tion was adequate. As shown in Table I, when androstenedione-1α-*d* was converted to estrone 85% of the label was retained. Since the 1β hydrogen is lost in aromatization,[2] the results confirm the assignment of the 1α-*d* configuration to the substrate and suggest that the stereoselectivity was 85% α and 15% β. This agrees with the conclusion from nmr determinations.[6] Estrone from incubation of androstenedione-1α,2α-*d* was 78% dideuterated, showing that the 2α hydrogen was *not* involved in the aromatization. The 15% d_0 molecules support the conclusion that the substrate was stereoselectively deuterated 85% α while the 7% d_1 molecules suggests that the distribution of deuterium (α/β) at C-1 and at C-2 is not exactly the same, perhaps due to slight racemization at C-2.

Table I. Per Cent Deuterated Species in Substrates and Products[a]

Substrate		Product estrone	Recovered substrate
Androstenedione-1α-*d*	d_0, 2	d_0, 15	d_0, 5
	d_1, 98	d_1, 85	d_1, 95
Androstenedione-1α,2α-*d*	d_0, 0	d_0, 15	d_0, 3
	d_1, 2	d_1, 7	d_1, 9
	d_2, 98	d_2, 78	d_2, 89
Androstenedione-2β-*d*	d_0, 12	d_0, 82	d_0, 26
	d_1, 78	d_1, 16	d_1, 65
	d_2, 10	d_2, 2	d_2, 9
	d_3, 0		
Androstenedione	d_0, 100	d_0, 100	d_0, 100

[a] Values are means ±3% from duplicate analyses of substrates or of compounds from duplicate incubations. Using a Varian M-66 mass spectrometer determinations were made by averaging relative peak heights on scans from 5 to 6 amu sweeps of the molecular weight range. Correction for ^{13}C content was made from analysis of standards. Conditions: sample weight, 20–30 μg; electron energy, 70 V; current, 50 μA; direct sample injection probe temperature, 110–125°.

Estrone from incubation of the 2β-*d* compound (78% d_1) was over 80% nondeuterated and showed a corresponding decrease from substrate in per cent of d_1 and d_2 molecules. This confirms that the 2β hydrogen is lost in desaturation in ring A. The recovered substrate lost 17% of the d_1 molecules, indicating some exchange had occurred. A lesser exchange was found for the 1α,2α-*d* substrate which, as noted, contained ~15% β-oriented deuterium molecules.

These results, together with our previous findings, establish that desaturation in ring A to produce the aromatic structure involves *cis* 1β,2β elimination and supports the general mechanism for estrogen biosynthesis from C_{19} and C_{18} steroids discussed.[2] Essentially this involves activation of the C-1 position for anion loss by formation of a $\Delta^{2(3)}$-enol. The 2β (axial) hydrogen would be stereoelectronically favored for elimination in this step, perhaps explaining the exchange of some deuterium from the C-2 labeled substrates. Thus it is possible that the substrate (or intermediate, *e.g.*, 19-hydroxyandrostenedione) could bind, enolize to some degree and be released, before conversion to estrone.

The oxidative step in the desaturation appears to involve the C-1β hydrogen in the rate-determining step and requires NADPH and oxygen.[2] In trying to evaluate the likely mechanisms, dehydrogenation or hydroxylation–dehydration,[2] we prepared the tested 2β-hydroxy-19-norandrostenedione recently and found it to be inactive as an estrogen precursor. Since the 2α-, 1β-, and 10β-hydroxy isomers are also poor substrates as compared to 19-norandrostenedione,[7] it does not appear that a *free* hydroxyl compound is an intermediate. *cis* desaturation, though not often demonstrated, has been shown to occur in the introduction of the Δ^5 bond in the conversion of Δ^7-cholestenol to 7-dehydrocholesterol and cholesterol in rat liver preparations.[8,9] The enzyme also requires molecular oxygen but not NADPH,[10] even for conversion of the possible intermediate, 3β,5α-dihydroxycholest-7-ene.[11] Evidence of an oxygen-dependent dehydrogenase for fatty acid desaturation has been recorded.[12]

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On the Deuteron Magnetic Resonance in Solutions of Paramagnetic Ions

Sir:

Although two of the nuclei of the stable hydrogen isotopes, the proton and the deuteron, have magnetic moments, only the proton has widely been used as a probe in nuclear magnetic resonance studies.

In the present communication we wish to show that the use of deuteron magnetic resonance (dmr) in studies of solutions of paramagnetic ions may lead to a more detailed understanding of the processes of nuclear relaxation in these systems. In addition to data derived from proton and oxygen-17 relaxation, dmr may provide new and important information on the rate constants and mechanisms of hydrogen exchange, on the electron–nuclear hyperfine coupling constants, and on the electron relaxation times.[1]

While being of similar chemical nature, deuterons as compared to protons have a smaller magnetogyric ratio (by a factor of about 6.5), and it is this difference that will be considered here. In the following discussion it is considered that conditions of both proton and deuteron magnetic resonance are attained in the same magnetic field and at an isotopic abundance of *ca.* 100%. The ratio γ_H/γ_D is defined as $f = 6.5$ and $f^2 = 42.5$.

