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Electronic Structures of Purine Bases Studied by Electrochemical-ESR Techniques. Spin Distribution in Purine Anion Radical

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We have investigated the ESR spectrum of anion radical of purine generated electrochemically using a helical electrode cell for low-temperature ESR measurements. The assignment of the hfcc was made with the aid of partial deuteration and MO calculations. The most notable observation is that about 45% of the spin density is localized at position 6. This correlates well with the preferential hydrogenation at this position. The McLachlan method reproduces the observed spin distribution well with $\beta_{N9C}/\beta_{CC}=1.0$, which indicates that the bonding between the nitrogen at 9 and carbons at 4 and 8 have large double bond characters. The INDO-MO calculations based on the two molecular geometries for 9*H*-purine also agree well with the experimental data. Finally the effect of tautomerism of purine on the spectrum is discussed.

Since Pullman-Pullman's first theoretical work[1)] a wide variety of experimental and theoretical investigations[2)] have been made on biologically important purine bases in order to elucidate their electronic strucutres. For example, the lowest excited singlet state has been identified as a $n\pi^*$ state from the electronic absorption spectrum of purine(I).[3)] Nuclear magnetic resonance (NMR) spectroscopy of H, ^{13}C and ^{15}N in purines[4,5)] has given useful information regarding their tautomeric behavior of the labile protons. Theoretical calculations using different methods have been made on excited state energies,[3,6)] electron distribution,[2,7,8)] dipole moments,[7,8)] and zero field splittings in triplet states.[9,10)]

In order to evaluate the results of various theoretical calculations it is important to have experimental data on the details of electronic wave functions, but such data have not been available on purines so far. Electron spin resonance (ESR) spectroscopy in solution is capable of providing the most direct information on the wave functions of π electrons through hyperfine studies, but their radical species in solution have not been detected before, presumably because of their instabilities. In 1974 Yao and Musha investigated the electrochemical reduction of I in nonaqueous solvents and reported an ESR spectrum which they ascribed to the purine anion radical($I^{\bar{\cdot}}$),[11)] but this is questionable because of its unusually large hyperfine coupling constants.

One of the present authors (H. O.) recently developed a two-electrode electrolysis cell using a helix for low-temperature ESR measurements which has several advantages over the commonly used electrolysis cell for ESR in obtaining the spectra of unstable anion radicals.[12)] Using this cell we have succeeded in obtaining an ESR spectrum which is assigned to $I^{\bar{\cdot}}$ with reasonable certainty. In this paper we first report the ESR spectra of the anion radicals of I, 8-deuteriopurine(I-*d*) and 6,8-dideuteriopurine(I-d_2) produced electrochemically at low temperatures and analyze them using the computer simulations. Secondly the hyperfine coupling constants (hfcc) obtained are compared with those calculated by McLachlan's method and INDO-MO method established by Pople and Beveridge.[13)] Both calculations are carried out based on the two tautomeric forms, 7*H*- and 9*H*-purines. Thirdly we discuss the effect of tautomerism of I in relation to the NMR results.[4)]

Experimental

Purine(Aldrich) was normally used without further purifications. Identical results were obtained by using purine purified by vacuum sublimation. I-*d* and I-d_2 were synthesized by heating purine in refluxing D_2O for 4 h and 72 h, respectively, according to the method by Schweizer *et al.*[14)] From the proton NMR spectra it was shown that I-*d* was produced completely, but I-d_2 sample was found to contain I-*d* by 35%. Acetonitrile(AN) and butyronitrile(BN) were purified according to the method of Van Duyne and Reilly.[15)] After being dried with molecular sieve 4A, *N,N*-dimethylformamide(DMF) was distilled over fresh molecular sieve 4A. Just before the preparation of the sample solution DMF was shaken with Na_2SO_4 to ensure complete dryness of the solvent and was transferred to the electrolysis cell with a pipet in an atmosphere of dry nitrogen gas. This process was effective of prolong the lifetime of the purine anion radical. As the supporting electrolyte tetrabutylammonium perchlorate(TBAP) or tetrabutylammonium tetrafluoroborate(TBFB) (Nakarai Chemical Ltd., SP grade for polarography) was used without further purifications. Electrochemical reduction of I, I-*d* and I-d_2 were carried out inside the cavity using the cell consisting of a helix and a gold wire going through inside the helix. The details of the cell construction were given previously,[12)] ESR spectra were taken with a JEOL PE-3 spectrometer equipped with a temperature controller.

