

ASSIGNMENT OF ^{29}Si NMR LINES AND DETERMINATION OF ^{29}Si - ^{13}C COUPLING CONSTANTS IN PERTRIMETHYLSILYLATED LIGNIN-RELATED PHENOL-CARBOXYLIC ACIDS BY SELECTIVE HETERONUCLEAR INADEQUATE METHOD

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Selective heteronuclear (Si-C) INADEQUATE method has been successfully used to assign all ^{29}Si chemical shifts in six pertrimethylsilylated lignin model compounds. Empirical assignment of the lines in the region $\delta = 23-25$ to trimethylsilyl esters of carboxylic compounds has been confirmed. Selectivity of the method permits unambiguous assignment to trimethylsiloxy groups which differ by their position on the benzene ring only. The assignment is possible despite that ^{29}Si - ^{13}C two-bond coupling constants (2.1-3.0 Hz) are of comparable magnitude with the three-bond coupling constants (1-2.3 Hz). The chemical shifts of trimethylsiloxy groups on the benzene ring are sensitive to substituent effect to such an extent that their assignment cannot rely on an empirical rule but requires experimental method such as the one used here.

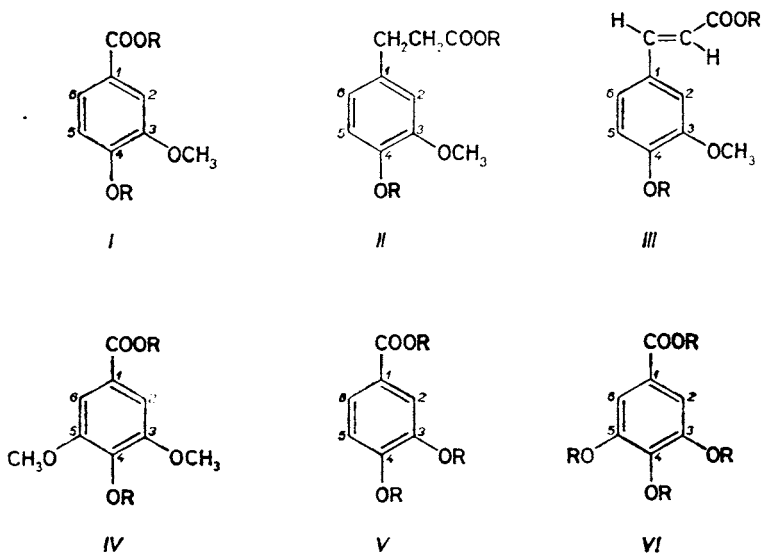
Silicon-29 NMR spectroscopy of pertrimethylsilylated samples holds a great promise for analysis of lignins¹⁻⁴. It can be used to identify various very similar polar functional groups which are present in a large amount and variety in lignins and their derivatives. With the present instrumentation and modern measuring techniques (INEPT and DEPT⁵) the ^{29}Si NMR spectroscopy is sensitive enough for practical applications. It provides semiquantitative information about the relative concentration of different polar functional groups present in the sample⁶.

The crucial step in all applications of NMR spectroscopy is the line assignment. Analysis of lignins or even lignin model compounds is especially demanding in this respect because, first, many of the functional groups are attached to carbon atoms which bear no hydrogen and, second, the functional groups are very similar (*e.g.* various phenolic OH groups that differ by their position or by the nature of other remote substituent on the benzene ring only). For the first reason one can choose among the methods which have been developed for ^{29}Si NMR line assignment (see the references in ref.⁷) only those which are based on ^{29}Si - ^{13}C couplings.

