Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 48 (2007) 7586–7590

Tetrahedron Letters

A new method for determining positions of phenolic hydroxyl groups through silylation and application of H(Si)C triple-resonance NMR experiments

Michal Maloň,* Shunya Takahashi and Hiroyuki Koshino*

RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Received 1 August 2007; revised 29 August 2007; accepted 31 August 2007
Available online 5 September 2007

Abstract—Two-dimensional triple-resonance H(Si)C NMR experiments have been applied at natural abundance to assign ^{13}C NMR signals in silylated phenols. The method showing its great potential in determining positions of hydroxyl groups is widely applicable to signal assignment and structure elucidation of synthetic and natural phenolic compounds.

In the course of studies on structural revision of some natural products,[1] including applications of the CAST/CNMR system,[2] which is a database-oriented ^{13}C NMR chemical shift prediction system, we found that the evidence of the hydroxyl group and assignment of its position in molecular structures is an important step in structure elucidation. In the last five years, there have been many reports on structural revision of aromatic compounds, especially focused on positional isomers of aromatic substitutions including phenolic hydroxyl groups.[3] There is an incredible structural diversity of phenolic compounds in natural products.[4] Highly substituted phenolic compounds contain many quaternary carbon atoms in their molecules and they lack protons suitable for long-range correlation HMBC experiments if a phenolic hydroxyl proton is not given a sharp signal in the ^{1}H NMR spectrum. Therefore several derivatization methods[5] including basic methylation or acetylation will help to analyze such compounds in some cases, but obtained methyl ethers or acetates of phenolic compounds are useless for assigning *ortho* carbon signals of the original hydroxyl group, meaning that accurate determination of the hydroxyl group position and connectivity is difficult or impossible. One interesting derivatization method is the introduction of phosphorus containing functional group such as phosphate[6] or phosphite.[7] To analyze phosphorus containing compounds, we have recently developed long-range H(P)C and H(C)P triple-resonance NMR experiments,[8] but there is a problem in specifying the number of multiple bonds (2–5) between phosphorus and carbon atoms. Another interesting approach is silylation and following the application of ^{1}H–^{29}Si HMBC.[9] This method is a powerful tool in structural studies of primary and secondary alcohols, but it usually fails in the case of tertiary alcohols and phenols.

Therefore, we have developed a new methodology for determining the position of phenolic hydroxyl groups and related functional groups, such as enol hydroxyl and carboxyl groups, using silyl protecting groups[5] and 2D long-range H(Si)C triple-resonance NMR experiments.

Multidimensional triple-resonance experiments employing proton detection are routinely used in structural elucidation of isotopically enriched biomacromolecules.[10] It has been shown that slightly modified 2D or 3D triple-resonance NMR experiments can be used at natural abundances in the study of polymers,[11] transition metal complexes[12] and organometallic compounds.[13] Such experiments usually use indirect proton detection in order to enhance sensitivity, and pulsed field gradients. Recently, it has been demonstrated that 2D triple-resonance H/Si/C experiments employing silicon detection can be used to assign carbon signals in

---

Keywords: Phenol; Double-INEPT; ^{29}Si–^{1}H long-range coupling; ^{29}Si–^{13}C long-range coupling.

* Corresponding authors. Tel.: +81 48 467 9361; fax: +81 48 462 4627; e-mail addresses: malon@riken.jp; koshino@riken.jp

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.08.111

silylated polyphenols through the measurement of $^nJ_{SiC}$ values.[14] Schraml et al. achieved a satisfactory sensitivity of the experiment at natural abundance by the application of a specially designed probe for silicon detection.[15,16]

We focused on proton detection experiments due to their superior sensitivity enhancement. We chose *tert*-butyldimethylsilyl (TBDMS) as a protective silyl group for several reasons. It contains two groups of chemically equivalent protons both of which may be very useful in the case of polyphenolic compounds and overlapping signals. Moreover, ^{1}H NMR spectrum of TBDMS contains two singlet signals, representing 6 and 9 equiv protons, thus the sensitivity enhancement is substantial. Furthermore, TBDMS ethers of phenols are usually stable and easy to handle. Finally, the TBDMS group can readily be removed after structural elucidation in order to recover a natural product for other studies.

The H(Si)C double-INEPT pulse sequence used in the study is described in Figure 1.[17] The experiment employs ^{1}H–^{13}C gradient selection, while additional pulsed field gradients Gs and Gp remove unwanted magnetization. Delays τ_1 and τ_2 are set according to heteronuclear long-range couplings $^nJ_{SiH}$ and $^nJ_{SiC}$, respectively. The $^nJ_{SiH}$ value was adjusted to 6.7 Hz according to the literature[18] and our measurements using ^{1}H–^{29}Si HSQMBC experiment.[19] Long-range H(Si)C correlations observed in H(Si)C double-INEPT spectra of **1**–**6** are summarized in Figure 2. Compound **1** was purchased, while **2**, **3**, **5** and **6** were prepared using TBDMSCl as an agent.[20] TMS ether **4** was prepared[21] for the purpose described below.