Results and Discussion

ESR Spectra. After the temperature of the solution was decreased as low as −50 °C the voltage applied between the two electrodes was increased until the ESR signal could be observed on an oscilloscope or a recorder with the modulation amplitude of 1.0×10^{-4} T. As the voltage reached −6.0 V an ESR signal with hyperfine structures appeared. In view of the results of a electrochemical study of purine by Yao and Musha[11)] the observed ESR spectrum is

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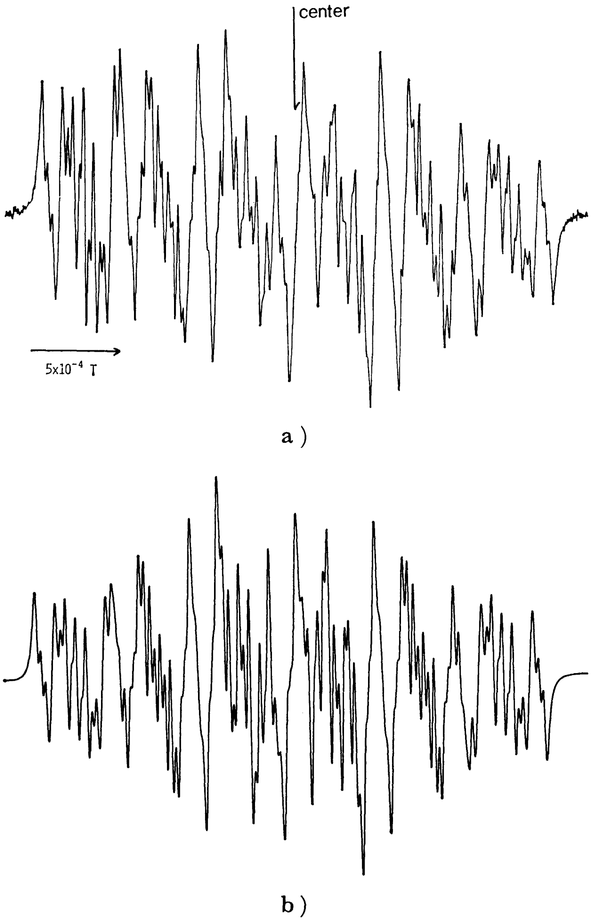
most likely due to $\mathrm{I}^{\cdot-}$. This appearance voltage(V_a) is appreciably lower than twice the half-wave reduction potential ($E^r_{1/2}$) of I measured by Yao and Musha (−2.30 V). However, the previous work[12] has shown that V_a is considerably lower than twice $E^r_{1/2}$, when the produced anion radical is unstable. Hence $\mathrm{I}^{\cdot-}$ is likely to be highly reactive. For the observation of high resolution spectra a voltage of −7.0 V or lower was applied during the ESR measurements. ESR measurements were made with low modulation amplitude of the order of 0.1×10^{-4} T. The spectrum intensity reached its maximum in several minutes and disappeared at most in 20 minutes.

A typical example of the spectrum taken with the modulation amplitude of 10^{-5} T is shown in Fig. 1a. It can be satisfactorily interpreted in terms of the three doublet splittings of 10.46, 4.80 and 1.75×10^{-4} T due to protons, and the three triplet splittings of 4.47, 1.14 and 0.28×10^{-4} T due to nitrogen nuclei. This interpretation of the spectrum was confirmed by the computer simulation made by using these splittings and the linewidths of 0.45×10^{-4} T (Fig. 1b). It should be noticed that the observed spectrum consists of the hyperfine splittings due to only one species.



