

Paramagnetic Relaxation Reagents. Alternatives or Complements to Lanthanide Shift Reagents in Nuclear Magnetic Resonance Spectral Analysis

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Abstract: Spin-lattice relaxation times (T_1 's), obtained from solutions containing nonshifting paramagnetic relaxation reagents, can be used for nmr spectral analysis complementing or replacing ^{13}C or other nuclei nmr data obtained with lanthanide chemical shift reagents. Chromium or iron tris(acetylacetonates) efficiently shorten ^{13}C T_1 's to 0.1–0.5 sec (at *ca.* 5×10^{-2} *M* metal concentration) without inducing any pseudocontact or contact shifts for nuclei being observed. Both $\text{Cr}(\text{acac})_3$ and $\text{Fe}(\text{acac})_3$ can interact with organic substrates, resulting in different experimental T_1 's for nuclei close to the site of complexation. It is possible to calculate complex geometry from the inverse sixth power distance dependence of the electron-nuclear dipole-dipole relaxation interaction; no angular dependence occurs, in contrast with the situation for pseudocontact shifts in lanthanide reagent complexes. Hydrogen-bonded $\text{Fe}(\text{acac})_3$ complexes with borneol and isborneol demonstrate that ambiguities present in ^{13}C LIS (lanthanide induced shift) studies do not occur with PARR (paramagnetic relaxation reagents) experiments. $\text{Cr}(\text{acac})_3$ and $\text{Fe}(\text{acac})_3$ also interact with non-hydrogen-bonding substrates such as 1-octyl nitrate, benzonitrile, and *n*-butylbenzene, resulting in preferential orientation of the organic substrates relative to the chelate molecules. For example, in 1-octyl nitrate the nitro group orients toward the metal complex.

During the past 3 years there has been a great deal of activity exploiting the use of lanthanide shift reagents to facilitate ^1H and ^{13}C nmr analyses.² The lanthanide reagents greatly simplify ^1H spectra by separating overlapping bands. Moreover it is, in principle, possible to calculate the time-averaged geometry of the substrate molecule from lanthanide-induced ^{13}C or ^1H shifts.

Paramagnetic spin-lattice relaxation additives that *do not induce chemical shifts in substrates* are known.^{3–6} These reagents are typically used to (1) shorten long spin-lattice relaxation times facilitating ^{13}C FT nmr observation of carbons that are remote from protons (*e.g.*, carbonyl carbons in organometallics); (2) remove the nuclear Overhauser effect (NOE) in proton decoupled ^{13}C studies, allowing quantitative ^{13}C analyses for small and intermediate sized molecules where the NOE's may be incomplete; and (3) suppress the generally unfavorable negative NOE in proton decoupled studies of nuclei with negative magnetogyric ratios (*e.g.*, silicon-29^{6a} and nitrogen-15^{6b}).

Tris(acetylacetonato)chromium(III)⁵ [$\text{Cr}(\text{acac})_3$] and $\text{Fe}(\text{acac})_3$ ^{6,7} have both been used as inert, organic-soluble relaxation reagents. At concentrations approaching 10^{-1} *M* (near the solubility limits for these reagents in many organic liquids), they will efficiently suppress $^{13}\text{C}\{^1\text{H}\}$ NOE's for compounds with ^{13}C T_1 's exceeding *ca.* 1 sec.⁷ Although lanthanide and non-

shifting paramagnetic relaxation reagents are generally chemically inert, they can interact physically with many organic substances, resulting in collision type or stronger complexations. This solution ordering of substrate molecules with relaxation reagents has been exploited with lanthanide reagents. Lanthanide-induced pseudocontact shifts depend on distance and angular relationships between the individual observed nuclei and the paramagnetic metal nucleus. Unfortunately, in ^{13}C nmr lanthanide experiments, contact-type shifts may be induced at nuclei close to the site of complexation. These contact shifts may be large and of opposite sign to coincident pseudocontact shifts. Separation of contact and pseudocontact terms is sometimes possible,^{8,9} but the utility of lanthanide reagents is obviously lowered by this complication.¹⁰

