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Nitroxide Radical Induced Nuclear Magnetic Resonance Contact Shift Studies.[1] Potential Utility of Specific Downfield ^1H Contact Shifts Induced by Hydrogen Bonding with Di-*tert*-butyl Nitroxide Radical

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Abstract: Utility of a nitroxide radical as a paramagnetic shift reagent in proton NMR spectroscopy is reported. DTBN (di-*tert*-butyl nitroxide radical) induces *upfield* contact shifts for the X-H proton in proton donor molecules and *downfield* shifts for C-H protons other than the X-H proton. This *downfield* contact shift, which is concerned in this study, is proved to be characteristic of protic molecules and shows conformational dependence. The proton lying on the zigzag path from the hydroxyl or NH group exhibits preferential DTBN induced downfield shift, obeying the "W letter rule". The origin of this contact shift is also discussed in terms of stereospecific electron spin transmission through the intervening bonds from the X-H proton donor group hydrogen bonded with DTBN. Potential utility of this downfield contact shift for structural elucidation around the proton donor group in organic and biologically important molecules is discussed. It is also revealed that the methyl protons in close spacial contact with N-H or O-H proton donor group exhibit substantial DTBN induced *downfield pseudocontact shift*, which is discussed in terms of anisotropy of the *g* value and the mode of hydrogen bond complex.

We wish to report here a novel downfield ^1H NMR contact shift induced by hydrogen bonding with a nitroxide radical, which is quite characteristic of protic molecules and shows geometrical or conformational dependence. This downfield contact shift is shown to provide potential utility as a sensitive tool for structural elucidation around the proton donor group and the mode of hydrogen bonding.

Our recent studies[2,3] on NMR contact shifts have shown that the nitroxide radical di-*tert*-butyl nitroxide, or DTBN, can be used to probe chemical phenomena associated with molecular interactions such as hydrogen bond[2] and charge transfer[3] interactions involving the free radical. In some of our early reports, we described[2a-f] that the X-H···DTBN hydrogen bond induces a strong *upfield* contact shift, characteristic of negative spin density, for the X-H proton, providing fruitful information on the intrinsic nature of this interaction. In the present study we are concerned with the specific DTBN induced *downfield* shift for the C-H proton other than the X-H proton in various proton donor molecules. We have measured here DTBN-induced proton contact shifts for various organic molecules with proton donor groups, such as aliphatic and aromatic alcohols, amines, and carboxylic acids and for some biologically important molecules.

Experimental Section

Materials. DTBN radical was prepared after the method of Briere and Rassat (see ref 2). Other chemicals used in the present study except for deuterated cyclohexanol derivative were commercially available and used without further purification. A deuterated cyclohexanol derivative such as *trans*-4-*tert*-butylcyclohexanol-d_4 was provided by Dr. T. Suzuki. Adamantanol was also supplied by Professor M. Kawanishi.

Proton NMR Measurement. Most of the proton NMR signals in the presence of DTBN radical exhibit substantial broadening. It is therefore preferable to use high-field NMR in order to avoid overlapping of the broadened signals. Proton NMR spectra were obtained with a Varian Associates HR-220 at 220 MHz at room temperature. DTBN was added drop by drop and linear plots of the DTBN-induced shifts vs. the concentration of added DTBN were obtained. Cyclohexane was used as an internal reference, since it was most insensitive to the DTBN-induced shift.

Results and Discussion

A. DTBN-Induced Downfield Shifts. Figure 1a shows the DTBN-induced spectral perturbation for diethylamine in CCl_4 solution. Addition of DTBN radical to the CCl_4 solution of the proton donor molecules caused a large *upfield* shift for the hydroxyl or amine proton, accompanied by strong line broadening, as reported previously.[2] Most of the other C-H protons in aliphatic alcohol or amine, however, experienced substantial *downfield* shifts, attenuating in magnitude along the aliphatic chain. Figure 1b shows the DTBN-induced downfield shifts for isopropyl alcohol, as an example. In Table I are presented the DTBN-induced downfield proton shifts for some proton donor molecules. These downfield shifts are proportional to the concentration of added DTBN radical and characteristic of the proton donating group (such as -OH, -COOH, $-NH_2$ and >N-H), although the X-H proton signals themselves showed remarkable broadening and upfield shift. On the other hand, when these X-H groups are replaced by the ones incapable of hydrogen bonding with DTBN radical (i.e., OCH_3, $COOCH_3$, $COCH_3$, $N(CH_3)_2$, $>NCH_3$, NO_2, etc.) or when a strong proton acceptor such as DMSO (dimethyl sulfoxide) is used as a solvent, the proton signals no longer exhibited the downfield shift, but rather experience a slightly upfield shift, implying that the C-H proton serves as a weak proton donor in the C-H···DTBN hydrogen bond.[2c] For example, the ring protons of pyrrole showed DTBN-induced down-

