J. Am. Chem. Soc. **1995**, *117*, 9547−9550

Application of a Gradient Enhanced Measurement for Carbon−Carbon Coupling Constants (GRECCO) to a Conformational Study of Geraniol and (*E*,*E*)-Farnesol

Thomas Fäcke and Stefan Berger*

Contribution from the Fachbereich Chemie der Philipps Universität, Hans-Meerwein-Strasse, D-35032 Marburg, Germany

Received May 3, 1995®

Abstract: A new technique gradient enhanced measurement for carbon−carbon coupling constants (GRECCO) is reported for the measurement of geminal and vicinal carbon−carbon coupling constants. The method is used for the conformational analysis of the di- and triterpenes geraniol and famesol. Both compounds were found to exist mainly in transoidal conformations.

Introduction

In order to obtain a better understanding of the interaction of molecules, it is very important to determine the arrangement of the atoms in space. Although there are many compounds which have essentially rigid geometry (e.g. steroids), many others can adopt a variety of conformations.[1]

$^3J_{HH}$ and $^3J_{CH}$ spin coupling constants in addition to NOE enhancements are frequently measured in order to assist in conformational assignments. Other NMR parameters like spin lattice relaxation times and chemical shift may be of additional use, however for small molecules these data are often not sufficient to provide enough restraints for the boundaries to be set in a molecular mechanics calculation.

Although the Karplus relation is usually used to obtain dihedral angles (and therefore conformational information) from vicinal H, H and H, C coupling constants, it can also be used in the same manner for vicinal C, C coupling constants.[2] Here we present an improved technique for structural investigations that is based on vicinal carbon−carbon scalar coupling constants. Several factors have up until now prevented their wider application. The measurement of $^{13}C-^{13}C$ coupling constants is considerably hindered by the low natural abundance of the ^{13}C nucleus. In addition earlier methods such as 1D- or 2D-INADEQUATE[3] and SELINQUATE[4] have been hampered by the residual signal of the isotopomers containing only one ^{13}C atom. Whereas this residual signal is not of importance for $^1J_{CC}$ measurements, it renders the evaluation of spin coupling constants smaller than 3 Hz nearly impossible for natural abundance measurements. Another important factor is that a sufficient high digital resolution is needed to measure accuratly $^3J_{CC}$ coupling constants. Therefore SELINQUATE, the 1D carbon selective version of 2D-INADEQUATE, was usually applied. With the advent of gradient selected NMR spectroscopy[5] the performance of the suppression of unwanted coherences has improved by an order of magnitude and in a very recent contribution it was shown that this principle also applies to INADEQUATE techniques.[6]

Results and Discussion

Description of the New Method. Our new technique for the measurement of small carbon−carbon coupling constants employs a different approach for central signal suppression. Double quantum filters are usually used for this purpose, which further reduce the sensitivity by a factor of 2. For measurements in natural abundance this is not very acceptable.

One principle of our approach will be discussed on the following sequence: ^1H: CPD; ^{13}C: $90°(x,-x,\text{sel})-\Delta-G_1-90°(x)-G_2-\text{acq}(x,-x)$ with gradient strength ratio $G_1/G_2 = -1$ and $\Delta + G_1 = 1/(2J_{CC})$. A frequency selected *single* quantum coherence (SQC) is allowed to evolve into an antiphase magnetization. This SQC is spatially labeled by the use of a gradient. A coherence transfer to the coupling partner is performed by a hard 90° pulse and the second gradient rephases all the magnetizations that stem from the coherence created by the selective pulse. All other magnetizations introduced by the hard 90° pulse will be dephased. The two pulsed gradients are directly positioned before and after the hard 90° carbon pulse so as to minimize diffusional effects. This pulse sequence has already been used to measure proton−proton[7] and carbon−carbon[6] coupling constants. However, the problem of central signal suppression was not addressed in both these publications, because in the case of protons the problem does not exist and in the case of carbon uniformly labelled compounds were used. The main signal of the *selected* carbon is still observable in our spectra of unlabeled compounds because it is also chosen by the selective pulse. This does not affect the experiment, since the analysis of the desired doublets is not disturbed by this signal. However, the observation of connectivities with ^{13}C resonances which are close in chemical shift is restricted by the selectivity of the soft pulse. We have found that a difference in resonance/frequency of at least 100 Hz is needed. In processing the spectrum every signal to be evaluated must be separately phased due to the evolution of chemical shift during the experiment.