With nonshifting paramagnetic relaxation reagents such complications do not arise. Resonance line positions are not affected, simplifying spectral interpretation. Only the nuclear spin-lattice relaxation times, T_1 's (and spin-spin relaxation times, T_2 's) are changed.¹¹ The effect of the paramagnetic reagent on T_1 's is to augment or replace existing relaxation pathways according to eq 1, where $1/T_1^{\text{obsd}}$ is the observed

$$\frac{1}{T_1^{\text{obsd}}} = \frac{1}{T_1^{\text{dia}}} + \frac{1}{T_1^{\text{e}}} \quad (1)$$

relaxation rate, $1/T_1^{\text{dia}}$ is the sum of all diamagnetic relaxation rates (from dipolar, spin-rotation, scalar, and chemical shift anisotropy mechanisms¹²), and $1/T_1^{\text{e}}$ is the

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(2) References cited in B. C. Mayo, *Chem. Soc. Rev.*, **2**, 49 (1973).

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(4) S. Barcza and N. Engstrom, *J. Amer. Chem. Soc.*, **94**, 1762 (1972).

(5) O. A. Gansow, A. R. Burke, and G. N. LaMar, *J. Chem. Soc., Chem. Commun.*, 456 (1972).

(6) (a) G. C. Levy, J. D. Cargioli, P. C. Juliano, and T. D. Mitchell, *J. Amer. Chem. Soc.*, **95**, 3445 (1973); *J. Magn. Resonance*, **8**, 399 (1972); (b) L. F. Farnell, E. W. Randall, and A. I. White, *J. Chem. Soc., Chem. Commun.*, 1159 (1972).

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(8) G. E. Hawkes, D. Liebfritz, D. W. Roberts, and J. D. Roberts, *J. Amer. Chem. Soc.*, **95**, 1659 (1973); G. E. Hawkes, C. Marzin, S. R. Johns, and J. D. Roberts, *ibid.*, **95**, 1661 (1973).

(9) O. A. Gansow, *et al.*, *J. Amer. Chem. Soc.*, **95**, 3390 (1973).

(10) Yb^{3+} chelates have been shown to be superior lanthanide reagents for ^{13}C nmr; contact shifts have not been observed with this metal; see ref 9 and J. Reuben, *J. Magn. Resonance*, **11**, 105 (1973).

(11) Along with $^{13}\text{C}\text{-}^1\text{H}$ nuclear Overhauser effects in $^{13}\text{C}\{^1\text{H}\}$ experiments.

(12) See references cited in G. C. Levy, *Accounts Chem. Res.*, **6**, 161 (1973).

relaxation rate due to electron–nuclear relaxation mechanisms.¹³ In heteronuclear nmr studies (such as ¹³C, ²⁹Si, ¹⁵N, etc.), each nucleus in a molecule may have an easily defined *individual* T_1 . The individual T_1^e term for any nucleus in a substrate complexed to a paramagnetic reagent depends on the time-averaged internuclear distance between that nucleus and the paramagnetic metal. Thus, nuclei close to the site of complexation will have the shortest T_1^e terms (fastest electron–nuclear relaxation rates). If the electron–nuclear dipole–dipole relaxation mechanism is completely dominant, a sixth power distance dependence is predicted.

It will often be possible to use the T_1^e data to facilitate nmr spectral assignments for compounds containing acidic hydrogens. Moreover, syntheses of other specific chromium or iron chelates¹⁴ should allow complexation with various types of organic substrates. These experiments are quite analogous to various “spin-labeling” methods used to identify binding sites in biological molecules.¹⁵ In this paper we present T_1^e data for several organic substrate systems complexed to the nonshifting paramagnetic relaxation reagents Fe(acac)₃ and Cr(acac)₃. Both specific (hydrogen-bonded) and nonspecific (electrostatic, dipolar) complexes have been observed.

