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Mobility of Triarylmethyl Radicals in Water–Glycerol and Water–Trehalose Media

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Abstract

In the recent years, triarylmethyl radicals are widely used as spin probes and tags, so the study of their properties is of great interest. In the present work, the magnetic resonance properties of triarylmethyl radicals (FT (Finland trityl) and OX063) labeled with 13C isotope in water-glycerol and water-trehalose solutions are studied using stationary and pulsed EPR within a wide temperature range. Their magnetic resonance parameters, electron spin relaxation times and rotation correlation times at different temperatures are measured. It is shown that 13C-FT radical forms aggregates in trehalose solution, while aggregates are not observed for 13C-OX063 radical.

Keywords: microviscosity, triarylmethyl radical, EPR

INTRODUCTION

Triarylmethyl radicals (TAM, trityl) are widely used as spin probes and labels [1, 2]. They differ from other stable paramagnetic materials (for example, nitroxide radicals), applied as spin probes and labels, by extremely narrow lines in the electron paramagnetic resonance (EPR) spectrum – not more than 5 μT [3], and a long time of electron spin relaxation in solutions [4]. Due to these properties, TAM are used as spin probes to measure oxygen concentration [5, 6], pH values [7, 8], the concentrations of phosphates [9] *in vitro* and in EPR tomography *in vivo* [10].

The time of electron spin relaxation determines the distance range that can be assessed by means of double electron-electron resonance (DEER). The measurements of this kind are usually carried out at helium temperature on frozen water–glycerol solutions. It has been shown in [11, 12] that the use of TAM as spin labels allows one to determine nanometer-scale distances in biopolymers at room temperature. For the dipole-dipole interaction to be not averaged, the measurements are carried out in the immobilised matrix, and the sample is localised either on silica gel surface [11, 13] or in trehalose matrix [11, 14].

As described in [15, 16], deuterated radical FT (Finland trityl) labelled with ^{13}C isotope at the central carbon atom was synthesised: ^{13}C-dFT (Fig. 1, *a*). The introduction of ^{13}C isotope at the indicated position leads to the appearance of two lines in the EPR spectrum, corresponding to splitting at the central carbon atom ^{13}C. Unlike for TAM with natural carbon content, for which the shapes of spectra are insignificantly dependent on motion, the EPR spectrum of ^{13}C-TAM has a hyperfine coupling constant (*hfc*) with substantial

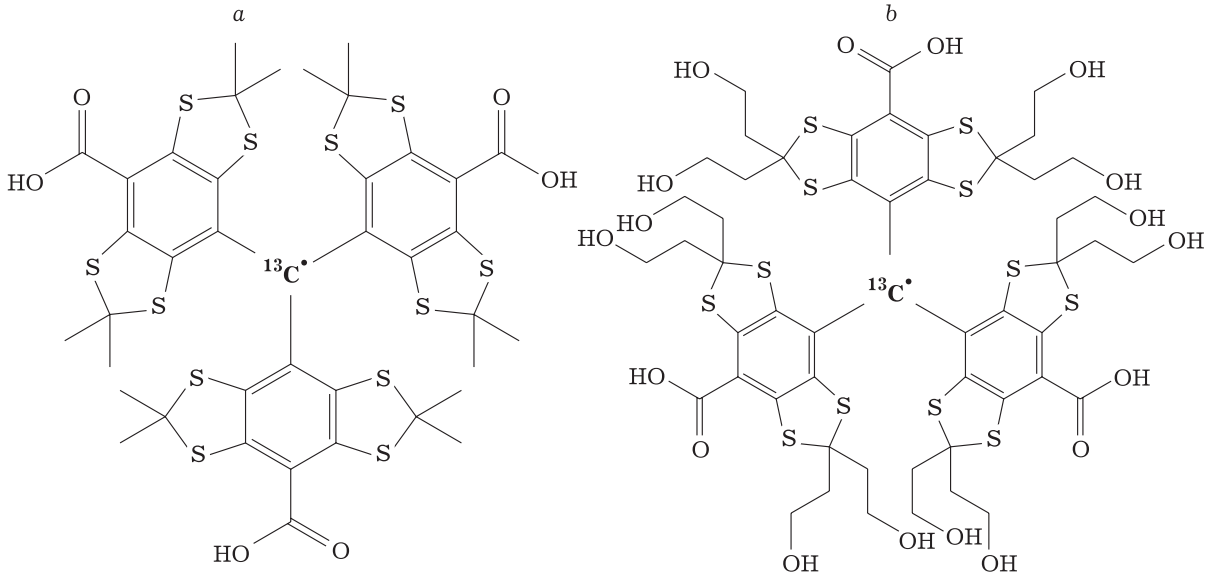

Fig. 1. Structure of triarylmethyl radicals under investigation: *a* – ^{13}C-FT, *b* – ^{13}C-OX063.