To demonstrate the better performance of this pulse sequence for unlabeled compounds, Figure 1 shows a comparison between

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® Abstract published in *Advance ACS Abstracts*, September 1, 1995.

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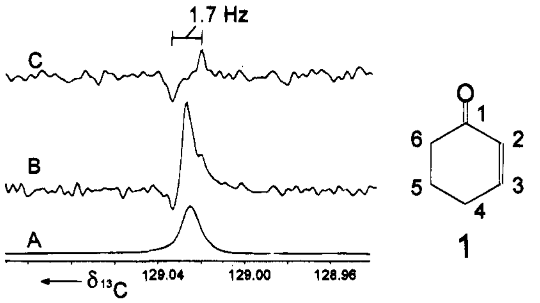

Figure 1. Measurement of $^{2}J_{C2C4}$ of cyclohexenone (**1**) (selection of C-4): (A) ^{13}C-NMR; (B) SELINQUATE (focused on 10 Hz); (C) magnetization transfer using gradients (focused on 10 Hz, Grad. (length 1 ms): 2.5 G cm^{-1}/−2.5 G cm^{-1}). Time domain 128k; processing 256k; exponential window function; line broadening 0.1 Hz; measurement time 8 h; 4k transients.

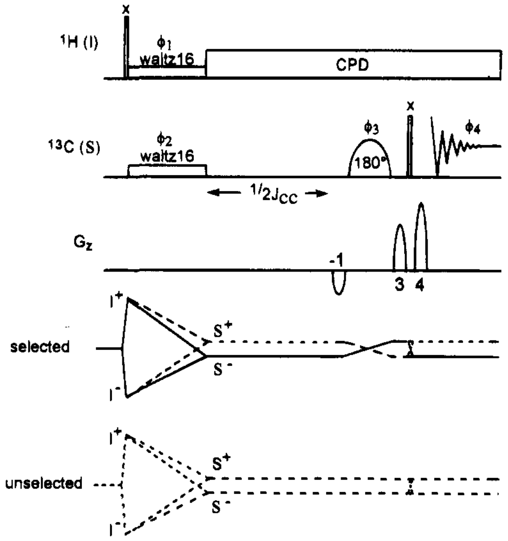

Figure 2. Pulse sequence of GRECCO (GRadient Enhanced measurement of Carbon COupling constants). Transversal proton magnetization is transferred via CP to carbon; thus the offsets should be set on the selected carbon and proton resonances. For $^{1}J_{CH}$ a CW spinlock (typ. ^{1}H, ^{13}C: 6 ms, $\gamma B_1 = \gamma B_2 = 167$ Hz; $\phi_1 = -y_8, y_8$; $\phi_2 = (-y_4, y_4)_2, (-x_4, x_4)_2$, $\phi_3 = x, y, -x, -y$; $\phi_4 = (x, -x)_2, (-x, x)_4, (x, -x)_2, (-y, y)_2, (y, -y)_4, (-y, y)_2$) and for $^{n}J_{CH}$ one cycle of a waltz16 composite pulse is applied on both channels (individual 90° pulse 1.5 ms; ^{1}H, ^{13}C: $\gamma B_1 = \gamma B_2 = 167$ Hz; $\phi_1 = \phi_2 = y$, $\phi_3 = x, y, -x, -y$; $\phi_4 = x, -x$). After an evolution time $1/(2J_{CC})$ (typ. 83 ms) a 180° Gaussian pulse (typ. 10−50 ms) is used to change the coherence order (see coherence pathway diagram). A hard 90° carbon pulse transfers the magnetization between coupled spins. The gradient ratio −1,3,4 (typ. 1.5 ms length, −2 G cm^{-1}, 6 G cm^{-1}, 8 G cm^{-1}) serves to select the magnetizations inverted by the Gaussian pulse. The corresponding coherence pathway is given signed with the label "selected". The coherence pathway below is valid for the other carbon coherences and is not refocused by the three gradients.

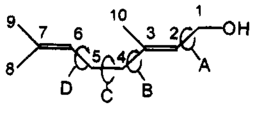

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Figure 3. Possible degrees of freedom of geraniol (**2**).

the spectra of cyclohexenone (**1**), obtained using the SELINQUATE pulse sequence (spectrum B) and the pulse sequence described above (spectrum C). The perfect signal suppression obtained using the latter pulse sequence makes the evaluation of the $^{2}J_{C2C4} = 1.7$ Hz coupling constant considerably easier.