Results and Discussion

The first molecule to be examined by ¹³C nmr with lanthanide shift reagents was borneol.¹⁶ We report here the ¹³C T_1 's for borneol before and after addition of $4.86 \times 10^{-2} M$ Fe(acac)₃. The results are given in Table I, along with ¹³C chemical shifts measured relative to the CH₃ carbons of TMS. The literature resonance assignments for borneol¹⁶ are used in Table I. Information available from the various T_1^e values supplements that obtained from the T_1 's measured without paramagnetic additives. The carbons closest to the hydrogen-bonded OH—C₁, C₂, and C₃—have particularly short T_1 's, $T_1^e \lesssim 0.3$ sec.¹⁷ For remote carbons C₄ through C₉, T_1^e exceeds ca. 0.4 sec. Within that group it is possible to distinguish the two seven-substituted methyl carbons since C₃ is expected to be closer to the iron nucleus. The present experiment confirms the previously tentative assignment. T_1^e for the remaining C₁₀ CH₃ carbon, 0.32 sec, easily distinguishes this carbon from the other, more remote CH₃ groups.

Another early ¹³C lanthanide shift paper was published by Gansow and coworkers,^{18a} reporting assignments for isborneol (the exo isomer of borneol). They reversed previous resonance assignments^{18b,c} for C₁ and C₇ based on an expected larger Eu(DPM)₃-induced

(13) Probably dipole–dipole interactions, but scalar relaxation may also contribute.

(14) For less strongly bound ligands Cr(III) is generally preferred since ligand exchange for chromium is less rapid than for Fe(III). In the case of the acetylacetonate chelate the Fe(III) compound is highly stable to typical organic media and substrates.

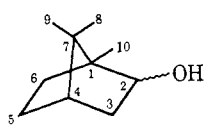
(15) ¹³C references: G. Kotowycz and K. Hayamizu, *Biochemistry*, **12**, 517 (1973); Y. K. Levine, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *ibid.*, **11**, 1416 (1972); R. E. Richards, 14th Experimental NMR Conference, Boulder, Colo., April 15–18, 1973.

(16) J. Briggs, F. A. Hart, G. P. Moss, and E. W. Randall, *J. Chem. Soc., Chem. Commun.*, 364 (1971).

(17) Line broadening is not evident in ¹³C spectra run with Cr or Fe(acac)₃ when $T_1 > 0.5$ sec ($T_1 \approx T_2$). Significant line broadening is observed when T_1 is as short as 50–100 msec.

(18) (a) O. A. Gansow, M. R. Willcott, and R. E. Lenkinski, *J. Amer. Chem. Soc.*, **93**, 4295 (1971); (b) H. Schneider and W. Bremser, *Tetrahedron Lett.*, 5197 (1970); (c) E. Lippmaa, T. Pehk, J. Passivirta, N. Belikova, and A. Plate, *Org. Magn. Resonance*, **2**, 581 (1970).

Table I. Carbon-13 Chemical Shift and Spin–Lattice Relaxation Data



borneol, OH endo; isborneol, OH exo

Carbon	δ , ¹³ C ^a	T_1 , sec ^a		T_1^e , sec ^d
		Soln I ^b	Soln II ^c	
A. Borneol (34% in Benzene- <i>d</i> ₆)				
1	49.7	40 ^e	0.30	0.30
2	77.0	4.7	0.16	0.17
3	39.2	3.3	0.26	0.28
4	45.6	5.4	0.39	0.42
5	28.6	2.7	0.40	0.47
6	26.3	2.0	0.34	0.41
7	48.0	40 ^e	0.44	0.44
8	18.8	5.6	0.39	0.42
9	20.3	4.8	0.45	0.50
10	13.5	3.3	0.32	0.35
B. Isborneol (~45% in Benzene- <i>d</i> ₆)				
1	49.1	~40 ^e	0.21	0.211
2	79.6	4.3	0.13	0.134
3	40.9	2.9	0.20	0.215
4	45.5	4.9	0.25	0.263
5	27.6	1.75	0.24	0.278
6	34.3	2.9	0.24	0.262
7	46.5	~40 ^e	0.28	0.282
8	20.4	5.4	0.255	0.268
9	20.7	5.4	0.285	0.301
10	11.5	3.42	0.176	0.186
C. 1-Octyl Nitrate (Neat)				
1	73.8	2.3	0.18	0.20
2	27.8	2.8	0.24	0.26
3	26.3	2.7	0.24	0.26
4/5 ^f	29.8	3.1	0.39	0.45
6	32.4	3.4	0.42	0.48
7	23.2	4.4	0.46	0.51
8	14.3	5.1	0.47	0.52