anisotropy (A_{zz} = 5.81 mT, $A_{xx} = A_{yy}$ = 0.607 mT) and a slight anisotropy of *g*-tensor ($g_x = g_y$ = 2.0031 and g_z = 2.0027), so the EPR spectrum can be used to obtain the information on viscosity [15]. Similarly, the authors of [17] proposed using chlorinated TAM (^{13}C-PTMTC) to study viscosity. The advantages of using ^{13}C-dFT to measure viscosity were later on demonstrated in [18]. Radical sensitivity to changes in the composition of the medium allowed evaluation of viscosity in human biological liquids (blood, tissues) and *in vivo* in mice. In turn, the synthesis of 99% enriched ^{13}C-PTMTC demonstrated a similar sensitivity of radical spectrum to its mobility and the differences between correlation times in the media with different composition [19]. The use of an isotopomer, deuterated analogue OX063 – ^{13}C-OX071 – to measure distances in proteins with the help of DEER was implemented with the orthogonal spin labelling with a label based on Cu(II) [20]. This label is biocompatible and does not form complexes with albumin during *in vivo* measurements of viscosity in tissues [16].

The goal of the present work is to study the magnetic resonance properties of triarymethyl radicals (^{13}C-FT and ^{13}C-OX063), enriched with ^{13}C at the central C^1 atom (see Fig. 1), in viscous solutions water–glycerol and water–trehalose at a temperature of 80–320 K by means of stationary-phase and pulsed EPR, and to measure the times of electron spin relaxation within the X band at low temperatures.

EXPERIMENTAL

Sample preparation

The stable radical ^{13}C-FT (Finland trityl) was synthesised according to the known procedure [21]. Trityl ^{13}C-OX063 was synthesised similarly to the non-labelled analogue [22] using di(*n*-butyl)carbonate-(*carbonyl*-^{13}C) [23] as the initial source of ^{13}C isotope (see Fig. 1). The solutions for the studies of EPR spectra were water–glycerol (50 : 50 v/v) and water–trehalose (80 : 20 by volume), because these mixtures are most frequently used to study biopolymer structures in experiments on distance measurements by means of DEER. To exclude line broadening because of the presence of atmospheric oxygen in the solutions, the capillaries with samples were evacuated at low temperature and then sealed in vacuum ($5 \cdot 10^{-2}$ Torr).

The samples of ^{13}C-TAM were prepared for measurements in dehydrated trehalose matrix by mixing 200 mg of trehalose, 600 μL of water, 10 μL of the aqueous solution of trityl (^{13}C-FT, ^{13}C-OX063) with the concentration of 10^{-3} mol/L. Then the mixture was dried for two days (10^{-5} Torr).

Experiment performance and data treatment

Stationary EPR spectra were recorded with an Elexsys E-540 EPR X-band spectrometer (Bruker, Germany). Temperature controller was used to determine and maintain temperature within the range of 120–320 K.

The samples were placed in glass capillaries of 0.5 mm in diameter. EPR spectra were recorded with the external magnetic field modulation amplitude and frequency of 0.5 G and 100 kHz, respectively, with the microwave radiation frequency equal to 2 mW. Modulation amplitude at low temperature was 2 G. EPR spectra were simulated using an EasySpin software package [24] in Matlab software environment, allowing simulations of various mobility modes for paramagnetic molecules and determination of their magnetic resonance parameters (*g*-tensor, hyperfine coupling (*hfc*) tensor, rotational correlation time (τ_c), *etc.*). Calculation of EPR spectra was performed in the approximation of slow molecular rotation (simulation mode: chili). Simulation is based on solving the stochastic Liouville equation on the basis of rotational eigenfunctions. The spin probe rotation was considered to be isotropic. The values of *g*-tensor and *hfc* tensor used in spectrum simulation (g_x = 2.0033, g_y = 2.0032, g_z = 2.0027; A_x = A_y = 0.6423, A_z = 5.7806) were measured previously in [15, 16, 18]. The values of *g*-tensor and *hfc* tensor of radicals in frozen solutions, determined by us from the best coincidence between the calculated and experimental data, coincide with the literature data published previously [15].