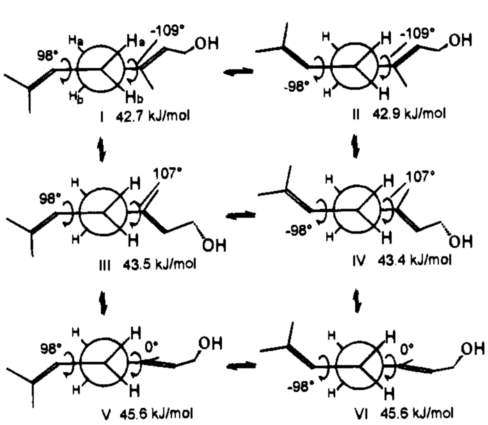

Figure 4. Energetically favored conformations of **2** obtained by force field calculations (MACROMODEL 4.5; MM3). The given dihedral angles refer to the longest carbon chain of **2**.

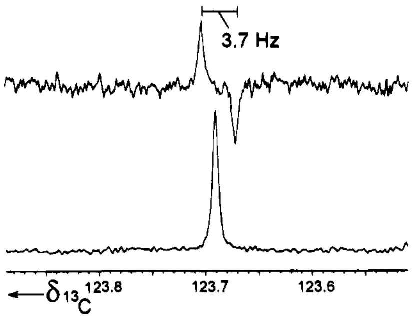

Figure 5. Bottom: Extension of ^{13}C-NMR spectrum of **2**; shown is the expansion of C-6. Top: C-3 selective GRECCO measurement using CP from H-1 to C-3 (more efficient than CP from H6 to C6; selection of C6 due to resonance of C2 is difficult). The antiphase doublet gives $^{3}J_{C3C6} = 3.7$ Hz; measurement time 11 h, 6k transients.

Despite this good signal suppression the overall sensitivity has to be significantly increased for a real performance at natural abundance. Obviously a polarization transfer would be beneficial, such as an INEPT or a heteronuclear cross-polarization (CP) method.[8] A long-range polarization transfer is required for quaternary carbons. INEPT experiments have disadvantages because of the evolution of homonuclear coupling during the evolution time.[9] Therefore we employ cross-polarization using a waltz16 composite pulse of the total length $1/^{n}J_{CH}$ on protons and carbons for long-range CP on quarternary carbons (enhancement of the selected ^{13}C resonance of the S/N ratio of 2). This has the additional advantage that the relaxation delay is dependant on the proton and not on the carbon relaxation time. The repetition rate of the experiment can be increased. The final sequence is shown in Figure 2 which we dub the GRECCO (GRadient Enhanced measurement of Carbon COupling constants) pulse sequence.

The main problem is to restrict the CP to the desired carbon only, and because of this we use a selective Gaussian 180° carbon pulse for selective inversion of the coherence pathway.[10] The set of three gradients provides a perfect suppression. This combination of semiselective CP and the frequency selective coherence pathway selection with a 180° pulse is necessary when selective polarization transfer from proton to carbon is

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Table 1. Carbon−carbon Coupling Constants of **2** Obtained with GRECCO (±0.3 Hz) at 300 K and ^{13}C Chemical Shifts

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
$\delta_{^{13}C}$	58.52	123.40	138.20	39.26	26.12	123.72	131.11	17.23	25.25	15.80
C1	–	48.1	1.7	4.8						4.4
C2	48.1	–	73.0	2.4	3.1					2.0
C3	1.7	73.0	–	42.1	2.3	3.7				41.9
C4	4.7	2.4	42.1	–	32.9	1.5	3.7			3.5
C5		3.1	2.3	33.9	–	44.3		3.7	4.7	1.7
C6			3.7	1.5	44.3	–	74.2	2.0	3.1	
C7				3.7		74.2	–	42.3	43.4	
C8					3.7	2.0	42.3	–	4.5	
C9					4.7	3.1	43.4	4.5	–	
C10	4.4	2.0	41.9	3.5	1.7					–

desired. For the case of protonated carbons we substitute the composite CP by a CW CP where its length is adjusted to $1/^1J_{CH}$ (enhancement factors 1−1.5).