Two such methods have been described^{7,8}. The first one combines difference spectroscopy with selective ²⁹Si decoupling to measure ²⁹Si satellites in the assigned ¹³C NMR spectra and thus identifies the signal arising from the nearest silicon atom. The second method⁸ combines for the same purpose selective (soft) ²⁹Si decoupling pulses with INADEQUATE pulse sequence and phase cycling⁹. The selective heteronuclear INADEQUATE method is superior to the first mentioned method in two aspects which are important for applications to lignin models: It is more selective and it works successfully even if the heteronuclear coupling constants involved are as small as 1–2 Hz. High selectivity is required because of the mentioned similarity of the functional groups in lignins. The capacity to solve the assignment problems in cases with small coupling constants is needed as only very little is known about two-bond silicon–carbon coupling constant values and it gives us the additional possibility to cross-check the assignment by showing the smaller couplings between the silicon atom and the next adjacent carbon atoms. In this paper we present the first application of the selective heteronuclear INADEQUATE method to solve such stringent assignment problems.

RESULTS AND DISCUSSION

We have chosen to study the following phenol-carboxylic acids which are related to lignins: vanillic (*I*), β-guaiacylpropionic (*II*), ferulic (*III*), syringic (*IV*), protocatechuic, (*V*) and gallic (*VI*) acids (R = H). The ²⁹Si NMR spectra of their pertrimethylsilylated *I*–*VI* (R = Si(CH₃)₃) represent assignment problems of increasing complexity.



In acids R = H, in trimethylsilyl derivatives R = Si(CH₃)₃.

While signals of the silicon atoms in COOSi and COSi groups can be differentiated by a comparison of the spectra of large model series of compounds, the lines belonging to different Si—O—C(arom) silicon atoms cannot be fully assigned except for the rare cases in which symmetry arguments can distinguish the signals according to their intensity (as *e.g.* in VI).

The chosen assignment method — selective heteronuclear INADEQUATE experiment — has been described in detail earlier⁸. In short, ¹³C NMR signal is detected after it has been excited by a simple pulse sequence with appropriate pulse and receiver phase cycling. The pulse sequence includes 90° and 180° hard ¹³C and soft (selective) ²⁹Si pulses, protons are decoupled throughout the experiment. After the registered signal is Fourier transformed, the resultant "spectra" contain only the ²⁹Si satellite lines of those carbon nuclei which are coupled with the selectively irradiated silicon nucleus.

Application of the selective heteronuclear (Si—C) INADEQUATE method to the assignment of ²⁹Si NMR lines requires assigned ¹³C NMR spectra. The carbon chemical shifts in compounds I—VI (Table I) were assigned according to the characteristic shift values (*e.g.* CH₂ or COO carbon atoms), according to the intensity in the broad-band decoupled spectra (differentiating the substituted aromatic carbon atoms from the unsubstituted ones), and according to the relative magnitude of long-range ¹³C—¹H coupling constants (three-bond couplings being larger than the two-bond couplings). The assignments given in Table I are consistent with the direct additivity of substituent effects.

Assignment of ²⁹Si NMR lines. The ²⁹Si NMR spectra of compounds I—IV consist of two lines only, the assignment considerations are similar for I—IV, they will be exemplified here on the spectrum of compound I. Assigning the line which occurs at $\delta = 24.41$ is no problem in the model compound with known structure. Selective pulse irradiation of this line in the INADEQUATE experiments gives the satellite spectrum of COO and aromatic C-1 carbon atoms. Hence the silicon must be the one which is bonded to COO group and the two couplings are two- and three-bond Si—C couplings. Comparable magnitude of these two coupling constants (2.6 and 2.2 Hz) would, however, forbid unambiguous assignment in a compound with unknown structure. It would not be clear whether the Si—O is attached to C=O or to C-1 atom. The selective INADEQUATE spectrum measured with the other silicon line irradiated shows silicon satellites at three carbon line positions (C-3, C-4, and C-5) with all couplings of comparable magnitude. Since the silicon atom can be coupled through a two-bond coupling with only one aromatic carbon atom and with two more aromatic carbon atoms through three-bond couplings, the satellite pattern can be explained only if the siloxy group is attached to aromatic C-4 atom and the couplings with C-3 and C-5 nuclei are the three-bond couplings. By such considerations silicon lines can be assigned even in compounds the structure of which has not yet been fully determined.

