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ASSIGNING ^{13}C-NMR RESONANCES OF NATURAL PRODUCTS

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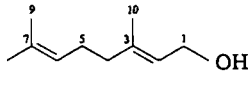
ABSTRACT.—The use of the nuclear relaxation agent Gd(fod)$_3$ with DEPT and INEPT pulse sequences has been investigated. It is shown that relaxation of specific ^{1}H nuclei is possible until they are no longer capable of transferring their polarization to ^{13}C nuclei. When this degree of nuclear relaxation is followed by observation of ^{13}C nuclei using the DEPT or INEPT pulse sequences, selected ^{13}C resonances become nulled. This technique has been applied to numerous natural products and demonstrates the usefulness of this technique in assigning ^{13}C-nmr chemical shifts of such compounds.

^{13}C-nmr experiments such as DEPT and INEPT (1,2) are now considered routine to the natural product chemist. These pulse sequences transfer the polarization of ^{1}H nuclei to ^{13}C nuclei in order to enhance the sensitivity of the ^{13}C-nmr signal. Successful ^{13}C signal enhancement using polarization transfer depends on various factors including the relaxation time of the ^{1}H (sensitive) nuclei (3). We have investigated the use of the nuclear relaxation agent tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)gadolinium [Gd(fod)$_3$] with DEPT and INEPT pulse sequences and have found it is possible to utilize the dependence of the polarization transfer mechanism on the ^{1}H relaxation rate in a predictable manner. The nuclear relaxation agent Gd(fod)$_3$ is capable of selectively inducing nuclear relaxation of ^{1}H nuclei to the point where they are no longer capable of transferring polarization to ^{13}C nuclei. When this degree of nuclear relaxation is followed by observation of ^{13}C nuclei using the DEPT or INEPT pulse sequences, ^{13}C resonances which are normally enhanced through polarization transfer are nulled.



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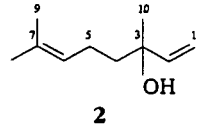

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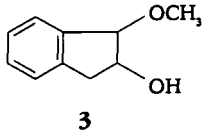

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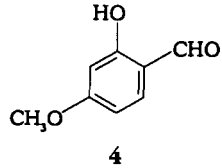

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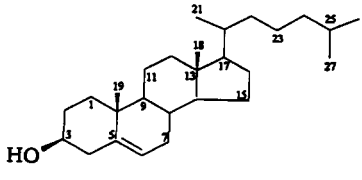

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RESULTS AND DISCUSSION

The usefulness of such a technique depends on $Gd(fod)_3$ selectively inducing ^1H relaxation in a predictable manner. This relaxation agent binds to specific functional groups that can act as a Lewis base such as the oxygen of an alcohol group (3). The extent of nuclear relaxation that is observed is augmented by increasing the concentration of relaxation agent relative to the sample. For example, it is possible to sequentially null individual ^{13}C resonances of the carbon chain of *n*-BuOH, beginning with the resonance closest to the oxygen bound Gd, by increasing the molar ratio of $Gd(fod)_3$ before each spectral acquisition.

The series of spectra of the primary alcohol geraniol [**1**], shown in Figure 1, was obtained by adding successive aliquots of $Gd(fod)_3$ and acquiring decoupled $^3/_4J$ refocused