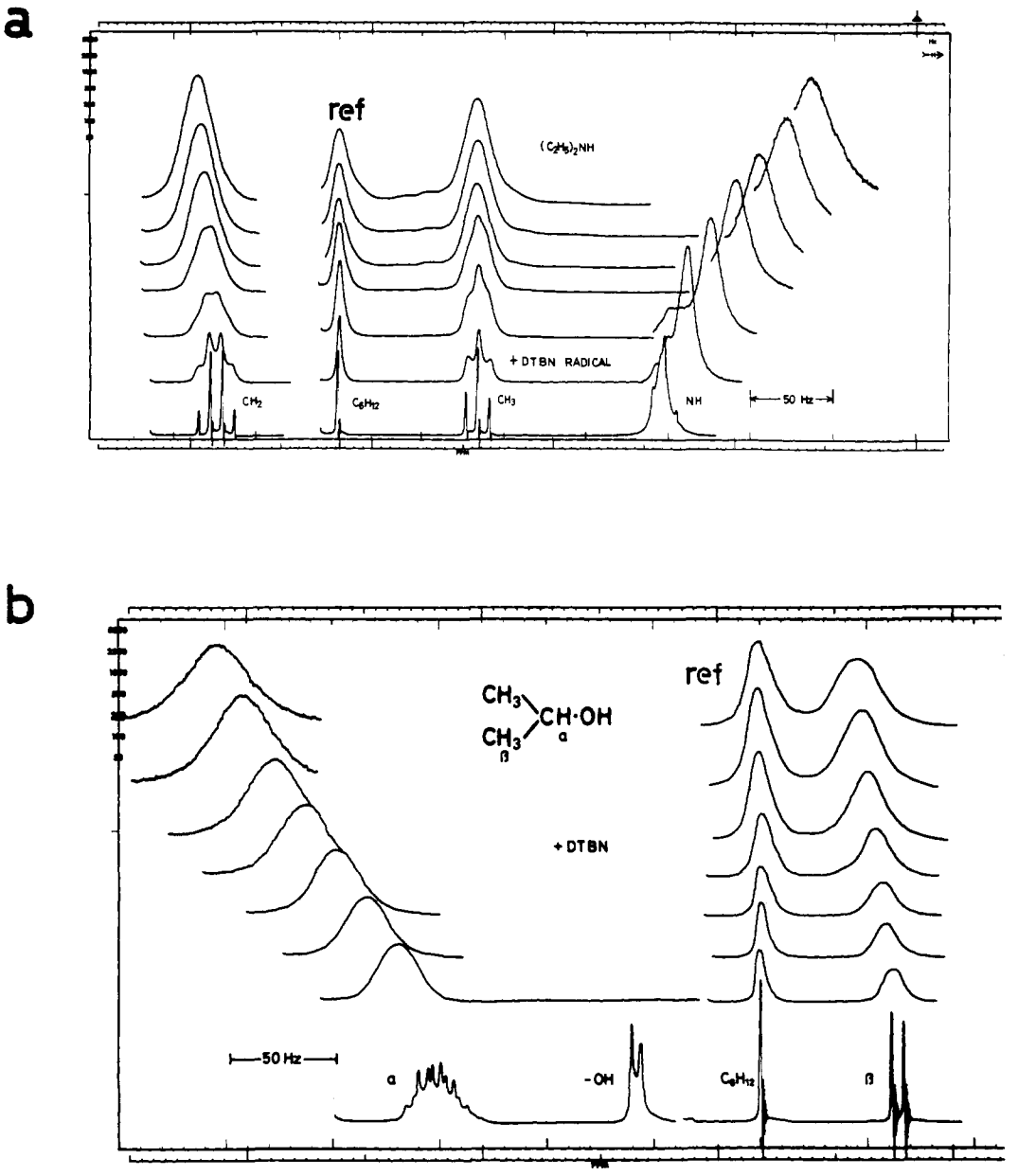


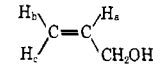
Figure 1. DTBN-induced spectral perturbations (at 220 MHz) for diethylamine (a) and isopropyl alcohol (b) in CCl_4 solutions. Each trace was obtained by the addition of 0.1 *M* DTBN to the solution ([solute] = 0.4 *M*). Cyclohexane was used as an internal reference.

field shifts, while those of methylpyrrole or furan experienced sizable upfield shifts. This has been further confirmed by observing substantial upfield shifts for the acidic C–H protons in the methyl group of nitromethane, in the methylene group of cyclopentadiene or indene (Figure 2), and of the keto form of acetylacetone.

The ring protons of phenol, a stronger acid than aliphatic alcohols, experience more pronounced DTBN-induced *downfield* shifts and broadenings (Figure 3). Signal broadening for the ortho proton of phenol was too large to be followed. For salicylaldehyde and *o*-nitrophenol, however, even the OH proton was hardly affected by the addition of the DTBN radical and the ring protons exhibited an *upfield* shift. The ring protons of *o*-chlorophenol in which a weak intramolecular hydrogen bond is involved showed normal downfield shift (Figure 4). A similar observation was also encountered for phenol in the DMSO solution (Figure 3). Strong intra- and intermolecular hydrogen bonding may prevent DTBN from forming a hydrogen bond with the OH group in phenol in DMSO solution or in salicylaldehyde, leading to upfield shifts of the ring proton due to the direct C–H···DTBN weak hydrogen bond. Since the DTBN-induced broadening and upfield shift demonstrated by the OH proton of *o*-nitrophenol is greater than that found for salicylaldehyde, the implication is that the intramolecular hydrogen bond is weaker in *o*-nitrophenol. This conclusion is based on the reasonable assumption that the O–H··· DTBN hydrogen bond is the same strength in both cases. In connection with this finding, it is also worth noting that all of the proton signals in acetylacetone (enol form) were hardly affected by the addition of the DTBN radical. It can be concluded from above results and discussion that the X–H···DTBN hydrogen bonding is responsible for downfield proton contact shifts for most of the C–H protons of the proton donor molecules.

It is therefore tempting to expect that DTBN-induced broadening of the X–H proton and downfield shifts for other C–H protons could serve as a sensitive probe to differentiate free and intramolecularly hydrogen bonded X–H groups in organic and biological molecules. Successful application of the present method to probe NH···O=C hydrogen bonding in amides, oligo peptides, and cyclic peptides

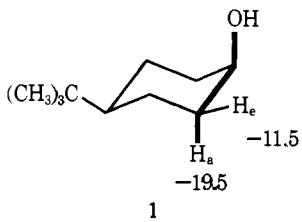
Table I. DTBN-Induced Downfield Shifts for C–H Protons in Some Proton Donor Molecules[a]