TABLE I
 ^{13}C NMR parameters of skeletal carbon atoms in I – VI

Compound	Parameter ^a	C-1	C-2	C-3	C-4	C-5	C-6	COO	C-a ^b	C-b ^b
I	C	124.78	113.22	150.33	149.04	120.13	123.81	166.37	—	—
	2J	—	—	—	2.2	—	—	2.6	—	—
	3J	2.2	—	-1.1	—	1.6	—	—	—	—
II	C	133.86	111.89	150.19	142.38	120.32	119.97	172.75	30.33	37.08
	2J	—	—	—	2.4	—	—	3.0	—	—
	3J	—	—	1.2	—	1.5	—	—	—	1.8
III	C	128.40	110.54	150.78	146.70	120.77	122.00	166.85	145.01	117.43
	2J	—	—	—	2.2	—	—	2.6	—	—
	3J	—	—	0.7	—	1.5	—	—	—	2.2
IV	C	123.47	107.17	150.97	138.65	150.97	107.17	166.49	—	—
	2J	—	—	—	2.4	—	—	2.5	—	—
	3J	2.1	—	1.2	—	1.2	—	—	—	—
V	C	124.90	122.51	146.09	151.03	120.19	124.40	165.95	—	—
	2J	—	—	2.1	2.1	—	—	2.6	—	—
	3J	2.2	1.5	1.8	1.8	1.5	—	—	—	—
VI	C	123.45	115.62	147.25	143.25	147.25	115.62	165.71	—	—
	2J	—	—	2.1	2.7	2.1	—	2.8	—	—
	3J	2.2	1.2	1.4	2.3	1.4	1.2	—	—	—

^a C denotes carbon chemical shift in δ -scale, 2J denotes two-bond ^{29}Si - ^{13}C coupling constant in Hz, and 3J denotes three-bond ^{29}Si - ^{13}C coupling constant in Hz; ^b carbon atoms of the side chain.

Assignment of COOSi lines in the spectra of compounds *V* and *VI* is straightforward, it is analogous to that described above for compound *I*. Assignment of the remaining lines in the spectra of these compounds is possible only thanks to selective arrangement of the experiments. In a non-selective (one-dimensional) heteronuclear INADEQUATE experiment the comparable magnitude of two- and three-bond couplings would prohibit unambiguous assignment. In the selective experiments undertaken here, the lines can be assigned by a simple extension of considerations described above though the satellites due to two- and three-bond couplings cannot be distinguished by the experiment.

The described assignments of ^{29}Si lines to the silicon atoms in COOSi and C(arom)-OSi groups is in agreement with the assignment deduced from a large collection of experimental data², detailed assignment of two SiOC(arom) lines in compound *V* is the first one so far reported.

As explained earlier⁸, the selective heteronuclear INADEQUATE experiments require exact calibration of the *rf.* field strengths and a precise knowledge of the centre frequencies of the ^{29}Si doublets. All this information is conveniently obtained from methyl ^{13}C spectra measured by non-selective (strong ^{29}Si and ^{13}C pulses) INADEQUATE experiments with weak CW irradiation of one line of the ^{29}Si doublet. A byproduct of these experiments is the assignment of the chemical shifts of methyl carbon atoms in the trimethylsilyl groups. Though of little practical use the assigned methyl ^{13}C chemical shifts are given in Table II for the sake of completeness.