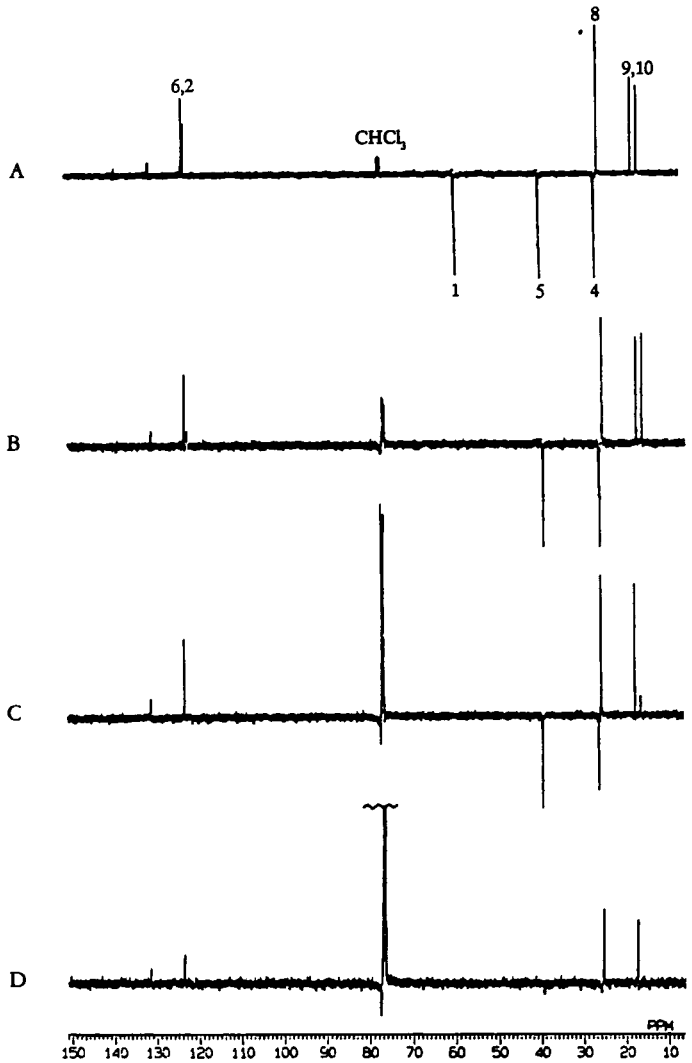

FIGURE 1. The decoupled $^3/_4J$ refocused INEPT spectrum of geraniol [**1**] ($CDCl_3$; 100 MHz for ^{13}C) with (A) 0.0, (B) 0.47, (C) 2.4, and (D) 9.5 mol % $Gd(fod)_3$. The appearance of the resonance at 77 ppm is due to relaxation of the $CHCl_3$ solvent by unbound relaxation agent.

INEPT spectra. The methyl and methine resonances are positively phased and the methylene resonances are negatively phased. The first aliquot of $Gd(fod)_3$ completely nulled the oxygenated C-1 resonance (59.5 ppm) and 75% of the C-2 resonance (123.5 ppm). The second aliquot resulted in almost complete nulling of the vinyl methyl attached at C-3 (C-10, 16.4 ppm). The last spectrum of the series contains only the resonances for the three carbon nuclei farthest away from the oxygen: the *gem* dimethyls (C-9, 17.8 ppm; C-8, 25.9 ppm) and the C-6 methine (123.9 ppm). The appearance of the resonance at 77 ppm is due to relaxation of the $CHCl_3$ solvent by unbound relaxation agent and is ignored. We used this set of spectra to correctly distinguish and assign the C-2 and C-6 methine resonances (123.5 ppm versus 123.9 ppm) and the C-9 and C-10 methyl resonances (16.4 ppm versus 17.8 ppm), even though both resonances had very similar shifts (4). Such accuracy is not always possible using calculations of substituent effects or by comparing the spectra of sample compounds to those of model compounds (5). A similar result can be observed in the decoupled $^3/_4J$ refocused INEPT spectra of the tertiary alcohol linalool [**2**] (Figure 2). As the quaternary carbinol carbon is not expected to be observed, the first resonance to lose intensity after the initial addition of $Gd(fod)_3$ is the methyl at C-3 (C-10, 27.8 ppm). The final spectrum of the series (Figure 2B) contains three intense resonances for the carbon nuclei most distant from the oxygen: the gem dimethyls (C-9, 17.5 ppm; C-8, 25.6 ppm) and the C-6 methine (124.1 ppm).