$^nJ_{SiC}$ values in *para*-substituted TBDMS-silylated phenols were extensively studied.[18] It is possible to conclude that $^2J_{SiC}$ (2.6 Hz) is significantly larger than $^3J_{SiC}$ (1.6–1.4 Hz) and $^{4,5}J_{SiC}$ (0.5–0.0 Hz). For that reason we decided to study compound **1** as a starting model compound.[22] We measured H(Si)C double-INEPT spectrum using $\tau_1 = 37.3$ ms ($^nJ_{SiH} = 6.7$ Hz) and $\tau_2 = 100$ ms ($^nJ_{SiC} = 2.5$ Hz)[23] to observe correlation signals of dimethylsilyl and *tert*-butyl methyl protons to a carbon directly bonded to TBDMSO group. The spectrum revealed correlation signals of TBDMS protons to a quaternary carbon (δ 149.3) and methine carbons (δ 120.8), as well. The signals were assigned as C-1 and C-2, respectively. After that, we measured another spectrum using $\tau_2 = 166.7$ ms ($^nJ_{SiC} = 1.5$ Hz)[23] in order to observe $^3J_{SiC}$ correlations. As expected, the correlation signal of C-1 was suppressed under this condition, while the correlation signal of C-2 (and C-6) became stronger. Those interesting observations suggest a method distinguishing between $^2J_{SiC}$ and $^3J_{SiC}$ correlations, based on the dependence of cross peak intensities on using τ_2 duration value. Surprisingly, signals of another methine and quaternary carbons based on $^4J_{SiC}$ and $^5J_{SiC}$ couplings, respectively, were also observed under the latter condition. Those signals were assigned as C-3 and C-4.

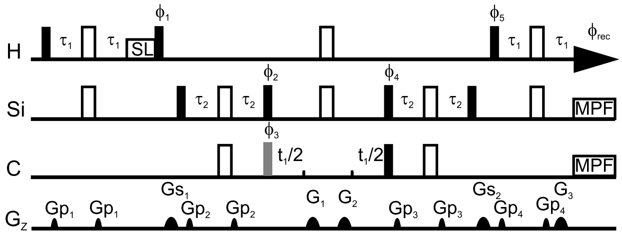

Figure 1. The H(Si)C double-INEPT experiment used in this study. Filled and open bars represent 90° and 180° pulses, respectively, while open boxes correspond to broadband decoupling (MPF) or spinlock pulse (SL). A grey bar in ^{13}C channel represents a pulse that may be used as band-selective. Composite π-pulses ($90°_x/180°_y/90°_x$) can be applied in ^{13}C and ^{29}Si-channels. Delays τ_n, phase cycles ϕ_n and the ratio of gradient strengths G_n are as follows: $\tau_1 = 1/(4^nJ_{SiH})$; $\tau_2 = 1/(4^nJ_{SiC})$; $\phi_1 = y$; $\phi_2 = y$; $\phi_3 = x, -x$; $\phi_4 = 2(y), 2(-y)$; $\phi_5 = y$; $\phi_{rec} = x, -x, -x, x$; G_1:G_2:G_3 = 1.988:1.988:1.000. Gradients Gs_n and Gp_n remove unwanted magnetization and their values are arbitrary. The experiments is performed in absolute mode.

Next, we applied the same method to TBDMS ether of ellagic acid (**2**). Two H(Si)C spectra obtained with 100 or 166.7 ms (τ_2) enabled us to distinguish between carbons directly bonded to TBDMSO groups and carbons in *ortho* positions. Thus C-2, C-3, C-4 and C-5 were assigned by means of triple-resonance experiments, solely. The spectra are shown in Figure 3.

Compound **3** was studied as a representative of flavonoid natural products including ketone carbonyl groups. Unfortunately, we did not observe any correlations to carbonyl carbon C-4 in either experiment. For a similar flavonoid compound, a TMS derivative of quercetin, the value of $^3J_{SiC}$ was reported to be 0.5 Hz.[14] For that reason, we prepared TMS ether **4** and measured its triple-resonance spectra. In this case, we obtained a correlation signal between trimethylsilyl methyl protons and C-4 when the second spectrum was recorded ($^nJ_{SiC} = 1.5$ Hz).[23] The value of $^3J_{Si\text{-}1a,C\text{-}4}$ in **4** is probably similar to the one found in TMS quercetin, therefore the correlation signal was observed. In the case of **3**, the bulky TBDMS group probably caused conformational change vanishing $^3J_{Si\text{-}1a,C\text{-}4}$. Thus we did not detect the desired correlation signal. Assignments of other ^{13}C NMR signals based on two typical conditions were straightforward in both compounds.

We decided to study TBDMS ether of kojic acid (**5**), γ-pyrone compound containing enol and primary alcohol hydroxyl groups. Two triple-resonance spectra were recorded.[24] In this case, carbonyl carbon C-4 was detected when $\tau_2 = 166.7$ ms ($^nJ_{SiC} = 1.5$ Hz),[23] only. This means that the $^3J_{SiC}$ value is also smaller than typical $^3J_{SiC}$, but its non-zero value allowed us to detect correlation at least in the second measurement.

Finally, we studied compound **6** as an example of TBDMS ester. Ester TBDMS protons were correlated to two carbon atoms of which C-1 was also correlated to ether TBDMS protons as shown in Supplementary data. Thus assignments of silyl ester carbonyl carbon C-7 and adjacent C-1 signals were possible. Discrimination between C-2 and C-3 was based on the comparison of the two spectra. In order to assign two TBDMS