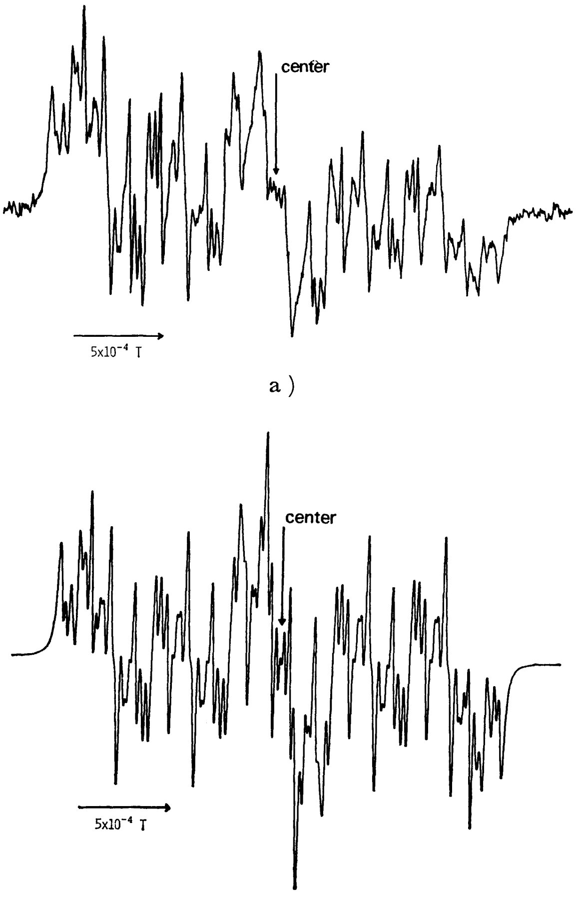
a)

b)

Fig. 1. a) ESR spectrum of $\mathrm{I}^{\cdot-}$ generated electrochemically in DMF with 0.1 M TBAP at −50 °C, the applied voltage between two electrodes being −7.0 V. b) The simulation spectrum based on the data in Table 1. Total width: 28.79×10^{-4} T, line width: 0.450×10^{-4} T.

Recent evidence based on ^{13}C NMR indicates that I in dimethyl sulfoxide (DMSO) is in an equilibrium between 7*H*- and 9*H*-purine at room temperature, the ratio of the former to the latter being 4:6.[4] We have not detected so far any evidence for the existence of such tautomerism in our system. The experimental conditions, such as temperature, solvent used, supporting reagent, purification steps of I, have been altered, but the same spectrum was observed. The *g*-value of the spectrum was 2.0028, being close to that of free electron.

In order to ascertain the identification of the anion radical and to assign the proton hfcc, we have also taken the ESR spectra of I-$d^{\cdot-}$ and I-$d_2^{\cdot-}$ in DMF at −50 °C. Their spectra were resolved well enough to allow the analysis of hfcc (Fig. 2 and 3), though the spectrum of I-d_2 was superposed with the spectrum due to 35% of I-*d*. In table 1 the observed hfcc are summarized with those calculated by Hückel molecular orbital (HMO) modified with McLachlan's method.[16] From the comparison of the coupling constants of $\mathrm{I}^{\cdot-}$ with those of I-$d^{\cdot-}$ and I-$d_2^{\cdot-}$ two coupling constants, 10.46 and 4.80G can be assigned unequivocally to positions 6 and 8, respectively. The others cannot be assigned experimentally but are tentatively assigned to the particular positions in the molecule



a)

b)

Fig. 2. a) ESR spectrum of I-$d^{\cdot-}$ generated with the same conditions as in Fig. 1a. b) The simulation spectrum based on the data in Table 1. Total width: 25.47×10^{-4} T, line width: 0.400×10^{-4} T.