It is often difficult to distinguish between carbons bearing hydroxy or methoxy groups on the basis of ^{13}C-nmr shifts unless closely related model systems are available. Inspection of the spectrum (Figure 3A) of the secondary alcohol 1-methoxy-2-indanol [**3**] clearly reveals that there is an oxygenated methyl at 57.22 ppm, an oxygenated benzylic carbon resonance at 90.44 ppm, and an oxygenated methine carbon resonance at 78.14 ppm. However, it is not obvious which of the later two resonances (90.44 ppm, 78.14 ppm) represents the ether and which resonance represents the free alcohol (i.e., 1-methoxy-2-indanol vs. 2-methoxy-1-indanol). Running a decoupled $^3/_4J$ refocused INEPT spectrum after the addition of $Gd(fod)_3$ results in a completely nulled resonance at 78.14 ppm (Figure 3B). This correctly suggests that the benzylic carbon bears the

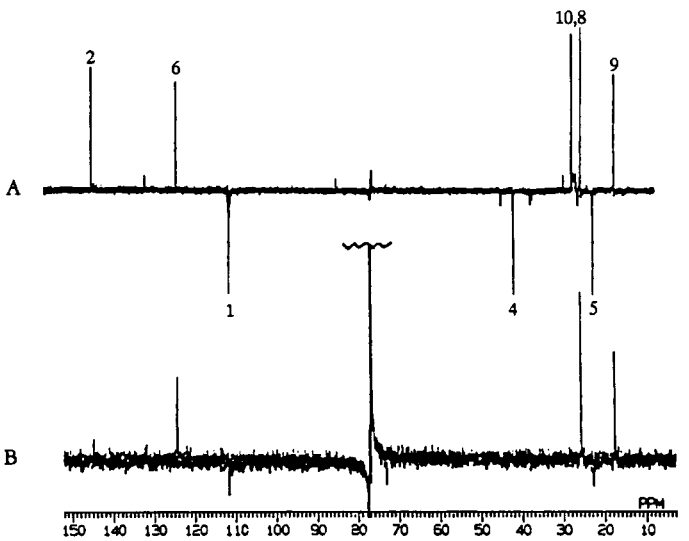

FIGURE 2. The decoupled $^3/_4J$ refocused INEPT spectrum of linalool [**2**] ($CDCl_3$; 100 MHz for ^{13}C) with (A) 0.0 and (B) 8.0 mol % $Gd(fod)_3$.