^a Chemical shifts downfield from internal TMS. T_1 's: 25.2 MHz and 38°, probable accuracies ± 5 –10% (the relative accuracies of the T_1 's are considerably better). ^b No paramagnetic materials added. ^c Fe(acac)₃ $4.86 \times 10^{-2} M$ (borneol), $6.0 \times 10^{-2} M$ (isborneol), and $4.2 \times 10^{-2} M$ (1-octyl nitrate). ^d $[T_1^e]^{-1} = (T_1(\text{II})^{-1} - [T_1(\text{I})]^{-1})$. ^e Estimated. ^f Unresolved.

shift at C₁. ¹³C T_1^e 's (see Table IB) for isborneol containing $6.0 \times 10^{-2} M$ Fe(acac)₃ are consistent with the earlier assignments^{18b,c} and in disagreement with the values obtained using Eu(DPM)₃. Gansow assigned the lower field line of the pair to C₇. We observed a shorter T_1^e for this signal, 0.21 sec vs. 0.28 sec for the high-field line. The location of the iron nucleus nearer to C₁ was confirmed by an even shorter T_1^e for C₁₀, 0.18 sec, and longer T_1^e 's for C₈ and C₉ (0.25 and 0.28 sec, respectively). Presumably, the Eu(DPM)₃-induced shift at the C₁ carbon that Gansow observed was smaller than that for C₇ because the C₁ shift resulted from addition of large and opposite contact and pseudo-contact shifts.^{2,8,9}

Since the electron–nuclear dipole–dipole relaxation rate exhibits no significant angular dependence, it should be feasible to determine the exact position of the paramagnetic metal nucleus in a given relaxation reagent–substrate complex from the experimentally determined T_1^e 's for carbons in rigid substrate molecules. Unfortunately, the quantitative situation is complex since there is always a large excess of substrate in typical ¹³C

nmr sample solutions. Moreover, the equilibrium constants and numbers of complexed substrate molecules per molecule of paramagnetic reagent are unknown. The observed T_1 's will reflect the time-averaged and species-averaged position of the metal atom. Recently, it has been shown¹⁹ that solution structure determinations using shift reagent data may be complemented by employing an additional lanthanide "relaxation" reagent. In this procedure the relative proton line widths induced by the reagent are fit to the relative distances to the paramagnetic center, assuming an r^{-6} dependence.²⁰ An analogous procedure can be employed using the ^{13}C T_1 's induced by the Cr and Fe acetylacetonates.

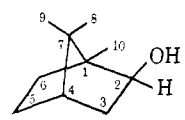
Proceeding directly to determine the location of the paramagnetic center using an r^{-6} dependence of T_1 's, it is found, taking isoborneol (Table IB) as an example, that the iron atom is predicted to be on the order of 20 Å away from the OH-containing carbon, a physically unreasonable result. This calculated distance arises from the fact that the various carbons of a given substrate molecule will experience considerable relaxation from reagent molecules that are not "complexed," resulting in a leveling of T_1 values across the molecule. Calculations must account for such "outer sphere" effects. Assuming fast exchange between "free" and "bound" states, one can write

$$\frac{1}{T_1^e} = \frac{X_f}{T_1^e(f)} + \frac{X_c}{T_1^e(c)} \quad (2)$$

where $1/T_1^e$ is the total relaxation rate at a given carbon due to the electron-nuclear dipolar interaction, $1/T_1^e(f)$ and $1/T_1^e(c)$ are the rates when the substrate is in the "free" and "complexed" forms, respectively, and X_f and X_c are the respective mole fractions. In general, X_f and X_c are not known (a fact which is of no consequence here since only relative rates are required). $X_f/T_1^e(f)$ is the total contribution to the relaxation rate for a given carbon from "outer sphere" or "translational" effects. It will be the same for all carbons in the molecule⁷ [$T_1^e(f) \equiv T_1^e$ of a carbon in a molecule that is completely noninteracting (e.g., cyclohexane), in the same solution, etc.]. Application of the above equation to data previously obtained⁷ for a 33% phenol, 0.035 M Cr(acac)₃ solution in a mixture of benzene, anisole, and toluene (all assumed to be noninteracting) yields a distance of approximately 5.8 Å from the paramagnetic center to C₁. The relative distances to the metal atom were determined using Dreiding models, and the time-averaged location of the Cr was assumed to be coplanar with the benzene ring. A comparison of the relative relaxation rates with the calculated relative distances to the Cr atom assuming a Cr-C₁ distance of 5.8 Å is given in Table IIB. Considering the size and nature of the molecules involved, this distance is physically reasonable for a hydrogen-bonded complex.