Molecules	Proton	DTBN-induced shift, Hz	Molecules	Proton	DTBN-induced shift, Hz
CH_3OH	CH_3	−148.8	CH_3CH_2**CH_2*NH_2	CH_2*	−24.0
CH_3CH_2OH	CH_2	−103.0		CH_2**	−6.0
	CH_3	−15.8		CH_3	−4.0
CH_3CH_2**CH_2*OH	CH_2*	−121.0		NH_2	+89.5
	CH_2**	−23.8	$(CH_3CH_2)_2NH$	CH_2	−12.2
	CH_3	−10.8		CH_3	−1.0
$(CH_3)_2CHOH$	CH	−86.3		NH	+88.0
	CH_3	−14.8	CH_3COOH	CH_3	−48.7
$(CH_3)_2CHCH_2OH$	CH_2	−124.3	CH_3CH_2COOH	CH_2	−8.0
	CH	−18.3		CH_3	−13.2
	CH_3	−7.3	CH_3CH_2**CH_2*COOH	CH_2*	−17.7
$(CH_3)_3COH$	CH_3	−8.0		CH_2**	−16.7
	CH_2	−150.0		CH_3	−5.0
	H_a	−19.0	$ClCH_2$**CH_2*OH	CH_2*	−209.5
	H_b	−6.2		CH_2**	−6.2
	H_c	−2.2	$ClCH_2$***CH_2**CH_2*OH	CH_2*	−169.5
$HC{\equiv}CCH_2OH$	CH_2	−183.0		CH_2**	−38.5
	CH	+73.7		CH_2***	0

[a] DTBN-induced shifts in hertz at 220 MHz (room temperature) at the concentration of [DTBN] = 0.4 *M* in the CCl_4 solution ([solute] = 0.4 *M*). The shifts were proportional to [DTBN] and the figures in this table are given as the representatives of the DTBN-induced shift. The plus sign designates the upfield shift.

and to probe the affinity of intermolecular hydrogen bonding involved in nucleic acid base pairs will be presented elsewhere.[4,5]

B. Stereospecific DTBN-Induced ^1H Contact Shifts. It is particularly worth noting that DTBN-induced downfield proton shifts showed marked conformational or geometrical dependence. The protons lying on a zigzag path from the OH or NH group exhibited preferential downfield shifts as evidenced from the studies of selected cyclohexanol derivatives, such as *cis*-4-*tert*-butylcyclohexanol (**1**), *trans*-4-*tert*-butylcyclohexanol-d_4 (**2**), adamantanol (**3**), and piperidine (**4**). DTBN-induced shifts at appropriate concentration of



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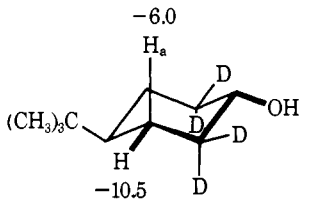

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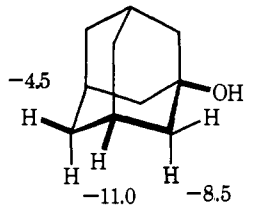

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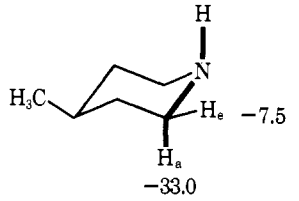

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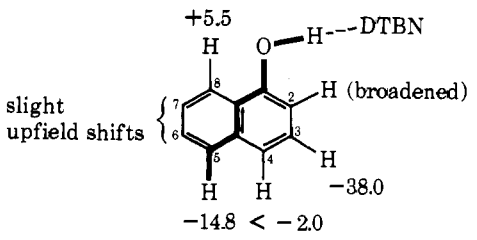
the radical are given below.[6] A trans relationship for a three-bond interaction and a trans-trans relationship for a four-bond interaction show the greatest effects in the examples given. Further evidence for this structural dependence of proton contact shifts was obtained for aromatic proton



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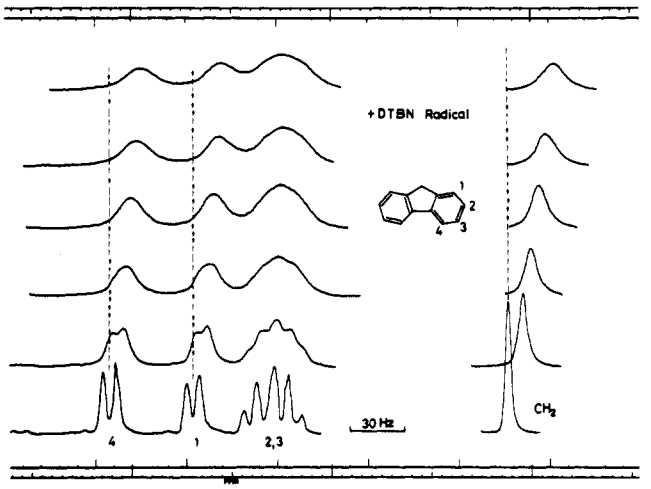
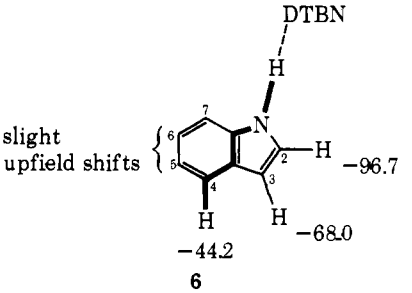


Figure 2. DTBN-induced shifts for indene in CCl_4 solution. Each trace was obtained by the addition of 0.1 *M* DTBN.

donor molecules. The proton H_5 in α-naphthol (**5**)[6] exhibited preferential downfield shift which is to be compared with that for H_4. Contrasting H_3 to H_8 in α-naphthol also makes a straightforward case, although H_8 exhibits an upfield contact shift. The proton H_5 lies on the zigzag route, favorable arrangement for electron spin transmission, from the hydroxyl group. The pronounced downfield shift for H_4 in indole (**6**) (below) and 5-methylindole (see Figure 5)



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may be also due to the favorable zigzag ("W letter") path from the N–H group capable of forming hydrogen bond with DTBN. The preferential downfield shift[6] of H_3, when compared with H_5, in *o*-cresol (**7**) or *o*-*tert*-butylphenol (**8**)