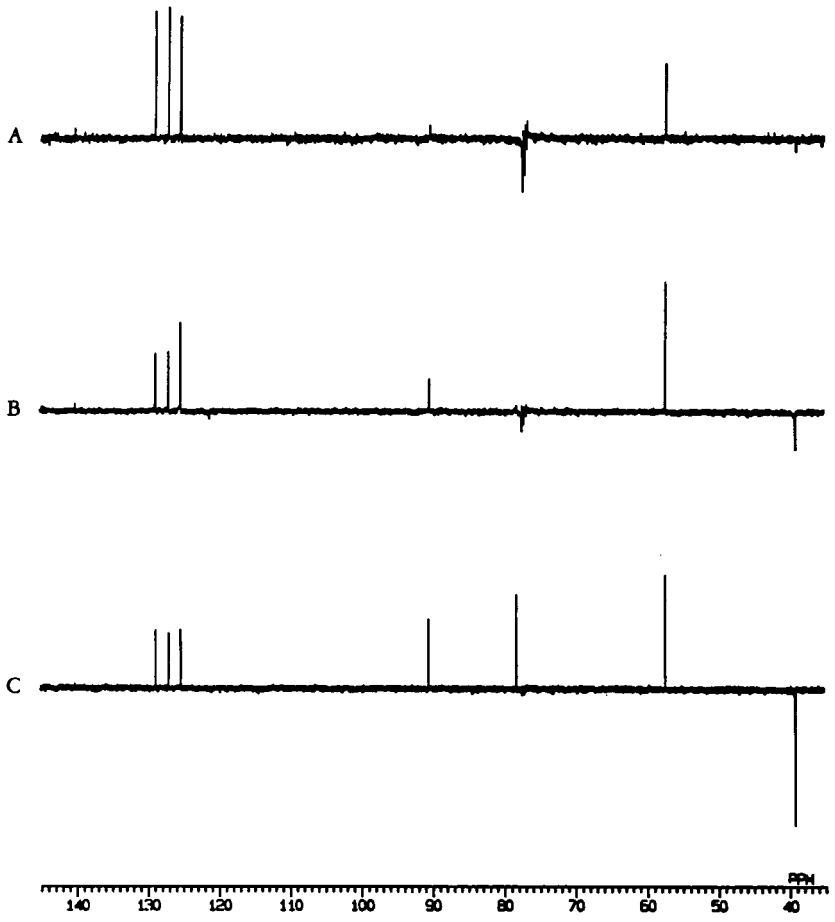

FIGURE 3. The decoupled ¾J refocused INEPT spectrum of 1-methoxy-2-indanol [3] ($CDCl_3$; 100 MHz for ^{13}C) with (A) 0.0, (B) 0.36, and (C) 0.72 mol % $Gd(fod)_3$.

methoxy group and that the nulled methine at 78.14 represents a site of hydroxylation. The nulling of the resonances at 90.44 and 39.13 ppm, best observed when an additional amount of $Gd(fod)_3$ is added, confirms that both sites are vicinal to the carbinol. The observation that the methyl resonance (57.22 ppm) remains nearly unaffected, relative to the resonances of C-1, C-2, and C-3, confirms that the methoxy and hydroxy groups of the five-membered ring are trans to one another.

The technique can also be used with phenols. Similar success was realized in distinguishing the site of hydroxylation of 2-hydroxy-4-methoxybenzaldehyde [4]. The decoupled ¾J refocused INEPT spectrum of this trifunctional benzene ring exhibits only resonances due to carbon nuclei proximal to the site of hydroxylation as the two oxygenated ring carbons (166.9 ppm and 164.5 ppm) are quaternary and are not observed. The aldehyde resonance (194.4 ppm) normally observed in the decoupled ¾J refocused INEPT is completely nulled upon the first aliquot of $Gd(fod)_3$ (Figure 4) while the protonated ring resonances are not significantly diminished in intensity. This correctly places the hydroxy group in close proximity to the aldehyde. It should be pointed out that while the aldehyde group and methoxy group of methoxy-hydroxy-benzaldehyde are potential sites of $Gd(fod)_3$ binding, the phenolic oxygen is well known to bind lanthanides much more efficiently than the oxygen of carbonyl or ether functional groups (3). Calculation of ^{13}C-nmr shifts using substituent constants is a possible alter-

