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Article

Carbon-13 Spin-Lattice Relaxation Times for *ent*-Kaurene Diterpenes - A Study of Molecular Motion in Solution

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Foram determinados os T_1 de ^{13}C em $CDCl_3$ e CD_3OD para quatro derivados do *ent*-kaureno. Comparação dos valores de T_1 permitiu uma avaliação qualitativa de reorientação molecular, bem como uma análise do movimento de rotação global da molécula nos dois solventes. Com base no tipo de rotação molecular é discutida a conformação de formas diméricas em solução.

The T_1 values of ^{13}C were measured in $CDCl_3$ and CD_3OD for four *ent*-kaurene derivatives. Molecular reorientations, molecular motion and the conformation of dimeric forms in solution are discussed.

Keywords: ent-*kaurenes, T_1 ^{13}C, molecular motion, conformation*

Introduction

Carbon-13 T_1 values can prove a considerable aid in the assignment of ^{13}C-NMR spectra, in particular for molecules containing large numbers of carbon atoms, since serious overlapping of the multiplet structures limits the use of off-resonance decoupling. For molecules with a rigid skeleton, $2T_1\ CH_2 \cong T_1\ CH < T_1\ CH_3 << T_1\ C$[1], and this can be used to assign the various carbons as either C, CH or $CH_2$2. In the limit of extreme narrowing ($\omega^2\tau_C 2 < 1$), T_1(DD) of a ^{13}C nucleus depends solely upon the number *N*, as shown by Eq. 1, on the condition that the molecule rotates isotropically. The symbol *N* represents the number of H atoms attached to the observed carbon, r_{C-H} is the C-H bond length, and τ_C is the rotational correlation time[1].

$$\frac{1}{T_1(DD)} = \frac{N\hbar^2 \gamma_C^2 \gamma_H^2}{(r_{C-H})^6} \tau_C \qquad (1)$$

The dipolar mechanism constitutes the dominant, and often the only significant relaxation mechanism for large molecules.

Unlike chemical shifts and coupling constants, T_1 values are dependent on molecular reorientation and can therefore act as powerful sources of information on molecular motion in solution[3,4]. This information can be used in studies of internal rotation (especially methyl groups), axes of rotation, and association. Besides intermolecular attraction in solution, many molecules can undergo interaction with the solvent, especially if those molecules present polar groups[5,6].

In this paper we describe the analysis of the T_1 values of ^{13}C in the qualitative study of the molecular motion in solution for four *ent*-kaurene (5) diterpenes. We discuss the possible solute-solute and solute-solvent interactions of the compounds *ent*-kaur-16-en-19-oic acid (1), 15α-acetoxy-*ent*-kaur-16-en-19-oic acid (2), methyl *ent*-kaur-16-en-19-oate (3), and *ent*-kaur-16-en-19-ol (4), as shown in Fig. 1, in $CDCl_3$ and CD_3OD.

The T_1 of ^{13}C has been found to depend on several factors such as the size of the molecule, solution viscosity and concentraction, and solute-solute and solute-solvent interactions. The T_1 values of the ^{13}C nuclei generally decrease with increasing molecular size, if the molecules are rigid. Medium-sized molecules (C_{10}-C_{50}) typically show T_1 values between 0.1 s and 2.0 s[7].

Table 1. Solvent dependence of spin-lattice relaxation times, T_1, of the ^{13}C atoms in *ent*-Kaurene derivatives (times in second).

