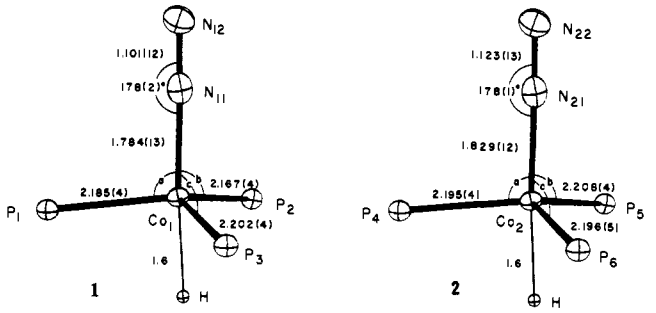


Figure 1. The inner coordination spheres of the two independent molecules of $Co(H)(N_2)(P(C_6H_5)_3)_3$. The N–Co–P angles are: molecule 1, $a$ = 97.5 (5), $b$ = 99.6 (4), $c$ = 98.4 (4)°; molecule 2, $a$ = 96.3 (4), $b$ = 99.3 (4), $c$ = 96.9 (4)°.

and a cycle of least-squares refinement on $F_o$ for the 3312 independent observations with $|F_o| > 3\sigma(F_o)$ converged to values of $R_1$ of 0.077 and $R_2$ of 0.074.

At this point difference Fourier syntheses were calculated around the cobalt atoms in an attempt to locate the coordinated hydrogen atoms. These were clearly visible at an electron density of 0.46 (5) e/Å$^3$ on molecule 1 and 0.42 (5) e/Å$^3$ on molecule 2. In a final cycle of refinement these hydride ligands were included as fixed contributions, the nitrogen atoms were allowed to vibrate anisotropically, and the refinement converged to values of $R_1$ and $R_2$ of 0.065 and 0.061. A final difference Fourier map showed no peaks of height greater than 0.37 (5) e/Å$^3$.

The geometry of the inner coordination spheres is shown in Figure 1, together with important bond distances and bond angles. The attachment of the nitrogen molecules is end on, the Co–N–N angles of 178 (2) and 178 (1)° representing an essentially linear arrangement. The N–N distances of 1.123 (13) and 1.101 (12) Å do not differ significantly and are comparable with the values of 1.0976 (2) Å in gaseous $N_2$[3] and 1.118 Å in $N_2^+$.[4]

The mode of attachment of the nitrogen molecules is strongly reminiscent of that of the isoelectronic molecule carbon monoxide. There is no significant lengthening of the N–N bond from that of gaseous nitrogen, just as no significant lengthening of the C–O bond is observed in coordinated carbon monoxide compared with the free molecule.

The Co–N distances of 1.829 (12) and 1.784 (13) Å do not differ significantly. The Co–N mean distance (1.806 (16) Å) is comparable with that for the Co–C bond of 1.75 (3) Å found in $(Co(CO)_3(P(n\text{-}C_4H_9)_3))_2$[5] and that of 1.797 (7) Å found for the axial Co–C bond in $Co(CO)_4(SiCl_3)$.[6] The Co–N distance is, however, significantly shorter than that of a typical Co–N bond distance of 1.96 Å found in various cobalt ammines.

This work provides unambiguous proof that the compound is a hydride of formula $Co(H)(N_2)(P(C_6H_5)_3)_3$ as suggested in our previous communication.[1] The positional parameters of the hydride ligands, as derived from interpolation of electron density peaks in the difference Fourier maps, result in Co–H distances of 1.67 and 1.64 Å. These values compare well with the distance predicted from the sums of covalent radii.

(3) P. G. Wilkinson and N. B. Houk, *J. Chem. Phys.*, **24**, 528 (1956).
(4) P. G. Wilkinson, *Can. J. Phys.*, **34**, 250 (1956).
(5) J. A. Ibers, *J. Organometal. Chem.*, **14**, 423 (1968).
(6) W. T. Robinson and J. A. Ibers, *Inorg. Chem.*, **6**, 1208 (1967).