Echo-detected EPR spectra were recorded and the time of electron spin relaxation was measured using a pulsed spectrometer developed at the NIOCh SB RAS (Novosibirsk) [25]. The times of electron spin relaxation and spin echo were measured through the pulse sequences $\pi/2-\tau-\pi-\tau-$echo and $\pi-T-\pi/2-\tau-\pi-\tau-$echo, where T and τ are variable delay times within the range from 100 ns to 5 ms. The duration of $\pi/2$-pulse was 20 ns. The resonator quality factor (Q) was ~80.

RESULTS AND DISCUSSION

Stationary EPR spectra of the isotopomers of triarylmethyl radicals at different temperatures

The stationary EPR spectra of the 0.1 mmol/L ^{13}C-FT solutions in the matrices water–glycerol (50 : 50) and water–trehalose (80 : 20) at different temperatures are shown in Fig. 2. One can see that the shape of ^{13}C-FT spectrum is substantially dependent on temperature.

The shape of the EPR spectrum changes from a doublet with the isotropic *hfc* constant at high temperatures (320 K) and rapid radical rotation to a completely immobilised spectrum (at 150 K). It may be noted that the mobility of ^{13}C-FT radical at the same temperature is higher for water–trehalose mixture than for water–glycerol mixture (at a temperature above 260 K).

Similar measurements were carried out for ^{13}C-OX063 radical in water–glycerol medium (50 : 50) (Fig. 3). Unlike for ^{13}C-FT, due to a large number of hydroxyl groups, the radical ^{13}C-OX063 is characterised by high hydrophilicity and solubility in water. Comparing the spectra of ^{13}C-FT (see Fig. 2, *a*) and ^{13}C-OX063 (see Fig. 3), one can see substantial differences in their shapes. At 280 K, in the case of ^{13}C-OX063, the transition between the fast and slow radical motion is already observed, while for ^{13}C-FT a similar spectrum shape is observed only at a temperature of 260 K. Differences in the mobility of radicals ^{13}C-OX063 and ^{13}C-FT are connected with the difference in their effective radii because of additional substituents in the radical ^{13}C-OX063. Besides, for ^{13}C-OX063, additional contribution to the change of rotational correlation time may be made by the interaction of OH groups with water and glycerol molecules.

Dependence of the radical rotational correlation time on viscosity of the medium

Comparison of experimental and calculated EPR spectra allowed us to determine rotational correlation time values τ_c for radicals at different temperature. The dependence of thus obtained TAM rotational correlation times on the viscosity (η) of water-glycerol solution, measured using a viscosimeter in [26], is shown in Fig. 4, *a*.

The temperature dependence of radical radii, calculated using the Stokes–Einstein–Debye equation, is presented in Fig. 4, *b*. The parameters were the values of τ_c for radicals, determined by us, and the known viscosity measured with a viscosimeter [26].

$$\tau_c = 4\pi R^2 \eta/(3kT)$$

where η is the viscosity of the medium, R is molecular radius, k is Boltzmann constant, and T is temperature.

One can see that the radii of radicals are independent of temperature: R_{eff}(^{13}C-FT) ≈ 7.5±0.3 Å; R_{eff}(^{13}C-OX063) ≈ 8.5±0.3 Å (see Fig. 4, *b*). The determined radii are in agreement with the values for ^{12}C-dFT (7.5 Å) and ^{12}C-OX063 (8 Å), reported in [27, 28].

