening bonds, on substitution both at silicon and carbon skeleton, etc.), the method could be used even more efficiently.

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