Silicon chemical shifts and coupling constants. The differences between the chemical shifts of the silicon nuclei in COOSi and C(arom)OSi groupings are analy-

TABLE II
 ^{29}Si and ^{13}C chemical shifts of trimethylsilyl groups in *I*–*VI*

Compound	^{29}Si Chemical shift ^a			^{13}C Chemical shift ^b		
	Si-3(5)	Si-4	Si—OOC	C(CH ₃)-3(5)	C(CH ₃)-4	C(CH ₃)—SiOOC
<i>I</i>	—	21.99	24.41	—	0.17	—0.32
<i>II</i>	—	19.92 ^c	23.26 ^c	—	—0.11	—0.75
<i>III</i>	—	21.60	23.60	—	0.12	—0.38
<i>IV</i>	—	22.87	24.69	—	0.21	—0.33
<i>V</i>	21.30	21.69	24.05	0.03	0.06	—0.42
<i>VI</i>	21.16	22.36	23.83	—0.05	0.37	—0.52

^a Silicon atoms are labelled by the number of the nearest skeletal carbon atom, δ -scale, approximate error ± 0.04 ppm; ^b carbon atoms of methyl groups on labelled by the number of the nearest skeletal carbon atom, δ -scale, approximate error ± 0.02 ppm; ^c concentration dependent.

tically significant, they can be used for line assignment. The chemical shift of the silicon atom in C(arom)OSi depends strongly on the number and nature of the other substituents on the benzene ring and, hence, it cannot be used to distinguish siloxy groups in different ring positions without some further experiments. Similarly the coupling constants cannot be utilized analytically as the observed ranges of values of two-bond couplings (2.1–3.0 Hz) and three-bond couplings (0.7–2.3 Hz) overlap in the most frequently occurring range of values. These findings stress the importance of experimental methods of line assignment.

Rather surprising observation is offered by a comparison of Si-4 chemical shifts in pairs of compounds I, IV and V, VI: *ortho* positioned methoxy and trimethylsiloxy groups have very similar (+0.9 and +0.7 ppm, resp.) *ortho* substituent effects despite their greatly different sizes. If this observation is confirmed by a detailed study of diluted solutions it would mean that the "sterical" *ortho* effect is essentially due to interaction of electrons on the *ortho*-positioned oxygen atoms.

EXPERIMENTAL

Model compounds. Gallic (VI) and protocatechuic (V) acids, were commercial products, acids I–IV were prepared by known procedures^{10–14}.

Trimethylsilylation. The procedure used was a modification of the method described by Klebe *et al.*¹⁵. The model compound (500–900 mg) was placed into a small reaction vessel equipped with a teflon septum seal. Approximately 20% stoichiometric excess of N,O-bis(trimethylsilyl)-acetamide (BSA) was added through a syringe. The mixture was vibrationally stirred and heated at 50–70°C for about 1 h. Excess reagents were distilled off *in vacuo* under a stream of dry nitrogen (50–60°C, 1.3 kPa).

NMR samples. The silylated products were dissolved in deuteriochloroform (Merck, Uvasol) in the reaction vessel. The (approximately 50%) solution was transferred by dry pipet into a dry 10 mm o.d. NMR tube and sealed.

¹³C NMR *line assignment.* The experiments needed to assign chemical shifts were carried out on a Bruker AM-500 spectrometer operating at 500.1 and 125.8 MHz for proton and carbon NMR, respectively. The experiments included measurements of decoupled, coupled (mono-resonance), and single-frequency off-resonance ¹³C NMR spectra. In some instances, measurements of ¹H NMR spectra and of 2D NMR correlations were needed to obtain unambiguous assignment.

Selective heteronuclear INADEQUATE experiments were performed on a Bruker CXP 360 spectrometer operating at 90.6 and 71.5 MHz for ¹³C and ²⁹Si, respectively. The necessary modifications of the standard CXP console and probe were described previously⁸.

References and precision. ¹³C NMR chemical shifts were referenced to the line of solvent (with $\delta = 77.00$) and are given with approximate error of ± 0.02 ppm. ²⁹Si chemical shifts were referenced externally since the samples did not contain any reference compound. They are precise within ± 0.04 ppm but a small systematic error of similar magnitude cannot be excluded. The heteronuclear coupling constants with the values in the range 2–3 Hz are accurate within $\pm 10\%$ (relative), the error in the values of smaller constants is considerably larger (c. 30%). The measurements were carried out at 295 K.

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