Compounds	1		2		3	4	
C	$CDCl_3$	CD_3OD	$CDCl_3$	CD_3OD	$CDCl_3$	$CDCl_3$	CD_3OD
1	0.40	0.86	0.24	0.50	1.11	1.14	1.09
2	0.37	0.81	0.22	0.45	1.05	1.05	1.11
3	0.36	0.70	0.20	0.39	0.81	0.74	0.74
4	5.98	11.10	3.70	6.18	-	11.60	11.90
5	0.68	1.58	0.40	0.89	2.18	2.36	1.94
6	0.41	0.97	0.25	0.51	1.15	1.19	1.00
7	0.42	0.90	0.26	0.53	1.13	1.12	1.15
8	5.27	9.77	4.31	7.35	13.79	10.90	-
9	0.76	1.56	0.44	1.00	2.18	2.24	1.95
10	-	-	3.12	6.14	13.50	12.00	9.85
11	0.33	0.65	0.23	0.43	0.98	0.98	1.00
12	0.33	0.72	0.20	0.42	0.94	0.87	0.88
13	0.85	1.84	0.54	1.00	-	1.97	-
14	-	-	0.22	0.42	1.00	-	1.97
15	0.42	0.92	0.47	1.00	1.16	1.19	-
16	5.88	12.30	3.64	7.54	14.60	14.90	7.63
17	0.39	0.87	0.22	0.48	1.13	1.15	1.29
18	0.68	1.01	0.53	0.77	1.20	1.20	1.40
19	7.53	20.00	4.56	-	-	1.26	1.04
20	0.57	0.86	0.47	0.60	1.00	1.16	1.10

Results and Discussion

As can be observed in Table 1, the polarity of the solvents has a strong influence on the T_1 values of compounds 1 and 2. Therefore, the T_1 values in CD_3OD are approximately twice the T_1 values in $CDCl_3$. This result is consistent with the presence of dimeric forms of the solute in $CDCl_3$ and the absence of dimers in the CD_3OD solution. The association leads to an increase in the effective molecular mass (and hence, of the correlation time), leading to a reduction in T_1[7].

Compounds 3 and 4 are unable to form such dimers, and this is reflected in their longer T_1 values. It should be noted that the small difference in magnitude of the T_1 values of compound 4 in the two solvents, usually shorter in CD_3OD, suggests the presence of solute-solute and/or solute-solvent interaction in CD_3OD and solute-solute interaction in $CDCl_3$, through hydrogen bonds.

In compounds 3 and 4, the T_1 value of the C_3 is considerably shorter than the other carbons of the compound (Table 1). From this we conclude that the molecules are undergoing anisotropic motion in solution, *i.e.*, the molecules have a preferred axis of rotation. The long diterpenoid axis passes through or very close to C_3[1].

Examination of Table 1 shows that compounds 1 and 2 also rotate anisotropically in CD_3OD solution. On the other hand, Table 1 shows that in $CDCl_3$, the C_3 in compounds 1 and 2 has an average T_1 value appreciably close to the others. This behavior suggests that the molecules rotate isotropically in $CDCl_3$ solution, *i.e.*, with equal facility in all directions.

Taking into account that approximately spherical bodies will preferentially undergo isotropic rotation[7], the *endo* conformation can be assumed for the dimeric forms previously deduced for compounds 1 and 2 in $CDCl_3$ solution. This inference is consistent with the results from X-ray crystallographic studies of the diterpene *ent*-kaur-16-en-19-oic acid (1). The tetrameric crystal structure of the compound exists as two pairs of hydrogen bonded dimers, wherein the COOH group C=O is *cis*, whereas C-OH is *trans* to the C_3-C_4 bond of the I ring[8].

The familiar framework model clearly shows that the orientations given above for the C=O and C-OH species are responsible for the *endo* conformation of the dimer.