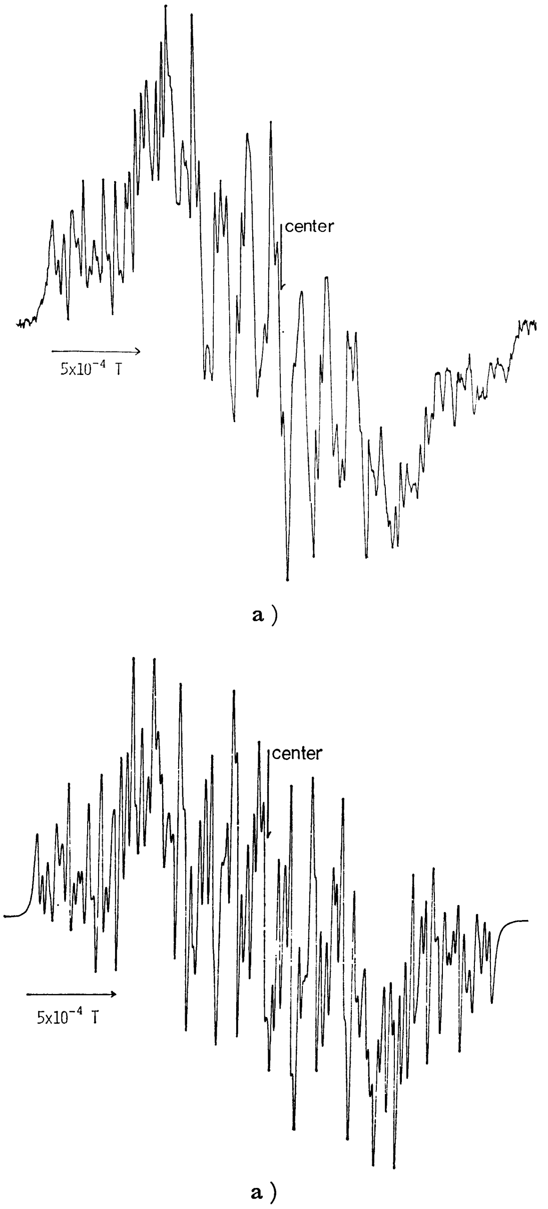
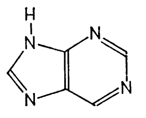


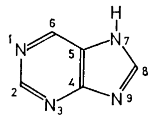
Fig. 3. a) ESR spectrum of I-$d_2^{-\cdot}$ including 35% I-$d^{-\cdot}$ generated with the same conditions as in Fig. 1a. b) The simulation spectrum based on the data in Table 1. Total width: 18.23×10^{-4} T for I-$d_2^{-\cdot}$ and 25.47×10^{-4} T for I-$d^{-\cdot}$, The ratio of their intensities: 65:35, line width: 0.400×10^{-4} T.

from the comparison of the experimental data with those obtained by the MO calculations (Table 1).

Comparison with Molecular Orbital Calculations.

The molecule of I is usually represented by the form in which the labile proton is attached to the N(9) nitrogen: 9*H*-purine. However, the chemical shift values in ^{13}C NMR spectra of I and its methyl derivatives in DMSO indicates that I in DMSO is a tautomeric mixture of 60% 9*H*-purine and 40% 7*H*-purine. It is necessary, therefore, to take these tautomers into account when we compare the experimental data with the calculated ones. We have calculated spin densities of these tautomers by McLachlan's methods, using the parameters derived empirically in pyridines and diazines by Talcot and Myers[17], except for the nitrogen to which the labile hydrogen atom is attached. The Coulomb integral of the nitrogen (α_{N}) in the NH fragment was estimated as $\alpha_{\mathrm{N}}=\alpha_{\mathrm{C}}+1.2\,\beta_{\mathrm{CC}}$, based on the analysis of hfcc of radical cations of the dihydro derivatives of some diaza-aromatic compounds[18]. The resonance integrals between the nitrogen atom in this fragment and the adjacent carbon atoms (β_{NC}) were adjusted so as to fit to the experimental data. Spin densities of the 9*H*-purine anion radical were calculated by changing $\delta=\beta_{\mathrm{NC}}/\beta_{\mathrm{CC}}$ and the hfcc were calculated using $Q^{\mathrm{N}}_{\mathrm{N}}=27.3$, $Q_{\mathrm{NC}}=-1.7$ and $Q_{\mathrm{H}}=-42.5$G.[17] The obtained hfcc are given in Fig. 4 as functions of δ. Here $\delta=0.5$ corresponds to a model in which the NH fragment can be regarded as a secondary amine group. Then I is adequately described as 4,5-disubstituted pyrimidine with a strongly mesomeric substituent at position 5. This model is characterized by the largest spin density (48%) at position 8, followed by the next largest (26%) at position 6. As δ increases, spin densities at positions 6 and 3 increase appreciably accompanied by the decreases at positions 8 and 2. The best fit to the experimental results, shown by the dotted lines in Fig. 4, is obtained at $\delta=1.0$, which means that the