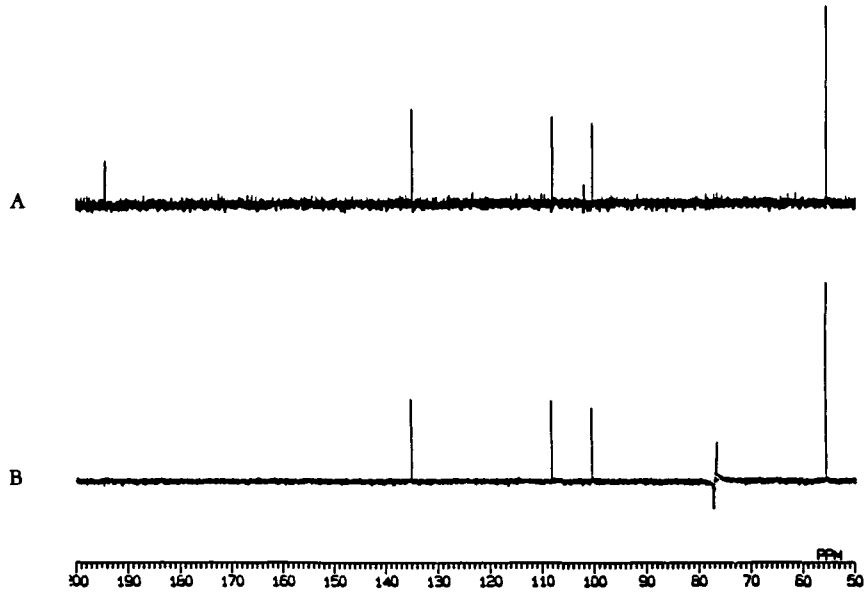

FIGURE 4. The decoupled $^{3}/_{4}J$ refocused INEPT spectrum of 2-hydroxy-4-methoxybenzaldehyde [**4**] ($CDCl_3$; 100 MHz for ^{13}C) with (A) 0.0 and (B) 0.96 mol % $Gd(fod)_3$.

native to the above procedure. However, these calculations are known to present difficulties when several substituents are involved in the calculation (5). Calculations using methoxy and hydroxy substituent effects (6) on the base shifts of benzaldehyde (7) (i.e., calculated shifts of 2-hydroxy-4-methoxybenzaldehyde versus 4-hydroxy-2-methoxybenzaldehyde) predict the shifts of the sites of methoxylation and hydroxylation of 2-hydroxy-4-methoxybenzaldehyde to be at 167.5 and 157.8 ppm, respectively. Similarly, the sites of methoxylation and hydroxylation of 4-hydroxy-2-methoxybenzaldehyde calculate to 162.7 and 162.6 ppm, respectively. With observed shifts of 166.9 and 164.5 ppm for these two resonances the use of substituent effects to assign the regiochemistry would in this case tend to favor the wrong regiochemistry.

This technique may also prove useful in assigning the ^{13}C-nmr resonances of large organic molecules such as steroids and triterpenes. It is common for such compounds to have several resonances of quite similar ^{13}C-nmr shifts. As can be seen in Figure 5A, the ^{13}C-nmr spectrum of cholesterol [**5**] appears complex even when observed at 125 MHz (500 MHz for ^{1}H), and two resonances overlap (C-7 and C-8, 31.90 ppm) even at this high field. The addition of an aliquot of $Gd(fod)_3$ (8.6 mol %) results in the complete removal of the resonances of the ^{13}C nuclei of the A ring (C-1, 37.26 ppm; C-2, 31.63 ppm; C-3, 71.77 ppm; C-4, 42.28 ppm) and the C-19 methyl group (19.38 ppm) of the A–B ring junction. The C-6 (121.66 ppm) and C-9 (50.15 ppm) resonances of the B ring are also completely nulled, and the overlapping methine (C-7) and methylene (C-8) resonances of the B ring are greatly diminished, as is C-11 of the C ring. [The diminishing of the methine and methylene resonances of the B ring is not clear in the spectra shown as the two resonances tend to cancel each other out due to opposite phasing in the decoupled $^{3}/_{4}J$ refocused INEPT experiment. In the decoupled $^{1}/_{4}J$ refocused INEPT experiment, methine and methylene resonances are both positively phased, and the resonance representing the two overlapping methine and methylene signals was nulled to a similar extent as the C-11 resonance.] The resonances of C-12 (39.80 ppm) and C-14 (56.77 ppm) in the C ring are at about 50% their normal intensity relative to the resonances that are unaffected, such as C-25. The nulling of a number of resonances