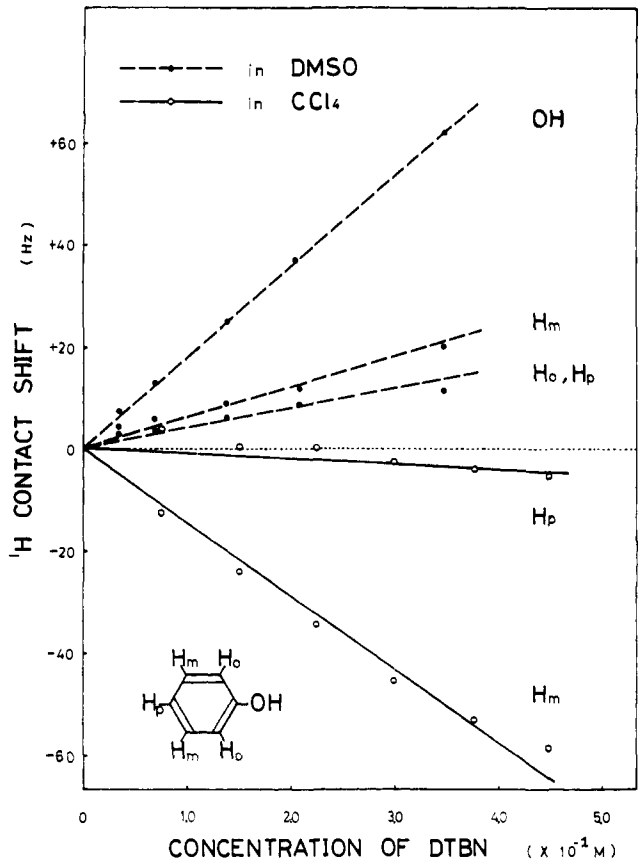
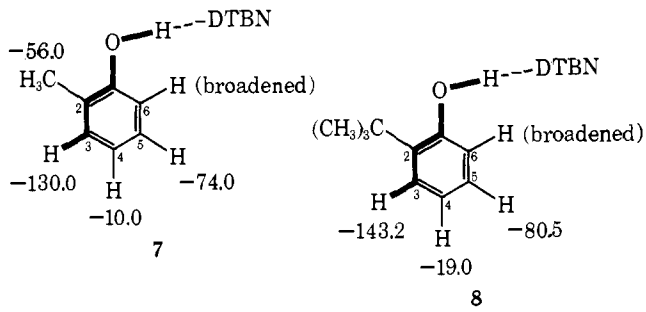


Figure 3. Linear plots of DTBN-induced shifts for phenol in CCl_4 and DMSO solutions.



may be explained in a similar way. The OH group is possibly required to be oriented trans with respect to the bulky ortho substituent, when it forms a hydrogen bond with DTBN. The stereospecific feature of DTBN-induced downfield proton shifts, obeying the "W letter rule", is also seen in allyl alcohol (see Table I).

The stereospecificity of the DTBN-induced shift appears to be characteristic of the contact shift and related to nuclear spin-spin coupling constants. It has been shown[2] that hydrogen bonding with DTBN radical induces negative spin density on the X-H proton by a spin polarization mechanism, namely by mixing of the excited triplet state of the X-H molecule.[7] This negative spin density would distribute itself through the bonds, most favorably through the zigzag or "W letter" path, onto C-H protons. This may allow us to expect a correlation between DTBN-induced ^1H contact shift and H-H nuclear spin coupling constants. The close similarity of conformational dependences of ^1H contact shift and H-H coupling would be due to this cause. According to the Pople's finite perturbation theory of nuclear spin coupling,[8] $H-H_j$ spin coupling is proportional to the electron spin density induced on the H_i when electron spin density is finitely placed on the H_j atom. This finite perturbation at the H_j atom possibly corresponds to the hydrogen bonding interaction between the $X-H_j$ proton and DTBN radical in which a small amount of electron spin density is induced on the $X-H_j$ proton. The configuration interaction (CI) treatment of X-H···DTBN biomolecular system[9] tells us that DTBN radical induces mixing of the local triplet excitation of the $X-H_j$ group, leading to appearance of the negative and positive spin density on the H_j and X atoms respectively, and the spin density on the proton H_i other than the X-H proton is proportional to the mutual polarizability, $\pi_{H_iH_j}$. Taking into account the well established relation between H_i-H_j spin coupling constant and mutual polarizability, it may be reasonable to deduce a correlation between DTBN-induced ^1H contact shift and H-H spin coupling. However, it should be noted that this relation holds only when the spin polarization mechanism plays an essential role in the transmission of electron spin density from DTBN radical to the X-H molecule.

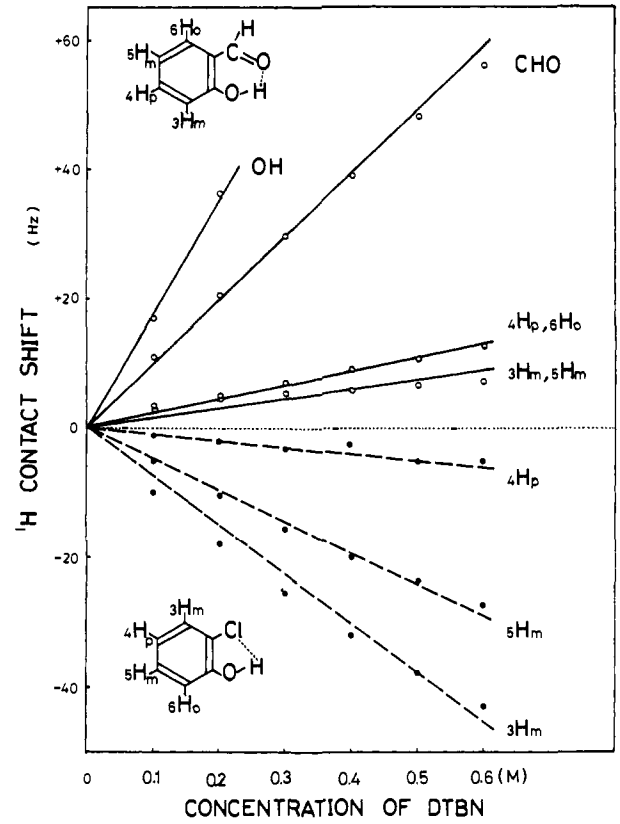
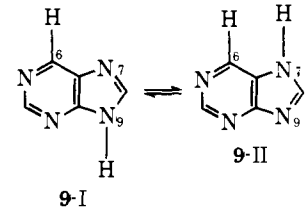


Figure 4. Linear plots of DTBN-induced shifts for salicylaldehyde and *o*-chlorophenol in CCl_4 solution ([solute] = 0.4 *M*).