According to the data obtained, the shape of EPR spectra of radicals ^{13}C-OX063 and ^{13}C-FT is

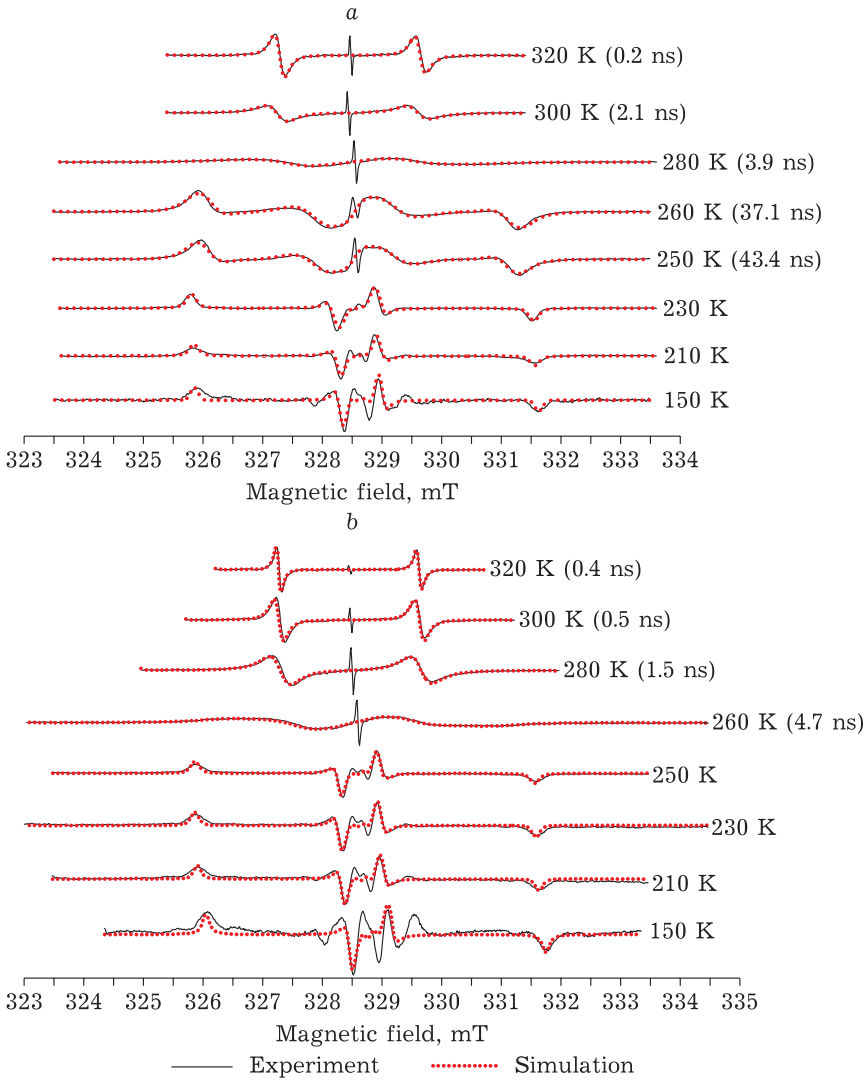

Fig. 2. Experimental and simulated EPR spectra of ^{13}C-FT in water-glycerol (50 : 50 by volume) (*a*) and water–trehalose (80 : 20 by volume) (*b*) at different temperatures. Designations here and in Fig. 3: temperature (K) and rotational correlation time (ns), simulated with the help of EasySpin package. Modelling parameters for ^{13}C-FT: $g_{13C\text{-}FT}$ = [2.0033, 2.0032, 2.0028], $A_{13C\text{-}FT}$(mT) = [0.575, 0.706, 5.711].

observed to be highly sensitive to the viscosity of the medium. It should be stressed that solvent viscosity was varied in our experiments by varying temperature, while in [15, 16] it was varied by varying water/trehalose ratio at room temperature.

Comparison of the obtained dependences of correlation time with similar results for frequently used nitroxide radical TEMPONE [29] demonstrates that ^{13}C-TAM is several times more sensitive to viscosity (see Fig. 4, *a*). The dependence for TEMPONE was plotted using τ_c values at different temperatures, measured previously by the authors of [29]. The values of anisotropy of *hfc* and g-tensor for the entire series of nitroxide radicals are very close, so it may be stated that this conclusion will be valid for the whole series of spin probes based on nitroxide radicals. It seems certain that the use of ^{13}C-labelled TAM as spin probes will allow substantial improvement of measurement accuracy and interpretation of the spectra in the studies of microviscosity and localisation of spin-labelled compounds in living organisms

The EPR spectra of radicals ^{13}C-FT and ^{13}C-OX063 in dehydrated trehalose

We also obtained the EPR spectra of both TAM in the dehydrated solution of trehalose. The experimental spectra exhibit substantial differences (Fig. 5). For ^{13}C-OX063, a typical EPR spectrum of immobilised radical was successfully ob-