7*H*-purine



9*H*-purine

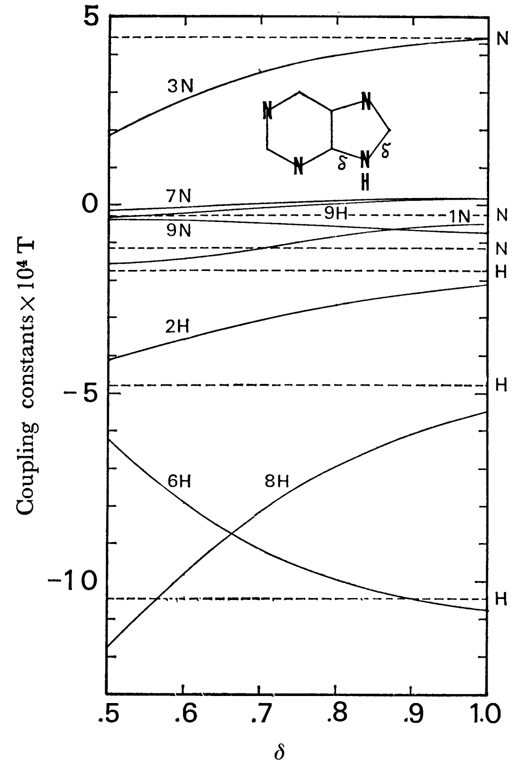


Fig. 4. Comparison of the hfcc of I$^{-\cdot}$ obtained experimentally (dotted lines) with those as functions of δ calculated by the McLachlan's method (see text).

TABLE 1. MEASURED HYPERFINE COUPLING CONSTANTS[a)] OF PURINE, 8-DEUTERIO- AND 6,8-DIDEUTERIOPURINE ANION RADICALS AT −50 °C, AND CALCULATED COUPLING CONSTANTS[a)] AND SPIN DENSITIES OF 7*H*- AND 9*H*-PURINE ANION RADICALS

Position	Purine		Purine-*d* in DMF	Purine-d_2 in DMF	Calculated hfcc's[c)] (McLachlan)	
	in AN+BN	in DMF			9*H*-Purine	7*H*-Purine
N1	0.28	0.28	0.28	0.28	−0.51 (0.014)	0.38 (0.043)
H2	1.79	1.75	1.75	1.75	−2.10 (0.086)	−1.07 (0.044)
N3	4.40	4.47	4.47	4.47	4.46 (0.172)	4.37 (0.170)
H6	10.75	10.46	10.46	D1.67	−10.76 (0.439)	−10.23 (0.418)
N7	<0.2[b)]	<0.2[b)]	<0.2[b)]	<0.2[b)]	0.16 (0.019)	0.26 (0.025)
H8	4.85	4.80	D0.74	D0.74	−4.55 (0.224)	−4.95 (0.243)
N9	1.18	1.14	1.14	1.14	−0.73 (−0.009)	−1.17 (−0.020)
Labile H	<0.2[b)]	<0.2[b)]	<0.2[b)]	<0.2[b)]	0.22	0.61

a) In unit of 10^{-4} T. b) Estimated from the line widths. c) Calculated by using the formula $a_H = Q_H\rho_C$, $a_N = Q_N^N\rho_N + Q_{NC}(\rho_C + \rho_{C'})$ and the parameters given in Ref. 17.

TABLE 2. HYPERFINE COUPLING CONSTANTS[a)] OF PURINE ANION RADICAL CALCULATED BY INDO METHOD BASED ON POSSIBLE RADICAL STRUCTURE[b)]

Position	7*H*-Purine		9*H*-Purine		Observed
	Watson	Inverted Spencer	Spencer	Inverted Watson	
N1	3.4999	3.0597	−0.0273	−0.3282	0.283
H2	0.2364	0.1089	−2.3398	−2.6486	1.750
N3	4.9997	4.9212	6.0115	6.2208	4.470
H6	−6.7342	−5.9061	−8.1522	−8.4456	10.46
N7	0.3073	0.4333	−0.1324	0.0677	<0.2
H8	−6.3173	−7.9999	−4.2455	−2.7192	4.80
N9	−2.4868	−2.4870	−1.6809	−1.5955	1.14
Labile H	−0.8193	−1.1284	0.7608	0.7699	<0.2
Total width	35.88	36.95	31.20	31.01	28.80

a) In unit of 10^{-4} T. b) See text.