C. Utility of the Stereospecific DTBN-Induced ^1H Contact Shifts. In order to gain further insight into the DTBN-induced proton contact shifts, we have studied here its application to structural elucidation of purine (**9**) and imidaz-



ole derivatives. There have been some debates over possible tautomerism of purine from experimental and theoretical points of view.[10] The problem inherent in this molecule concerns the assignment of the labile proton in the imidazole ring to N_7 or N_9. In general the proton has been believed to

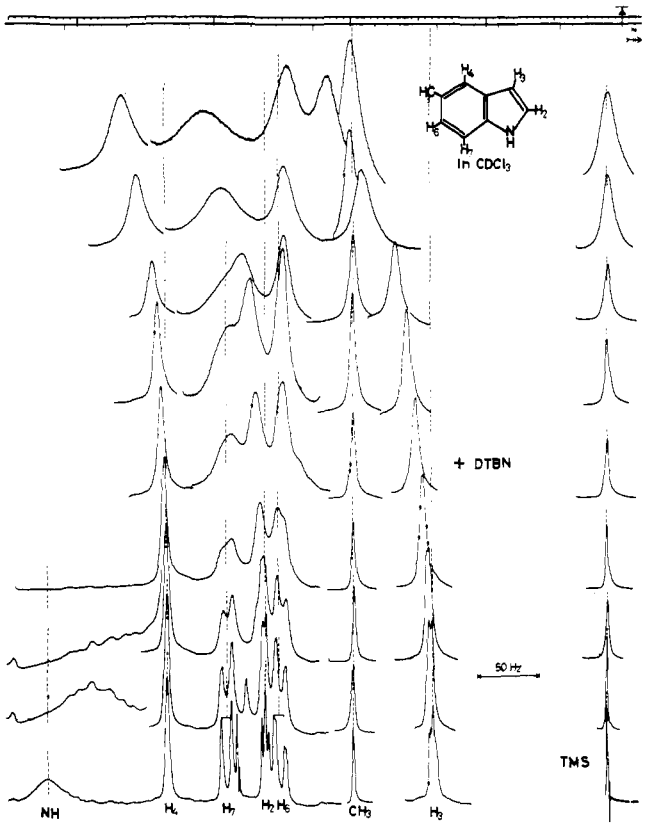
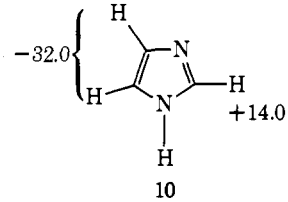


Figure 5. Proton NMR spectral perturbation (at 220 MHz) for 5-methylindole in $CDCl_3$ (0.5 *M*) induced by the addition of DTBN.

be at N_9, but no direct evidence has been presented to exclude the assignment on N_7 until the crystallographic study was presented by Watson et al.[11] They reported that the structure of purine is in tautomer II, in the crystal state at least, where a proton is attached to atom N_7 rather than to N_9. Although ^{13}C NMR has been used to disclose the structure of purine in solution,[12] this problem is still in dispute.

The proton H_6 lies on the zigzag path from the N-H group in the N_9-H tautomer I, but on the folded path in the N_7-H tautomer II. This may allow us to expect preferential downfield contact shift for the H_6 proton induced by the N_9-H···DTBN hydrogen bonding for the structure I. If the tautomer II were preferred in solution, there should be no substantial DTBN-induced contact shift for the H_6 proton.

We have observed DTBN-induced proton contact shifts for purine (Figure 6) and related molecules in $CDCl_3$ solution.[13] The most striking feature in Figure 6 is the preferential DTBN-induced downfield shift for the H_6 proton of purine. Another interesting feature is the slight downfield contact shift for the proton H_8. This proton, which is more acidic than the H_2 proton, can serve as a weak proton donor in the C_8-H···DTBN hydrogen bond, and senses an upfield contact shift, as is always the case for imidazole (**10**) derivatives. This upfield contact shift for H_8 appears to be compatible with the downfield shift arising from the NH···DTBN hydrogen bond, which is essentially important in indole derivatives (see Figure 5).



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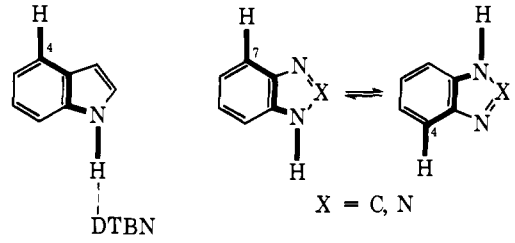
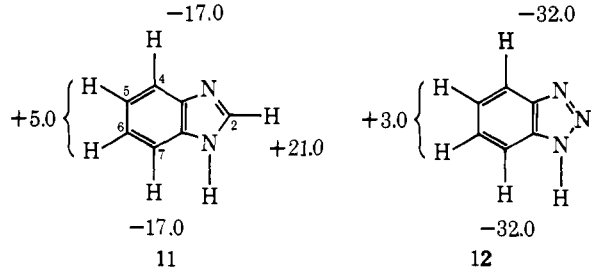
The finding in Figure 5 that in 5-methylindole the H_4 proton exhibits sizable downfield shift and H_7 is insensitive to this DTBN-induced shift seems to serve as an aid in interpretation of the results for purine. The proton H_4 in 5-methylindole senses substantial positive spin density, favorably transmitted through the zigzag intervening bonds from



the N-H group which is hydrogen bonded with DTBN radical. The sizable downfield contact shifts[6] for the H_4 and H_7 protons in benzoimidazole (**11**) and benzotriazole (**12**),