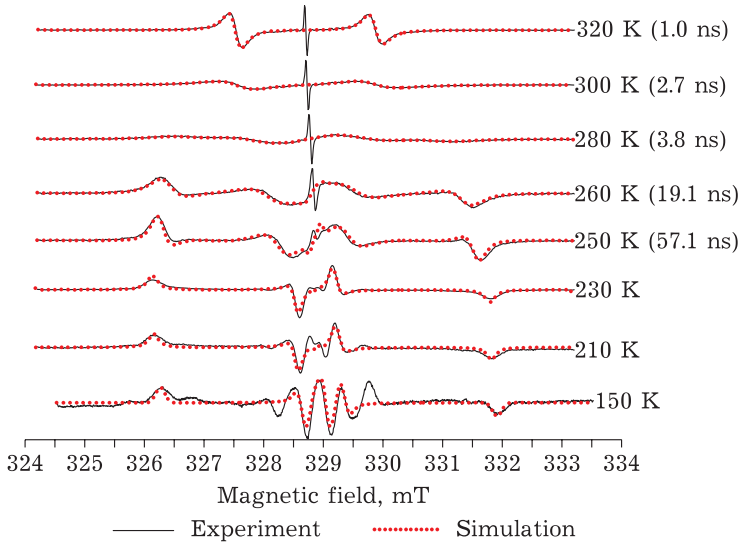

Fig. 3. Temperature dependence of stationary experimental and simulated EPR spectra of ^{13}C-OX063 in water–glycerol (50 : 50). Modelling parameters for ^{13}C-OX063: $g_{13C\text{-}OX063}$ = [2.0033, 2.0032, 2.00275], $A_{13C\text{-}OX063}$ (мТ) = [0.5714, 0.6147, 5.7092]. For designations, see Fig. 2.

tained. For ^{13}C-FT, one exchange-broadened line is observed in the spectrum. We suppose that the reason is the formation of aggregates as a consequence of FT hydrophobic nature. It is known that OX063 is a hydrophilic radical, while, according to literature data, FT forms aggregates when its concentration is high, which has been demonstrated previously for ^{12}C-TAM [30, 31].

Pulse measurements for ^{13}C-FT and ^{13}C-OX063

The times of electron spin relaxation and echo-detected EPR spectra were measured for deoxygenated ^{13}C-FT and ^{13}C-OX063 samples with the concentration of 0.1 mmol/L. The obtained inversion curves of spin echo recovery and decay were approximated with the exponent and stretch-exponent, respectively [32] (Table 1). In water–glycerol solution (2 : 1), the times of longitudinal and transverse relaxation were T_1 = 2.3/2.6 ms (^{13}C-FT/^{13}C-OX063) and T_2 = 3.7/5.2 μs (^{13}C-FT/^{13}C-OX063) at 80 K. Temperature rise causes a decrease in relaxation time (see Table 1), which is known to be due to an increase in mobility and the corresponding contribution into relaxation from the modulation of an anisotropic component