Application of GRECCO to Geraniol (2). The performance of this new pulse sequence is demonstrated for the two terpenes geraniol (**2**) and farnesol (**3**). Both compounds are important precursors for the biological cholesterol synthesis. Some aspects of their conformations have been previously discussed.[11] These compounds were selected because the H, H and C, H spin coupling constants cannot be used for the determination of dihedral angle *C* because an equilibrium between several complex spin systems has to be taken into account. The $^3J_{C3C6}$ coupling constant provides a conformational analysis because a simple AX spin system is observed. The determined coupling constant provides direct information about the equilibrium between the participating conformations. In these compounds the inclusion of vicinal C, C spin coupling information would significantly contribute to the soundness of a conformational analysis.

The diterpene **2** contains two nonconjugated double bonds that are separated by an ethylene bridge. There are four degrees of freedom resulting from single bonds as shown in Figure 3. Our interest will focus on the ethylene bridge with three dihedral angles *B*, *C* and *D*. Ab initio[12] and our own force field calculations have shown that the dihedral angle *C* mainly exists in *trans* and *gauche* conformations. Eclipsed conformations therefore can be excluded. Figure 4 gives the lowest energy conformations of **2** obtained using MACROMODEL.[13] Our calculations predict a dominant *trans* dihedral angle *C* populated to 85% according to the Boltzmann distribution law at 300 K. Because the relative energies computed by force field calculations were recently questioned,[12] the conformations of Figure 4 have therefore been used to build an ensemble of conformations which should be correlated with our results from the NMR measurements.

Figure 4 gives six conformations of **2** in Newman projections of the dihedral angle *C*. The other conformations (>4.4 kJ/mol higher than **I**) have a gauche dihedral angle *C*. The dihedral angles *B* and *D* are governed by a 1,3 allylic strain. To minimize this steric interaction we expect the dihedral angles of C2−C3−C4−H4 and C7−C6−C5−H5 to be ~0°. There are two different conformations possible in both cases because of the presence of two protons (H4a, H4b and H5a, H5b). The same situation applies for dihedral angle *A*, so there are also two conformations possible. **I**, **II**, **III**, and **IV** represent the four diastereotopic conformations. **V** and **VI** have a 1,3 allylic strain with respect to dihedral angle **B** and should therefore be disfavored (2.9 kJ/mol higher than **I**).

A $^3J_{C3C6}$ of 3.7 Hz was measured with the GRECCO sequence. The spectrum is shown in Figure 5. As mentioned earlier only the *gauche* and *trans* conformations of dihedral angle *C* are expected. The Karplus relation of C, C coupling constants for aliphatic systems gives 5 Hz for a transoidal and 1 Hz for a gauche structure. Although there is some uncertainty in these numbers, a value of 3.7 Hz gives direct evidence for the dominance of transoidal conformations around the dihedral angle *C*. The measured carbon−carbon coupling constants of **2** are given in Table 1.

For dihedral angle *B* $^3J_{C5C10} = 1.7$ Hz and $^3J_{C5C2} = 3.1$ Hz were measured. The first number supports the calculations, that dihedral angle C5−C4−C3−C10 is ~90°. With the latter number, one has to take into account that if there is a double bond present, interactions between π-orbitals and the orbitals of the terminal carbon result in additional contributions to the coupling constant.[14] The same situation applies for dihedral angle *D* ($^3J_{C4C7} = 3.7$ Hz). Thus $^3J_{C2C5}$ and $^3J_{C4C7}$ cannot be used to obtain conformational information. In conclusion carbon−carbon coupling constants are especially useful for conformational analysis in aliphatic substructures, where no such π-contributions are observed.

However, a $^3J_{CH}$ coupling exists which could be used for examining the dihedral angle *D* of **2**. The proton selective carbon *J* resolved method introduced by Bax and Freeman[15] was applied and gave a $^3J_{C4H6}$ value of 3.3 Hz that indicates the dihedral angle *D* should be around 55 or 120°. A value of ~9 Hz should be expected for a transoidal H6−C6−C5−C4 structure. A cisoidal structure would give ~7 Hz. It is therefore reasonable to conclude that there are a considerable amount of conformations populated with a perpendicular H6−C4 dihedral angle. This is in agreement with the calculations.

NOESY spectra of **2** also support this conformational analysis. There is one small cross-signal (H_1-Me_8) which indicates that *gauche* (dihedral angle *C*) conformations are also populated. Further analysis of the NOE results is mainly dependent on the number of conformations allowed in the model. In summary, the experimental results indicate that dihedral angle *C* exists mainly in a *trans* rather than in a *gauche* conformation. The $^3J_{C5C10}$ and $^3J_{H6C4}$ coupling constants indicate that the dihedral angles *B* and *D* should adopt a conformation as given in Figure 4.