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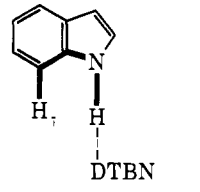
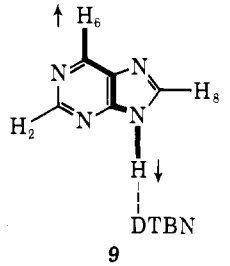
as compared with those for H_5 and H_6, may be explained along similar lines. The H_7 proton in indole is separated by the folded skeleton from the N-H group, unfavorable for electron spin transmission, and no appreciable contact shift is expected. This "W letter rule" could be also the case for purine. Therefore, presence of a certain amount of the tautomeric form I is possibly responsible for the preferential downfield shift for the H_6 proton in purine, where the zigzag path is formed between H_6 and the N-H group. In other words, the negative spin density induced on the N-H proton by hydrogen bonding with DTBN radical distributes itself on the proton H_6 favorably through the W shaped intervening bonds, as is always the case for long range H-H spin coupling. However, some contribution from the tautomer II cannot be entirely excluded, since the presence of II would induce no appreciable shift for the H_6 proton. Accordingly, the present result affords direct evidence for the presence of the N_9-protonated form of purine in solution, at least in the presence of hydrogen bond at the N_9-H group.





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In a similar way, the presence of one (IV) of the two possible tautomers (III and IV) in 4-(or 5-)methylimidazole (**13**) and 3-(or 5-)methylpyrazole (**14**) is confirmed by the

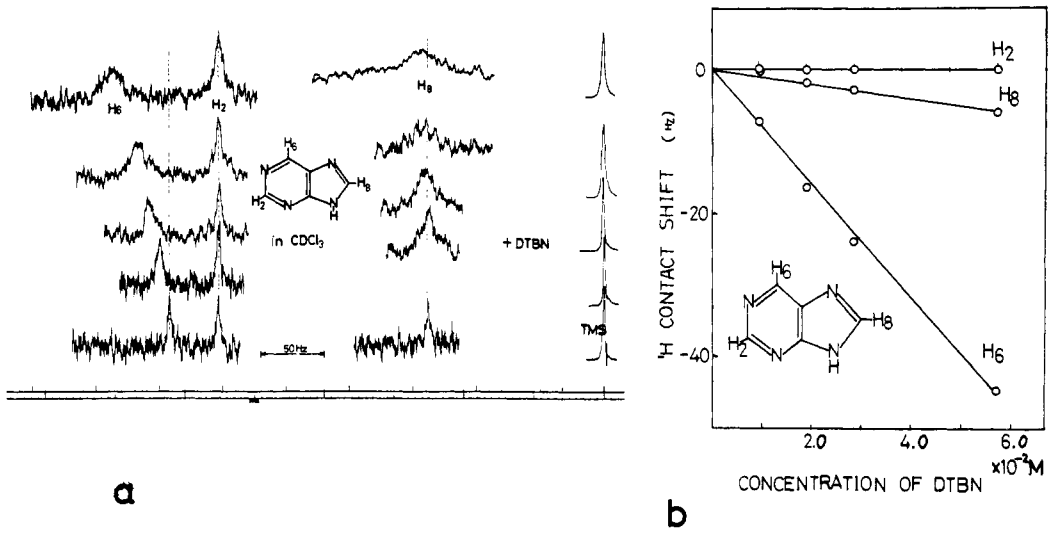
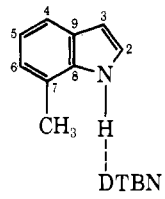


Figure 6. Proton NMR spectral perturbation for purine in $CDCl_3$ solution (solute concentration = saturated) induced by the addition of DTBN.



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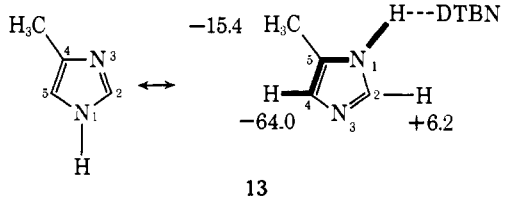
observation of sizable downfield contact shifts[6] for the H_4 (or H_5) of methylimidazole and for the H_3 (or H_5) of methylpyrazole.[14]

D. Contribution of DTBN-Induced Pseudocontact Shifts. In the preceding sections we described the DTBN-induced *contact* shift which exhibits specific conformational dependence. In this section we note the DTBN-induced *pseudocontact* shift and its potential utility in the structural studies.

It is well documented[15] that contribution of pseudocontact shift is essential in the isotropic shift resulting from a paramagnetic metal complex such as a lanthanide shift reagent in which anisotropy of the g value is quite large. However, the C-H protons which are spacially very close to the hydrogen bonded DTBN radical are expected to show DTBN-induced pseudocontact shifts even if the g tensor anisotropy of DTBN radical is small.[16] In this respect, we tried to observe DTBN-induced pseudocontact shifts for the proton which is spacially close to the X-H proton donor group but separated by several intervening bonds. For this purpose we have chosen 7-methylindole (**15**) as a model molecule.



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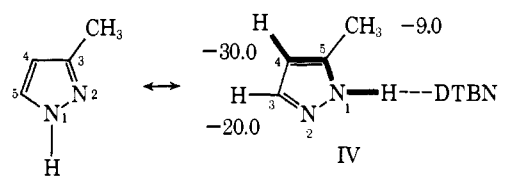

III IV

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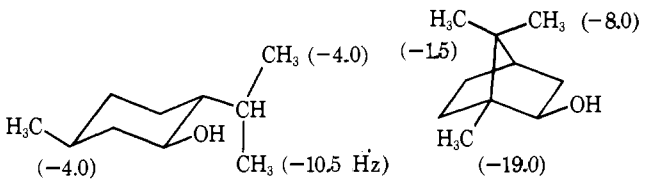
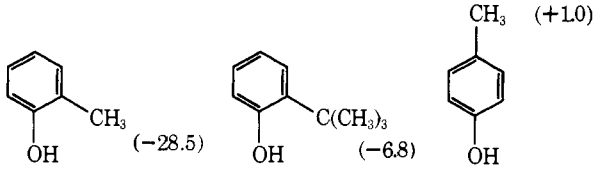
DTBN-induced spectral perturbation for 7-methylindole in $CDCl_3$ solution is shown in Figure 7. It is particularly worth noting that 7-methyl protons exhibit a sizable downfield shift, comparable to the shifts of H_2, H_3, and H_4 ring protons. However, a downfield shift was not detected for the methyl protons in 5-methylindole in which the methyl group is far apart from the N-H group. The preferential downfield shifts for H_2, H_3, and H_4 in indole derivatives are well recognized as resulting from contact shift origin. The unexpectedly large downfield shift of the 7-methyl protons, which are separated by the folded type of several intervening bonds and are possibly in close contact with the hydrogen bonded DTBN radical, could be attributable to pseudocontact interaction.