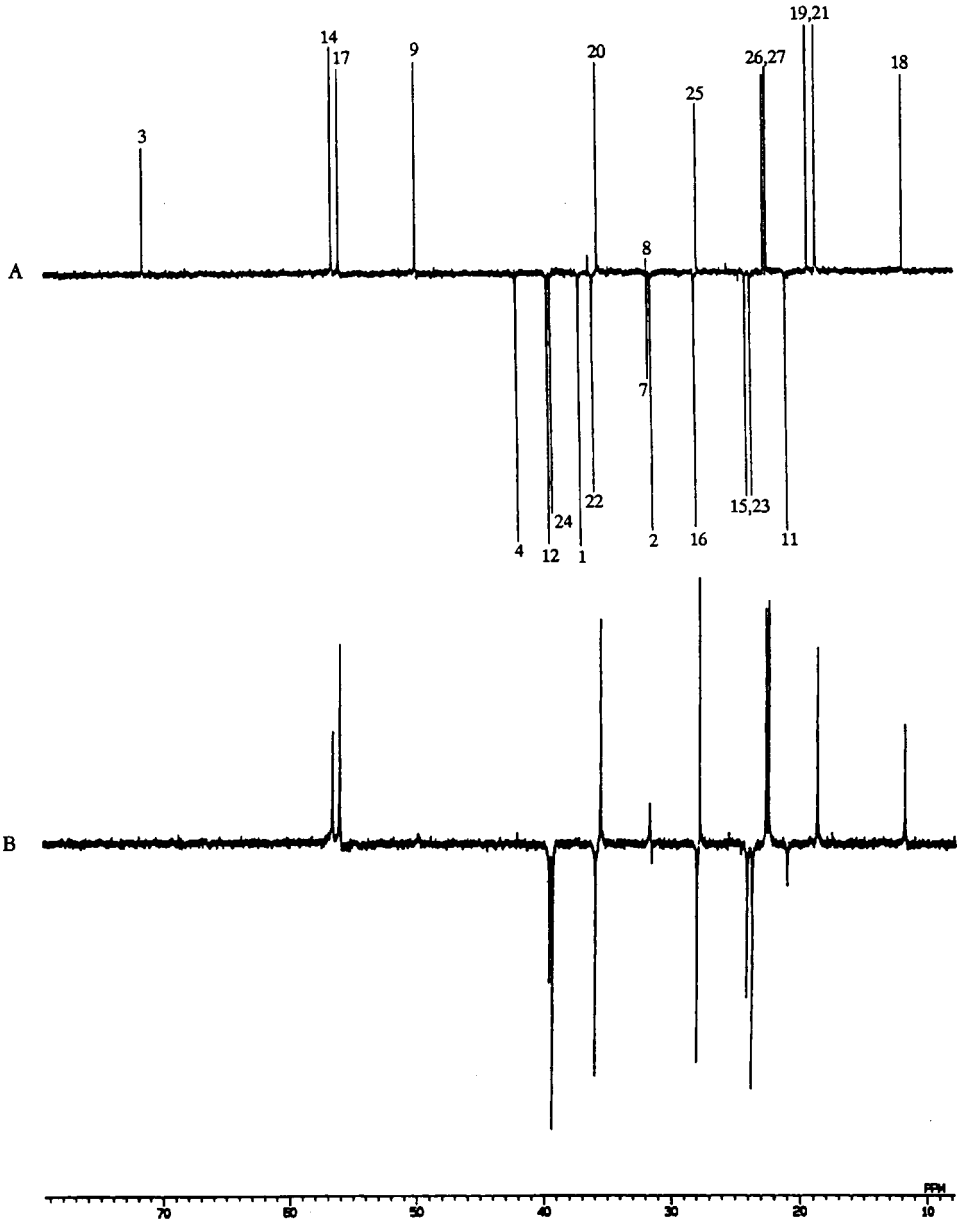

FIGURE 5. The decoupled $^{3}/_{4}J$ refocused INEPT spectrum of cholesterol [**5**] ($CDCl_3$; 125 MHz for ^{13}C) with (A) 0.0 and (B) 8.6 mol % $Gd(fod)_3$.

reduces the complexity of the spectrum. The portion of the spectrum that remains may be scrutinized as if it originated from a compound half the size of the original parent molecule. Resonances such as C-14 and C-17 (56.77 ppm, 56.18 ppm), C-15 and C-23 (24.28 ppm, 23.84 ppm), and C-12 and C-24 (39.80 ppm, 39.51 ppm) can be distinguished with a high confidence level and without dependence on a series of model compounds. It is interesting to note that the initial literature assignment of cholesterol that we used (8) had incorrectly reversed the assignments of C-12 and C-16. It was not until we noticed that what we believed to be the C-12 resonance was half the intensity expected, and that what we believed to be the C-16 resonance was twice what we ex-

pected, that we went back to the literature for the correct assignment (9) of these two resonances (C-12, 39.80 ppm; C-16, 28.21 ppm).