Application of GRECCO to (*E*,*E*)-Farnesol (3). **3** is the triterpene analogue of **2** and contains an additional ethylene bridge which causes three more degrees of freedom. We have chosen **3** because we want to show that it is also possible to measure vicinal carbon−carbon coupling constants in a C15 compound at natural abundance. Figure 6 gives the normal ^{13}C-NMR spectrum of **3** (A) and the spectrum obtained using the

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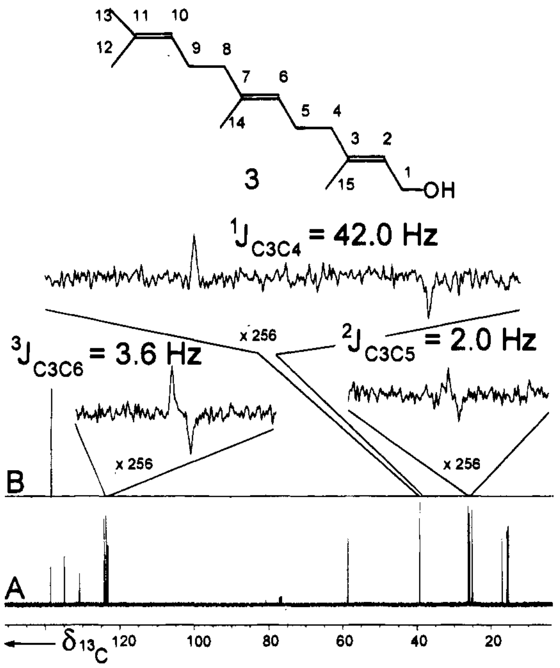

Figure 6. (A) Extension of the ^{13}C-NMR spectrum of **3**; (B) C-3 selective carbon–carbon coupling measurement with GRECCO using CP from H-1 to C3 with three expansions magnified by a factor of 256. The antiphase doublets give $^{1}J_{C3C4}$ = 42.0 Hz, $^{1}J_{C3C15}$ = 41.8 Hz (not shown), $^{2}J_{C3C5}$ = 2.0 Hz, and $^{3}J_{C3C6}$ = 3.6 Hz; measurement time 7.5 h, 6144 transients.

GRECCO pulse sequence (B). The $^{3}J_{C3C6}$ = 3.6 Hz coupling constant is important for our discussion.

An identical measurement for C-7 was also performed and a $^{3}J_{C7C10}$ of 3.4 Hz was obtained. These vicinal carbon–carbon coupling constants were found to be slightly smaller than that for **2**. We conclude that once again transoidal structures are favored, though perhaps to a somewhat lesser extent as for **2**.

Conclusion

Vicinal carbon–carbon coupling constants can be determined in natural abundance for small organic compounds. Additional conformational information is obtained and thus $^{3}J_{CC}$ values are useful when other coupling constants cannot be applied.

Experimental Section

All experiments were performed at 300 K on a Bruker AMX-500 spectrometer equipped with a multinuclear inverse probe with self-shielded gradient coils and a Bruker z-gradient accessory, which delivered sinusoidal gradients up to 8 G cm^{-1}. All compounds are commercially available 70% solutions in CDCl$_3$ of **1**, **2**, and **3** and for the GRECCO measurements of **2** and **3** nondegassed samples were used. The spectra were referenced to the solvent signal and calculated with δ_C(TMS) − δ_C(CDCl$_3$) = 77.0.

Some proton decouplers change their phase with an attenuation. The relative phases of the excitation 90° hard pulse and the following CP must therefore be adjusted with respect to each other. For a successful experiment a signal-to-noise ratio >30 must be achieved in a one transient carbon spectrum. The signals must have a line width smaller than 2.5 Hz. If $^{1}J_{CC}$ coupling constants should be measured the selective 180° carbon pulse must be sufficiently short to irradiate the desired doublet. For further research in this field we suggest using a carbon optimized gradient probe, which would additionally increase the sensitivity of this experiment.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (SFB-260) and the Fonds der Chemischen Industrie. T.F. thanks the federal state of Hessen for a graduate fellowship. We thank Prof. Dr. D. Leibfritz and Dr. W. Willker for experimental details in the carbon detected PFG experiments.

JA951438F