The chemical shift of nuclei in paramagnetic complexes

(1) The difference between proton and deuteron relaxation in solutions of paramagnetic ions has been investigated by G. Laukien and F. Noack, *Z. Physik*, **159**, 311 (1960). However, these authors have not studied the effects of temperature and have not considered the effects of chemical exchange.

due to the Fermi-contact part of the hyperfine Hamiltonian is given by[2]

$$\Delta\omega_M/\omega = -Ah(\gamma_e/\gamma_n)S(S+1)/3kT \quad (1)$$

where the hyperfine coupling constant is

$$A = -(4/3)\gamma_e\gamma_n\hbar|\psi(0)|^2 \quad (2)$$

Thus the shift is independent of γ_n and will have the same value in units of parts per million for protons and deuterons, being f times as small for the latter if expressed in hertz. The same conclusion is reached for the pseudo-contact shifts originating from the dipolar part of the Hamiltonian.[3] Small isotope effects on $|\psi(0)|^2$ may be ignored.

It is well known that protons in diamagnetic solutions give rise to sharp resonance lines. In this respect, it should be pointed out that the deuteron line width in pure water is about 1 Hz,[4] although in this case the main relaxation mechanism is due to interactions of the nuclear quadrupole moment with intramolecular electric field gradients.

The nuclear relaxation rate, $1/T_{2M}$, of a nucleus in a paramagnetic complex usually originates from interactions with the unpaired electrons and is composed of two main terms: dipolar and hyperfine. Assuming that $T_{1e} = T_{2e}$, e designating electron, and $\omega_e\tau_c < 1$, $\omega_e\tau_e > 1$, the correlation times being $1/\tau_e = 1/T_{1e} + 1/\tau_M$ (τ_M is the residence time of a nucleus in the complex) and $1/\tau_c = 1/\tau_e + 1/\tau_r$, the nuclear relaxation rate is given by[5]

$$T_{2M}^{-1} = (20/15)S(S+1)\gamma_n^2g^2\beta^2r^{-6}\tau_c + (4/3)S(S+1)(\pi A)^2\tau_e \quad (3)$$

(In the case that $\omega_e\tau_e < 1$ the second right-hand term should be multiplied by a factor of 2.)

Since A is proportional to γ_n (eq 2), it is seen that T_{2M}^{-1} is proportional to γ_n^2, and the line width of a deuteron in a paramagnetic complex will be smaller by a factor of f^2 than that of a proton in the same position.

Chemical exchange occurs *via* two most commonly encountered mechanisms: exchange of whole ligand molecules and reactions of hydrogen ion transfer. The latter are generally acid catalyzed. Nmr is sensitive to the faster of the two processes. A deuterium isotope effect is expected in protolytic reactions, *i.e.*, $\tau_M^D > \tau_M^H$.

The effects of chemical exchange on nuclear relaxation in solutions of paramagnetic ions have been considered in detail by Swift and Connick.[6] Under conditions of slow exchange the "excess" relaxation of the solvent nuclei is given by $1/T_{2p} = P_M/\tau_M$, P_M being the population ratio in the two environments. In this case isotope effects on the rate constant $1/\tau_M$ may become apparent. Two limiting conditions may exist for fast exchange: (a) $\tau_M^2 \ll \Delta\omega_M^{-2} \ll \tau_M T_{2M}$, $1/T_{2p} = P_M\tau_M\Delta\omega_M^2$; (b) $\tau_M T_{2M} \ll T_{2M}^2, \Delta\omega_M^{-2}$, $1/T_{2p} = P_M/T_{2M}$. In both cases the relaxation rate of deuterons will be f^2 times as small as that of protons. It is also seen that conditions of fast exchange for deuterons will be attained at τ_M values f times longer than those for protons.

If $1/T_{2M} \gg |\Delta\omega_M|$, another case is obtained and $1/T_{2p} = P_M/(T_{2M} + \tau_M)$. Since T_{2M} of deuterons is f^2 times longer than that of protons, it will have a much greater influence on the observed "excess" relaxation. Thus the use of dmr offers an excellent probe for studying electron-spin relaxation (eq 3). Moreover, by comparing the relaxation times of protons and deuterons it should be possible to separate the contributions of T_{2M} and τ_M.

The use of dmr offers in general a reduction of line widths by a factor of f^2 leading to an improvement of the over-all resolution of the spectrum by a factor of f. Thus it will be easier to resolve the dmr absorption of nuclei in the vicinity of paramagnetic ions or to measure more accurately isotropic shifts under conditions of fast exchange. If there are detection difficulties due to low signal-to-noise ratio, these may be amplified by a factor of 2.35, where the relative sensitivity and line widths have been taken into account. This is the only apparent disadvantage.

We have made use of dmr for studying aqueous solutions of vanadyl(IV). For the first time it was possible to obtain the temperature dependence of the electron relaxation time from nmr measurements. The electron relaxation time is of the order of 10^{-8} sec, and its variation with temperature is in good agreement with the theoretical predictions of Kivelson, *et al.*[7] A detailed account of this work will be presented in a forthcoming publication.

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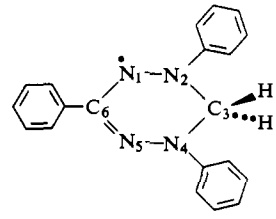
Structure of 3,4-Dihydro-2,4,6-triphenyl-*s*-tetrazin-1(2H)-yl Free Radical by Crystal-Packing Analysis and X-Ray Diffraction

Sir:

The title compound, also referred to as 1,3,5-triphenylverdazyl (TPV), is a very stable free radical based on the *s*-tetrazine ring system.[1] The radical has a paramagnetic susceptibility corresponding to one unpaired electron per molecule. The electron paramagnetic resonance spectrum indicates that the unpaired electron density is about equally distributed among the four nitrogen atoms. The radical is even more stable than 2,2-diphenyl-1-picrylhydrazyl (DPPH), where the unpaired electron is primarily distributed between two nitrogens. An investiga-



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