Similar appreciable downfield pseudocontact shifts were also encountered for the methyl protons in L-menthol (**16**), isoborneol (**17**), *o*-cresol (**7**) and *o-tert*-butylphenol which involve methyl groups located in the proximity of the OH group, leading to close contact with the hydrogen bonded DTBN radical. DTBN-induced shifts (in hertz) for these methyl protons at the concentration of [DTBN] = 0.28 M in $CDCl_3$ solution (solute concentration = 0.40 M) are as follows. The methyl protons far from the OH group in *p*-



16 17



7 8 18

cresol (**18**) exhibited slight upfield shifts due to direct weak hydrogen bonding between methyl protons and DTBN.

In order to get further insight into DTBN-induced pseudocontact shift, we tried to estimate it theoretically by the use of McConnell and Robertson's equation.[17] The experimental value of the nitroxide g tensor, g_{zz} = 2.0027, g_{xx} = 2.0089, and g_{zz} = 2.0061 (π-orbital direction is taken as the

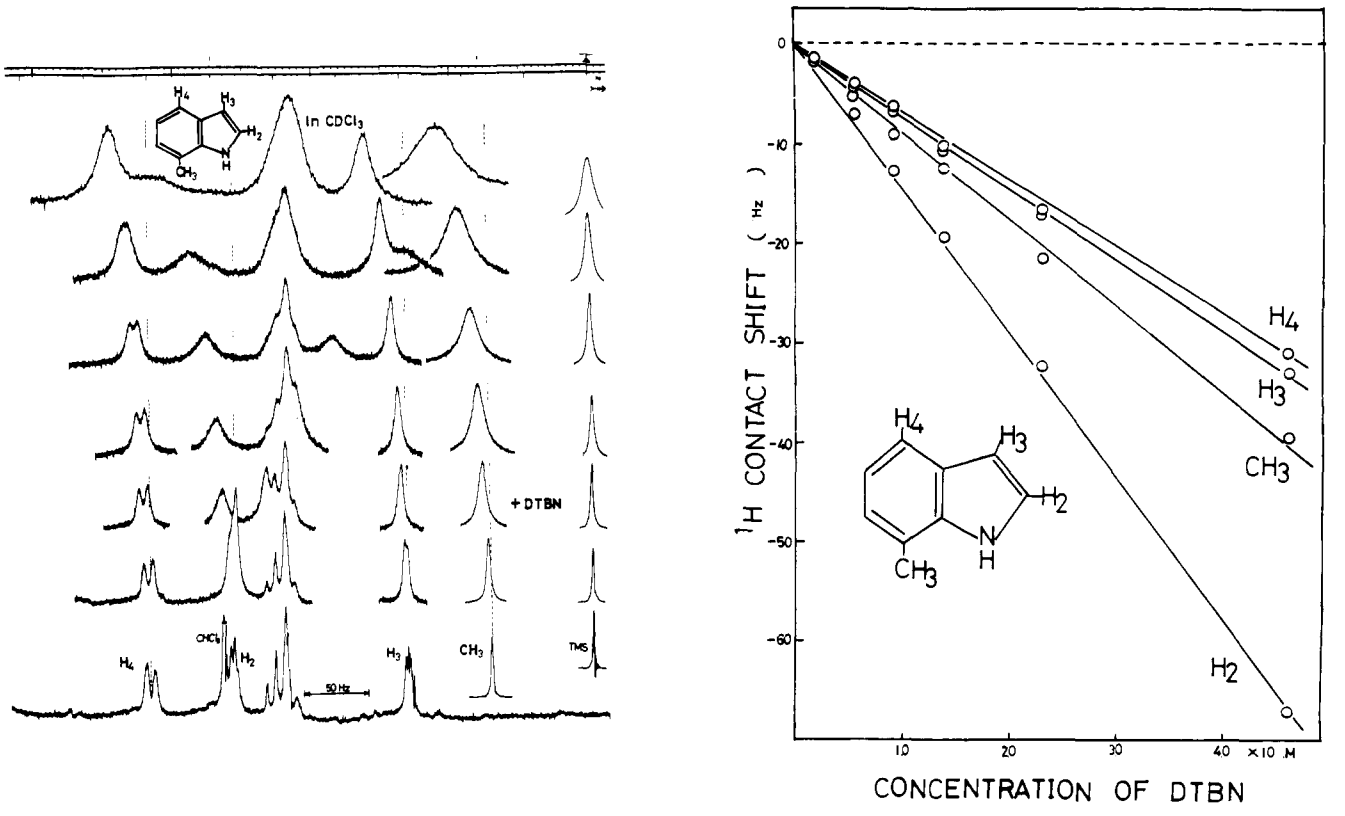
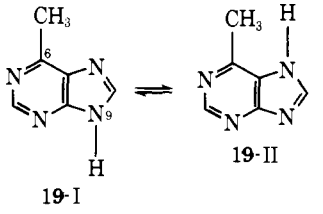


Figure 7. Proton shifts induced by the addition of DTBN for $CDCl_3$ solution (0.46 *M*) of 7-methylindole.