electronic structure of $I^{\overline{\cdot}}$ should be regarded as one of a 10π-electron heterocyclic aromatics rather than that of a substituted pyrimidine with a strongly mesomeric substituent. In other words, the bonding between the nitrogen at 9 and carbons at 4 and 8 have double bond characters as much as an aromatic –C–C– bonding. This choice of δ is also supported by the result of the X-ray study by Watson *et al.*,[19)] which showed that the bond lengths between N(9) and C(4), and N(9) and C(8) are 1.37 and 1.31 A, respectively, indicating double bond characters of these bonds. The hfcc of 7*H*-purine anion radical was also calculated using the same parameters as in the case of 9*H*-purine, which are also shown in Table 1. It should be noticed in Table 1 that the calculated coupling constants of two tautomers are quite similar to each other and are in good agreement with the experimental results except for that of the labile proton.

In order to further compare the experimentally determined hfcc with those calculated by MO methods we have also made INDO calculations. As is well known, the results of the calculations carried out by this method depends on the geometries adopted. Here we used the same geometries as those adopted by Pullman *et al.*;[7)] Watson and inverted Spencer geometries for 7*H*-purine, and inverted Watson and Spencer geometries for 9*H*-purine. The Watson geometry was derived from the crystallographic data of purine which has 7*H*-form in crystal.[19)] The Spencer geometry means the structure established by Spencer on the basis of a general study of purine derivatives which are in 9*H* forms.[20)] The inverted Watson and the inverted Spencer geometries were obtained by inverting the lengths of the 7–8 and 8–9 bonds, those of the 5–7 and 4–9 bonds, and the corrsesponding angles, respectively. The hfcc of $I^{\overline{\cdot}}$ calculated with these geometries are listed in Table 2.

It is seen from the table that the calculated hfcc for 9*H*-purine are rather different from those for 7*H*-purine and are in better agreement with the experimental hfcc. The best agreement between the experimental and the calculated hfcc seems to be obtained in the case of Spencer geometry of 9*H*-purine. The inverted Watson model can predict a large hfcc for H6, but the agreement with the experimental results is somewhat poorer. The total width of the ESR spectrum calculated for 9*H*-purine also agrees reasonably well with the observed total width. On the other hand, the hfcc calculated for 7*H*-purine do not reproduce the observed features of hfcc. The total width of the spectra calculated are much larger than the observed one. Thus the results of the INDO calculations seem to be in favor of the 9*H*-form for I^{-}.

The most remarkable feature of the spin distribution of the purine anion is that about 45% of the spin density is localized at position 6. Such a large localized spin density is very rare in a π electron system of the size of I and should have an important implication to its chemical reactivity. It is known that the reaction such as hydrogenation takes place preferentially at position 6.[11] This was rationalized from the result of MO calculation.[21] According to Fukui's frontier electron theory[22] such a reaction should take place at the position where the frontier electron density is the largest. Our result clearly shows a large electron density in the frontier orbital (LUMO) at position 6.

It is interesting to compare the unpaired electron spin distribution in LUMO of I with that of indole. Although its electron spin distribution is not known experimentally, the result of a semiempirical calculation[23] may be used for comparison. The spin densities at positions 2, 3, 6, and 8 of indole, the position numbers of which correspond to those of I, are 0.136, 0.220, 0.212 and 0.155, respectively. From the comparison with the data of I it may be deduced that aza-substitution to the particular ring carbons enhances the localization of the spin density to position 6.

Tautomerism of Purine. Sometimes in the ESR spectra the dynamic behaviors of radical species can be revealed from line-width alternation, or line-width variation which can be related to the lifetimes of the radical species under consideration. If we take into account the difference in the calculated coupling constants between two stable tautomers, two possibilities can be considered from the observed spectrum: one is the case that the anion radical formation stabilizes one of the two tautomers (7*H*- or 9*H*-purine) at the temperatures around -50 °C, and the other is that the exchange of the labile proton between the two tautomers is very rapid compared with the ESR time scale, 10^6 Hz s^{-1}. It is difficult at present, to say definitely which is appropriate for the interpretation of the observed spectrum, but the former case is more likely when we take into account the ^{15}N NMR result that only one very broad signal for N7 and N9 could be observed because of slow hydrogen exchange of the order of 10^2 Hz sec^{-1}.

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