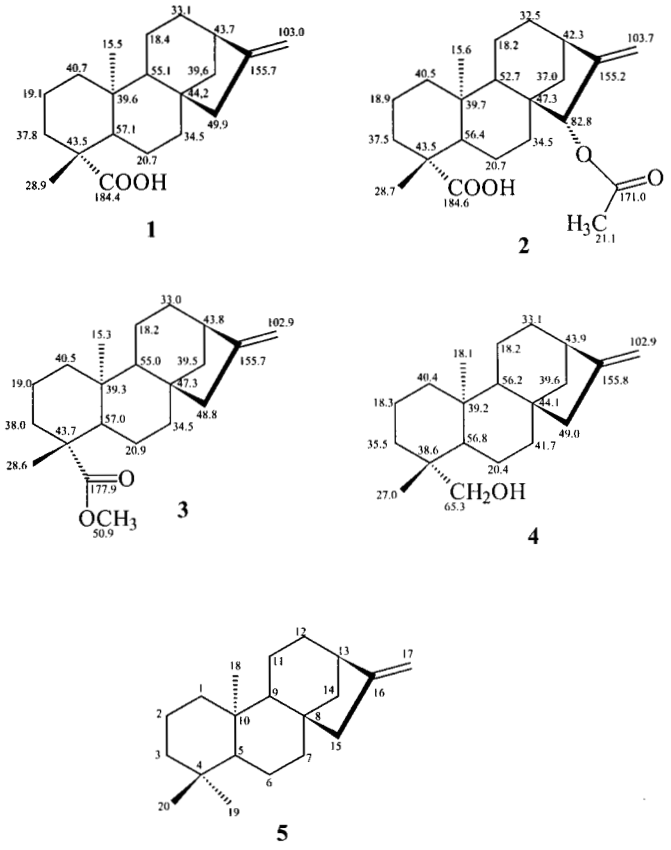

Figure 1. ^{13}C Chemical Shifts δC of *ent*-Kaurene Derivatives ($CDCl_3$, TMS).

Experimental

ent-Kaur-16-en-19-oic acid (1) and *ent*-kaur-16-en-19-ol (4) were isolated from *Annona squamosa* Linn. (Annonaceae)[9,10].

Methyl *ent*-kaur-16-en-19-oate (3) was obtained from acid 1 by treatment with CH_2N_2 in Et_2O, mp 87-89 °C (MeOH) lit[11]. 87 .°C, ^{13}C-NMR data, Fig. 1.

15α-Acetoxy-*ent*-kaur-16-en-19-oic acid (2). Starting from acid 1, 15α-hydroxy-*ent*-kaur-16-en-19-oic acid was prepared by a procedure adapted from procedures described for allylic oxidation[12,13]. Selenium dioxide (9.82 mmol) in 95% ethanol (40 mL) was added in drops (4 h) to acid 1 (8.42 mmol) in 95% ethanol (30 mL) and refluxed for 24 h. The mixture was filtered on a silica gel layer, and this was washed with ether (3 x 10 mL), giving a red oil. The addition of benzene (15 mL), followed by slight stirring, gave a precipitate, which was collected by filtration. Crystals of 15α-hydroxy-*ent*-kaur-16-en-19-oic acid were obtained in a 70% yield, mp 227-230 °C (EtOH - Benzene) lit.[14] 228-230 °C, ^{13}C-NMR data, Fig. 1. Acetylation with acetic anhydride-pyridine yielded acid 2, mp 171-173 °C, lit.[15] 173-174 °C, ^{13}C-NMR data, Fig. 1.

Samples for ^{13}C-NMR were prepared in $CDCl_3$ and CD_3OD (conc. Table 2) and submitted to a stream of dry N_2 for 20 min. The ^{13}C-NMR spectra (50.33 MHz) with complete decoupling were recorded on a Bruker AC-200 spectrometer in 5 mm (OD) tubes, generally at 30 °C. Figure 1 shows the chemical shifts for compounds 1 to 4.

Table 2. Concentrations of the $CDCl_3$ and CD_3OD solutions of the *ent*-Kaurene derivatives 1 to 4.

Compound	1	2	3	4
CD_3OD (mol/L)	0.26	0.22	-	0.17
$CDCl_3$ (mol/L)	0.26	0.22	0.25	0.21

The T_1 values were obtained from (180°-τ-90°) 25 s pulse sequence (Inversion-Recovery Method), using at least 17 τ values, varying in the range of 0.01-12 s for compounds 1 and 2, and 0.01-15 s for compounds 3 and 4 (generally three to six values recorded). A 90° pulse corresponded to a pulse time of 5.91 μs. Normally, spectral widths of 10416 Hz were used, acquisition time 1.57 s, relaxation delay 20 s, number of spectra (NS) 128, and digital parameters: line broadening (LB) 2.0, size (SI) 64 K.

Conclusion

It is clear from this work that T_1 data can be used as an aid in structural and conformational analysis. The results

obtained for four compounds of diterpenic origin constitute interesting examples of isotropic and anisotropic motions, molecular reorientations, association and conformation being determined by the measurement of ^{13}C relaxation times in two solvents.

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