z axis),[16] allows us to estimate the pseudocontact shift for the methyl protons of 7-methylindole. When we assume that the hydrogen bond length between the N–H proton and the oxygen atom of the nitroxide radical is 1.5 Å and that the hydrogen bond complex is axially symmetric with respect to the N–H···O–N hydrogen bond axis, the geometric factor, $(3\cos^2\theta - 1)/r^3$, is estimated approximately as 4.74×10^{-23} cm^{-3} and $(g_\parallel - g_\perp)$ is +0.0045 for the σ type of hydrogen bond[18] where the O–H group is in the N–O bond axis, and −0.0048 for the π-type hydrogen bond[18] where the O–H group is directly over the oxygen π orbital of the nitroxide radical. These values yield pseudocontact shifts of −0.75 ppm for the σ type of hydrogen bond and of +0.80 ppm for the π type one. This is to be compared with the experimental limiting shift (−0.15 ± 0.02 ppm) obtained by the computer simulation method[19] of simultaneous determination of equilibrium constant and limiting isotropic shift for the X–H···DTBN hydrogen bond complex formation. The σ type of the hydrogen bond appears to be responsible for the observed downfield pseudocontact shifts, although both types of hydrogen bond would be involved in reality.[20]

Finally we will show an example of the application of DTBN-induced pseudocontact shifts for structural study of 6-methylpurine (**19**) in which a tautomeric shift of the hydrogen between the two imidazole nitrogens seems possible *a priori*, as stated in section C. We examined DTBN-in-



19-I 19-II

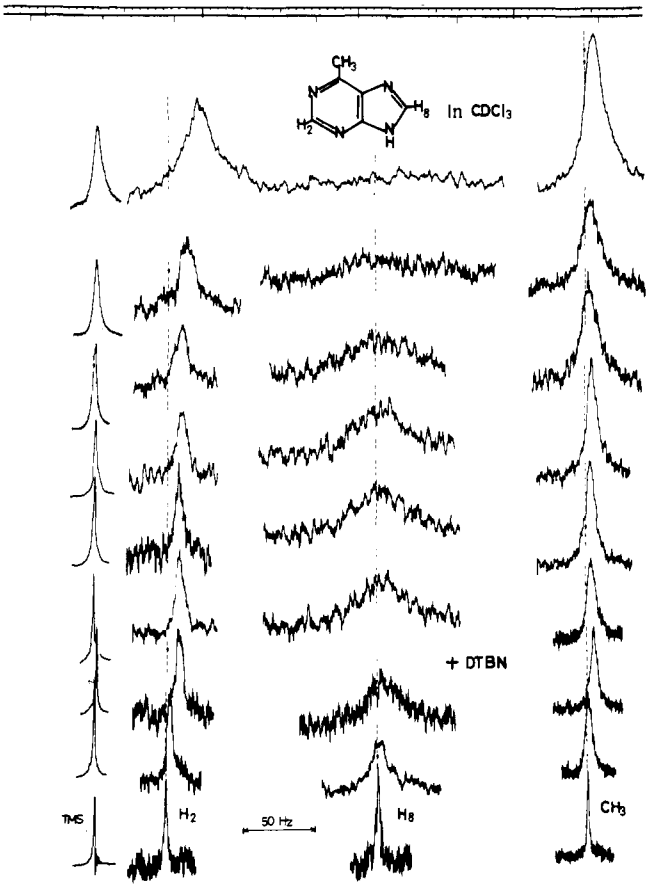


Figure 8. Proton NMR spectral perturbation induced by DTBN for $CDCl_3$ solution (0.0 *M*) of 6-methylpurine. Methyl and internal TMS signals are recorded with an appropriate sweep offset.

duced shift for the methyl protons in 6-methylpurine in $CDCl_3$ solution. By analogy with 7-methylindole in which location of the methyl group with respect to the N–H group is the same as that in tautomer II of purine, observation of no appreciable DTBN-induced downfield shift for 6-methyl protons (see Figure 8)[21] may allow us to see that 6-methylpurine is not principally in the tautomeric form II. This is in accord with the conclusion obtained from the DTBN-induced *contact* shift discussed in the preceding section.

Concluding Remarks

From the above results and discussion the following conclusions may be drawn. (1) The X–H···DTBN hydrogen bond induces quite sensitive *upfield* contact shifts for X–H protons, as was well established by our previous works.[2] (2) This hydrogen bond also induces a *downfield* contact shift for other C–H protons in the proton donor molecules. This downfield contact shift is quite useful in probing the hydrogen bonding involving various organic molecules; it is quite sensitive to the presence of free or solvated or intramolecularly hydrogen bonded states of the X–H proton donor group. (3) The DTBN-induced downfield contact shift exhibits conformational dependence; the protons lying on the zigzag path from the X–H group show preferential downfield shifts, obeying the "W letter rule", the generalization established for nuclear spin-spin coupling. The close similarity between the DTBN-induced contact shift and the nuclear spin–spin coupling constant is also substantiated theoretically by using finite perturbation theory. (4) The methyl protons in close spacial contact with X–H proton donor groups exhibit appreciably DTBN-induced downfield *pseudocontact* shifts. (5) The present study demonstrates the potential utility of DTBN-induced proton contact shifts as a sensitive tool for structural elucidation of proton donor molecules and also for studying hydrogen bonding involved in various organic and biologically important molecules.

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(6) The figures designate DTBN-induced shift in hertz at 220 MHz at the concentration of [DTBN] = 0.3 *M* in the CCl_4 solution ([solute] = 0.4 *M*). The minus sign means the downfield shift. DTBN-induced shifts were in linear proportion to the concentration of added DTBN radical. Therefore, these figures should be taken as representatives of the shift.

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(13) For the present study, an inert solvent such as CCl_4 or $CDCl_3$ is essential to observe DTBN-induced shifts because in aqueous solutions or in dipolar aprotic solvent N–H···DTBN hydrogen bond formation is hindered by strong solvation of the N–H proton donor group.

(14) 4-Methylimidazole is taken as a model of histidine.

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(20) This result does not contradict our previous conclusion on the mode of X–H···DTBN hydrogen bond obtained from DTBN-induced contact shifts of the X–H proton (see ref 2e).

(21) Upfield bias of H_2 proton signal of 6-methylpurine by the addition of DTBN radical is caused by the direct C_2–H···DTBN hydrogen bond.