It is possible to observe the contribution each proton gives to signals in the refocused INEPT experiment by examining heteroscalar correlated 2D nmr spectra with and without $Gd(fod)_3$. As a two-dimensional version of the decoupled $\frac{1}{4J}$ refocused INEPT experiment it relies on the same principle of ^{13}C sensitivity enhancement. In this experiment, two correlations are normally observed for the C-12 methylene resonance of cholesterol with one correlation for each of its two protons (1.13 ppm, 1.98 ppm). Only one correlation is observed when $Gd(fod)_3$ is added to the sample (Figure 6). This suggests that the decrease in intensity of the C-12 carbon resonance observed in

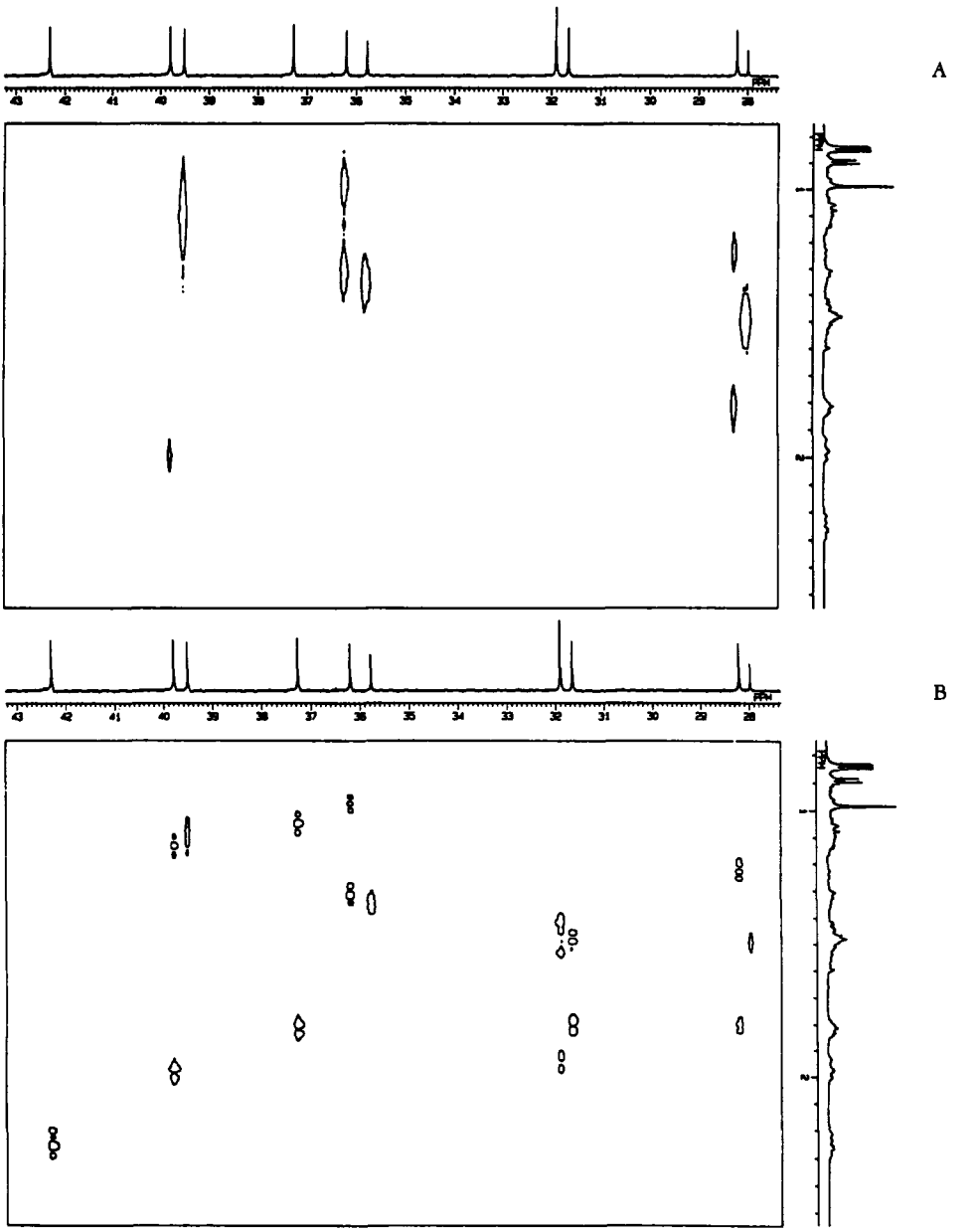

FIGURE 6. The heteroscalar correlated 2D nmr spectrum of cholesterol [**5**] ($CDCl_3$; 125 MHz for ^{13}C) with (A) 0.0 and (B) 8.6 mol % $Gd(fod)_3$.

the 1D experiment shown in Figure 5, about 50% its normal value relative to the C-25 resonance, comes about because one of its two protons (1.13 ppm) is no longer contributing via the polarization transfer mechanism.

All one-dimensional spectra presented thus far were acquired using the $^{3}/_{4}J$ decoupled INEPT experiment. Spectra we acquired using the analogous 135° DEPT experiment gave essentially the same results. What has been presented so far has been a display of the qualitative use of induced relaxation to control the degree of polarization transfer. As with other lanthanide shift or relaxation experiments, this technique works with primary, secondary, and tertiary alcohols as well as with phenols. It should be transferable to other classes of natural products capable of binding the reagent in a predictable manner, such as amines. We make no attempt to quantitate the effects of $Gd(fod)_3$-induced nuclear relaxation on the degree of polarization transfer. However, the preceding spectra clearly show the value of using a relaxation agent in concert with a polarization transfer pulse sequence. The technique should be particularly useful in distinguishing resonances of alcohols from ethers, assigning ^{13}C-nmr shifts, and in simplifying the interpretation of the ^{13}C-nmr spectra of large organic compounds such as steroids and triterpenoids.