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**Betty R. Davis, Nicholas C. Payne, James A. Ibers**
*Department of Chemistry, Northwestern University*
*Evanston, Illinois 60201*
*Received December 12, 1968*

## Studies on the Mechanism of Estrogen Biosynthesis. VI. The Stereochemistry of Hydrogen Elimination at C-2 during Aromatization

*Sir:*

In previous publications on the mechanism of estrogen biosynthesis we reported that in the aromatization of androstenedione[1,2] (androst-4-ene-3,17-dione), 19-nor-androstenedione,[2] and 19-hydroxyandrostenedione[3] by placental tissue, the 1β hydrogen is eliminated. We now wish to report on the stereochemistry of loss of hydrogen at C-2 during the aromatization of androstenedione.

Androstenedione stereoselectively deuterated at 1α or 2β or 1α, 2α was used in the study. The 2β-*d* compound was prepared by equilibrating androstenedione for 16 hr in 10% deuterium oxide–diglyme containing 2.2 mmol of deuterated sodium hydroxide.[4] The compound (0.995 atom of deuterium per molecule) had essentially the deuterium distribution previously found (2β/2α = 10) as judged by combustion analysis,[5] mass, ir, and nmr spectra, and enzymatic dehydrogenation.[4]

Androstenedione-1α,2α-*d* was prepared by α-face reduction of androsta-1,4-diene-3,17-dione with deuterium and tris(triphenylphosphine)rhodium(I) chloride.[6] The product showed deuterium absorption at 2155 and 2195 cm$^{-1}$ and little at 2145 cm$^{-1}$ (2β-*d*). Equilibration with base gave androstenedione-1α-*d* with absorption at 2155 cm$^{-1}$ (1α-*d*).

The deuterated compounds and nondeuterated androstenedione, mixed with androstenedione-4-$^{14}$C of negligible mass, were incubated separately in duplicate as previously described[2] using 300 μg of substrate (40,000 dpm), placental microsomes equivalent to 80 g wet weight of tissue, and an NADPH generating system suspended in 12 ml of 0.05 *M* phosphate buffer, pH 7.2. After incubation for 1 hr the steroids were extracted and separated by tlc in 65% ether–hexane to give estrone and androstenedione. The products were chromatographed in the Bush $B_3$ and A paper systems, respectively, and were further purified on silica gel columns using ethyl acetate–benzene mixtures for elution. The yield of each material was 45–65 μg.

Product estrone and recovered androstenedione from incubation of undeuterated substrate both gave mass spectra with quantitatively similar peak ratios in the M − 1 to M + 5 area as those obtained with purified standard materials, showing that the method of purifica-

(1) T. Morato, K. Raab, H. J. Brodie, M. Hayano, and R. I. Dorfman, *J. Am. Chem. Soc.*, **84**, 3764 (1962).
(2) J. D. Townsley and H. J. Brodie, *Biochemistry*, **7**, 33 (1968).
(3) H. J. Brodie, G. Possanza, and J. D. Townsley, *Biochim. Biophys. Acta*, **152**, 770 (1968).
(4) S. K. Malhotra and H. J. Ringold, *J. Am. Chem. Soc.*, **86**, 1997 (1964).
(5) Combustion analysis by J. Nemeth, Urbana, Ill.
(6) C. Djerassi and J. Gutzwiller, *J. Am. Chem. Soc.*, **88**, 4537 (1966).

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 $\Delta^5$ bond in the conversion of $\Delta^7$-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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(7) Details to be published.
(8) M. Akhtar and S. Marsh, *Biochem. J.*, **102**, 462 (1967).
(9) A. M. Paliokas and G. J. Schroepfer, Jr., *Biochem. Biophys. Res. Commun.*, **26**, 736 (1967).
(10) M. E. Dempsey, *J. Biol. Chem.*, **240**, 4176 (1965).
(11) S. M. Dewhurst and M. Akhtar, *Biochem. J.*, **105**, 1187 (1967).
(12) M. I. Gurr and K. Bloch, *ibid.*, **99**, 16C (1966).
(13) This work was supported by U. S. Public Health Service Grant AM-6894 and Training Grant T01-CA-5001 (G. P.).

**H. J. Brodie, K. J. Kripalani, G. Possanza**
*The Worcester Foundation for Experimental Biology*
*Shrewsbury, Massachusetts 01545*
*Received December 12, 1968*

## 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 $\gamma_H/\gamma_D$ 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.