A crude analysis for isoborneol similar to the above (Table IIA) (using a $T_1^e(f)$ of 0.37 sec, estimated on the basis of earlier data^{7,21}) reveals that the time-averaged

Table II. A Comparison of Carbon-Metal Distances with "Complexed" Electron-Nuclear Relaxation Rates



A. Isoborneol ^a		
Carbon	$[T_1^e(c)]^{-1}$ ratio (C ₂ /C _X)	$(r_{\text{Fe-X}}/r_{\text{Fe-C}_2})^6$
1	2.9	3.7
3	3.6	2.5
4	7.5	9.1
5	11.4	11.4
6	7.5	7.5
7	11.4	9.8
8	9.0	8.3
9	45 ^c	26 ^c
10	2.2	2.4

B. Phenol ^b		
Carbon	$[T_1^e(c)]^{-1}$ ratio (C ₁ /C _X)	$(r_{\text{Cr-X}}/r_{\text{Cr-C}_1})^6$
2 (ortho)	10.6	9.8
3 (meta)	1.7	2.0
4 (para)	5.5	6.2

^a From T_1^e data in Table IB, using a value of 0.37 sec for $T_1^e(f)$ and assuming an Fe-C₂ distance of 5.2 Å. All rates are calculated relative to C₂. See text for further details. ^b From the T_1^e data in ref 7 for a 33% phenol, 0.035 M Cr(acac)₃ solution in a benzene, anisole, toluene mixture. $T_1^e(f)$ was 1.15 sec, and a Cr-C₁ distance of 5.8 Å was assumed. All rates are calculated relative to C₁. See text for further details. ^c Since $[T_1^e(c)]^{-1}$ is a small difference between two large numbers, this value is subject to considerable error.

position of the iron atom in the isoborneol-Fe(acac)₃ complex is about 5.2 Å distant from the OH-containing carbon (C₂), about 3 Å below the plane containing carbons 2, 3, 5, and 6.

Of course, there is not necessarily a single point that will exactly define the time-averaged locus of the iron nucleus. That will only be true if the motions around the iron-hydroxyl group axis are symmetrical. The fit obtained from the present data does indicate that the single-point locus is not a bad approximation.

In many cases it is not necessary to have an acidic group such as OH to perform these spin-labeling experiments. Electrostatic or dipolar interactions can result in preferential orientation of the polar ends of molecules toward the metal chelates. Thus, Fe(acac)₃ "complexes" with the polar end of 1-octyl nitrate.²² ^{13}C T_1 's for this compound are summarized in Table I. Quantitative calculations are impossible for this non-rigid molecule. However, the average distances between the first seven carbons (unresolved carbons 4 and 5 are treated as a unit) and the metal chelate are differentiated by the T_1^e data for the compound (Table I). It should be noted that the variation in T_1 's for the undoped 1-octyl nitrate sample results from internal or segmental motion¹² along the chain. In this particular case the undoped T_1 's can thus give the same information derivable from the electron-nuclear T_1 's.

Other examples can be observed indicating preferential orientation of substrates relative to Fe or Cr(acac)₃. In alkyl benzenes and benzonitrile, for example, the ring substituents orient away from the metal chelate (the phenyl rings themselves orient toward the chelate).

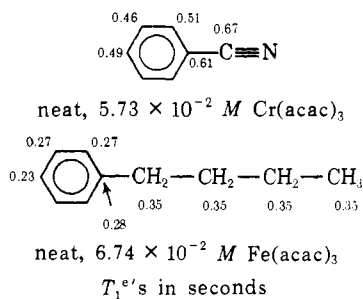
(22) G. C. Levy and W. D. Clossen, unpublished results.

(19) G. N. LaMar and J. W. Faller, *J. Amer. Chem. Soc.*, **95**, 3817 (1973).

(20) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).