As in any study involving the use of lanthanide metals, an obvious limitation of the technique lies in the necessary restriction of its use to compounds where the binding of the metal is clearly predictable. Applications to unknown and complex structures and to structures where the metal binding is not clear must be deemed unreliable at the very least. In an investigation into the metal-binding properties of several sugars using $Gd(NO_3)_3$ in D_2O where each compound had several hydroxy groups, we found that while the observation of binding sites was straightforward it was quite difficult to predict the preference of any one of the multiple binding sites in advance. Experimental precautions to be exercised are essentially the same as those involved in lanthanide shift experiments. These precautions have been thoroughly discussed in detail in several review articles (3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All spectra were acquired on a JEOL GSX-400 or JEOL GSX-500 nmr spectrometer operating at 100 MHz and 125 MHz, respectively, for ^{13}C and equipped with a 5-mm tunable probe. All spectra were acquired in $CDCl_3$ and all shifts were reported using $CDCl_3$ (77.0 ppm) as an internal reference. All test compounds, including $Gd(fod)_3$, were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin and used without further purification. Addition of the relaxation agent was similar for all experiments. $Gd(fod)_3$ was dissolved in $CDCl_3$ to form a 0.1 M solution. A series of small aliquots of this solution were added to an nmr sample with successive decoupled $^{3}/_{4}J$ refocused INEPT spectra acquired after the addition of each aliquot. Amounts of $Gd(fod)_3$ added were reported as the mol % of $Gd(fod)_3$ present with respect to the substrate. All decoupled $^{3}/_{4}J$ refocused INEPT spectra have methyls and methines positively phased and methylenes negatively phased. Any assumptions we have made as to the binding preference and resulting geometry of the reagent have been those commonly accepted in standard lanthanide shift and relaxation nmr experiments (3).

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