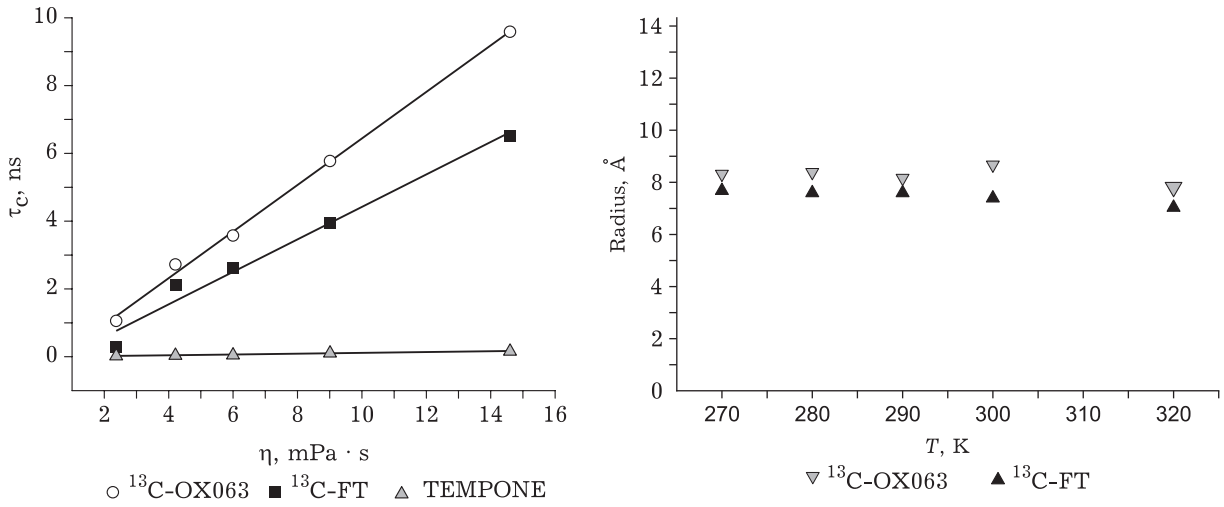

Fig. 4. *a* – Dependence of correlation time (τ_c) of nitroxide radical TEMPONE (data from [29]) and triarylmethyl radicals (^{13}C-FT and ^{13}C-OX063) on the viscosity (η) of water-glycerol solution, measured with a viscosimeter in [28]; *b* – dependence of the radii of isotopomers ^{13}C-FT and ^{13}C-OX063 on temperature (*T*).

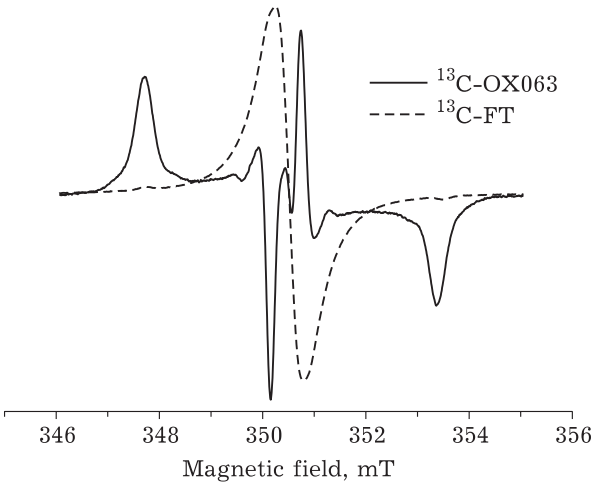

Fig. 5. Spectra of ^{13}C-FT and ^{13}C-OX063 in anhydrous trehalose solution at room temperature. Composition of mixture before drying: trehalose 200 mg; water 600 μL; TAM (^{13}C-FT or ^{13}C-OX063) 10 μL, with concentration 1 mmol/L.

of *hfc* and *g*-factor anisotropy [6, 11, 33]. According to the data (see Table 1), the times of spin relaxation for ^{13}C-OX063 are longer than for ^{13}C-FT. It should be stressed that a similar dependence was previously observed for non-labelled forms of FT and OX063 radicals [34].

CONCLUSION

The results of stationary and pulsed EPR studies of the magnetic resonance properties of triarylmethyl radicals (^{13}C-FT and ^{13}C-OX063), enriched with ^{13}C at the central C^1 atoms, in viscous water-glycerol and water-trehalose media at a temperature of 80–320 K are described in the work. The times of electron spin relaxation at X band at low temperature are measured. Analysis of experimental data allowed us to obtain the temperature dependence of rotational correlation times for both radicals. It has been discovered that ^{13}C-FT radical forms aggregates in anhydrous trehalose, while no aggregates are observed for ^{13}C-OX063 radical. The measured time of electron spin relaxation of radicals ^{13}C-FT and ^{13}C-OX063 at a temperature of 80 K only slightly differs from the values for ^{12}C-FT and ^{12}C-OX063, which makes ^{13}C-FT and ^{13}C-OX063 promising for application as orthogonal labels in the studies of biomolecules by means of double electron-electron resonance (Pulse ELectron DOuble Resonance, PELDOR).

TABLE 1

Times of electron spin relaxation (T_1 and T_2) of isotopomers ^{13}C-FT and ^{13}C-OX063

Compound	Temperature, K	T_1, ms	T_2, μs
^{13}C-FT	80	2.3	3.7
	100	1.0	3.0
	150	0.2	0.9
^{13}C-OX063	80	2.6	5.2
	100	1.0	4.3
	150	0.4	4.1

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