(21) T_1^e is generally ca. 20% longer than the observed T_1^e for remote carbons in a complexed molecule such as phenol or isoborneol. In a very large complexed molecule $T_1^e(f)$ would be approximately equal to the observed T_1^e for carbons remote to the site of complexation.

This can be seen from the electron–nuclear T_1 's for two benzonitrile and *n*-butylbenzene solutions.



It is not known what kind of interaction exists between these compounds and the metal chelates. It is certain that the interactions are very weak; they may be dipolar or electrostatic in origin. No differences have thus far been observed between the complexing ability of Cr(acac)₃ and Fe(acac)₃. C₁–C₄ T_1 's ratios for phenol containing either compound are equal. On the other hand, unrelated paramagnetic materials exhibit differing selectivities. Preliminary results with organic free radicals such as galvinoxyl indicate little interaction with molecules such as phenol.

Conclusions

Spin–lattice relaxation data obtained from solutions containing nonshifting paramagnetic relaxation reagents can be used for nmr spectral analysis, complementing or replacing ¹³C or other nuclei nmr data obtained with lanthanide chemical shift reagents. Table III lists some *pros* and *cons* for the two methods.

Nonshifting paramagnetic relaxation reagents clearly do not broadly obviate the need for LIS reagents. Nevertheless, they do constitute another powerful weapon in the chemists' arsenal for attacking organic structure problems.

Experimental Section

Spin–Lattice Relaxation Measurements. ¹³C spin–lattice relaxation times (T_1 's) were obtained at 38° and 25.2 MHz from inversion–recovery Fourier transform nmr experiments, as modified by Freeman and Hill.²³ The pulse sequence utilized was

$$(T - 90^\circ_\infty - T - 180^\circ - t - 90^\circ_i)_x$$

(23) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **54**, 3367 (1971).

Table III. Use of Nonshifting Paramagnetic Relaxation Reagents as Alternatives to Lanthanide Shift Reagents

Pros	Cons
1. No necessity for locating shifted resonance lines	1. Spin–lattice relaxation measurements require significant spectrometer instrumentation and experimental time
2. T_1 's(c) has no angular dependence, only inverse sixth power dependence on distance between the nucleus and the electron source	2. Observed T_1 's must be separated into diamagnetic T_1 's ^a and T_1 's(f) and T_1 's(c) (see text)
3. No ambiguity due to two competing and opposite effects (as with pseudo-contact and contact shifts) ^b	3. In very large molecules electron–nuclear relaxation may not be able to compete with efficient ¹ H dipolar relaxation

^a Not necessary when $T_1^e \ll T_1$ (diamagn). ^b In cases studied to date.

where t is experimentally varied and T is set greater than four times the longest T_1 to be measured.

In the Freeman–Hill modified program, the log of ($S_\infty - S_t$) is plotted against t , where S_∞ and S_t are the transformed signals from the 90°_∞ and 90°_{*t*} pulses, thus yielding only positive peaks in the spectral display. T_1 is the time, t , at which ($S_\infty - S_t$) reaches 0.368 ($\cong 1/e$) of $S_\infty - S_t$ for $t = 0$ ($\cong 2S_\infty$). Because the effective ¹³C radiofrequency field of the XL-100-15 is under 2 kHz (90° pulse $\cong 125 \mu\text{sec}$), spin–lattice relaxation experiments were restricted to spectral widths $\lesssim 1500$ Hz. When larger widths were required, separate experiments were run on each spectral region. For each determination eight to ten sets of measurements were taken. Reproducibility of T_1 values was considerably better than $\pm 10\%$ in most cases. Accuracy limitations depend in each instance on solute concentration and the length of data acquisition. In these studies very high signal–noise ratios were achieved (>100).

¹³C Chemical Shifts. All chemical shifts are reported to 0.1-ppm downfield from internal TMS. For borneol and octyl nitrate 8K transforms were computed giving 4K data points in the phase-corrected spectra (at 25.2 MHz). The spectrum of isoborneol was obtained at 67.9 MHz (Brüker HX-270) using a 32K transform.

Samples. Commercially available materials were used without additional purification. Nmr samples were not degassed; relaxation contributions from dissolved O₂ at 0.2 atm are insignificant when $T_1 < 5$ –10 sec.

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