Applications of Rare Earth Nuclear Magnetic Resonance Shift Reagents. III.[1,2] Graphical Analysis of Paramagnetic Shifts for Systems Having Two Coordination Sites. Testosterone and 17α-Methyltestosterone

C. C. Hinckley,* M. R. Klotz, and F. Patil

Contribution from the Department of Chemistry, Southern Illinois University, Carbondale, Illinois 62901. *Received October 10, 1970*

Abstract: Paramagnetic shifts were obtained in the pmr spectra of testosterone (T) and 17α-methyltestosterone (MT) by the addition of the dipyridine adduct of tris(dipivalomethanato)europium(III). Log–log plots of observed paramagnetic shifts *vs.* the europium–steroid hydrogen distances provide a means of separating the various contributions to the shifts, and are the basis of a technique of graphical analysis which is sensitive to molecular conformation. The induced paramagnetic shifts of pmr signals in the spectra of steroids are due largely to the pseudocontact shift. Contact-shift contributions for protons near the sites of coordination may be separated from the pseudocontact shifts by this graphical method. The relative contributions to induced paramagnetic shifts resulting from metal coordinated at the two coordination sites of the steroid may also be separated graphically. Relative association constants for the europium chelate and hydroxyl and carbonyl group of T and MT are calculated.

Shift reagents[1,3] are paramagnetic metal complexes which may associate with a wide variety of organic substrates and through association induce paramagnetic shifts in the proton magnetic resonance (pmr) spectra of the organic compounds. Recently a number of studies of pmr shift reagents have been published which indicate that such reagents have wide application in high-resolution pmr spectral analysis.[1-4] Metal complexes are useful in this context only if the paramagnetic electron relaxation time of the metal is short enough so that the induced shifts are not accompanied by serious broadening of the pmr spectrum. Applications include the assignment of resonance absorptions, separation of overlapping lines, and molecular conformation studies. In this study the dipyridine adduct of tris(dipivalomethanato)europium(III), $Eu(DPM)_3 \cdot 2py$, was used as the shift reagent.

In the association of europium complexes with aliphatic systems, paramagnetic shifts are dominated by the pseudocontact interaction[5-7] (eq 1), where

$$\Delta\nu_i = K/R_i^3 \qquad (1)$$

$\Delta\nu_i$ is the paramagnetic shift induced in the resonance of the ith proton and R_i is the average distance from the Eu^{3+} ion (radius 0.95 Å) to the ith proton in the metal chelate–organic substate complex. This simplified form of the equation reflects the finding that the distance parameter, R, dominates the relative magnitudes of the observed pseudocontact shifts induced by the shift reagent $Eu(DPM)_3 \cdot 2py$. In what follows, K is assumed constant for a particular solution composition and temperature. To examine this approximate relationship for systems which have more than one coordination site available on the substrate molecule, pmr spectra of solutions containing testosterone and 17α-methyltestosterone were obtained and paramagnetic shifts measured as functions of $Eu(DPM)_3 \cdot 2py$ concentration.

Experimental Section

Preparation of Tris(dipivalomethanato)europium(III). The method of Eisentraut and Sievers[8] was followed. A solution of europium nitrate was prepared by dissolving 1.76 g of Eu_2O_3 with stirring (1 hr) in 30 ml of water to which 5.0 ml of 6 *N* HNO_3 had been added and then diluting with 30 ml of 95% ethanol. A second solution was prepared by dissolving 1.2 g of NaOH in 50% ethanol and adding the solution to 0.55 g of 2,2,6,6-tetramethylheptane-3,5-dione (dipivalomethane, HDPM) dissolved in 50 ml of 95% ethanol. The resulting mixture was added to the europium solution. A light yellow solid precipitated and the mixture was stirred under reduced pressure for 2 hr. Ethanol was removed by vacuum distillation until the liquid volume was reduced to about half. Water (350 ml) was added and the crude $Eu(DPM)_3$ was separated by filtration and stored in a desiccator.

Preparation of the Dipyridine Adduct. Crude $Eu(DPM)_3$ was added with warming to 50 ml of pyridine until the solution was saturated. After filtering, an additional 10 ml of pyridine was added to the filtrate and the solution was refrigerated. Colorless $Eu(DPM)_3 \cdot 2py$ crystals were obtained.

Nmr Solutions. Testosterone and 17α-methyltestosterone (58.1 and 6.14 mg, respectively) were dissolved in 1.0 ml of deuterated chloroform, $DCCl_3$. An approximately 0.1 *M* solution of $Eu(DPM)_3 \cdot 2py$ was prepared by dissolving 0.43 g of europium complex in 5.0 ml of carbon tetrachloride.

Pmr Spectra. Spectra were obtained with a Varian HA-100 nmr spectrometer for solutions of 40 drops of steroid solution and 7 drops of tetramethylsilane reference, TMS. Spectra were recorded after successive dropwise additions of the europium complex solution.

Distance Measurements. Dreiding models (scale 1 Å/in.) of testosterone and 17α-methyltestosterone were constructed[9] and distances measured from the metal ion (radius Eu^{3+} = 0.95 Å) on coordination at the hydroxyl and carbonyl groups to protons having assigned pmr resonances (Table I). Metal–hydrogen distances for the conformations (Table II) were measured from models in which the 17-hydroxyl group was in the α (equatorial) conforma-

(1) Part I: C. C. Hinckley, *J. Amer. Chem. Soc.*, **91**, 5160 (1969).
(2) Part II: C. C. Hinckley, *J. Org. Chem.*, **35**, 2834 (1970).
(3) J. K. M. Sanders and D. H. Williams, *Chem. Commun.*, 422 (1970).
(4) J. Briggs, G. H. Frost, F. A. Hart, G. P. Moss, and M. L. Staniforth, *ibid.*, 749 (1970).
(5) Recent reviews include: M. Base, *Progr. Nucl. Magn. Resonance Spectrosc.*, **4**, 335 (1969).
(6) See also: D. R. Eaton, "Physical Methods in Advanced Inorganic Chemistry," H. A. D. Hill, and D. Pay, Ed., Interscience, New York, N. Y., 1968, p 462.
(7) For a detailed examination of the pseudocontact interaction see: B. R. McGarvey, *J. Chem. Phys.*, **53**, 86 (1970), and references quoted therein.
(8) K. J. Eisentraut and R. E. Sievers, *J. Amer. Chem. Soc.*, **87**, 5254 (1965).
(9) Texts containing structural information of steroids include: (a) C. W. Shoppe, "Chemistry of the Steroids," Butterworths, Washington, D. C., 1966; (b) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959.

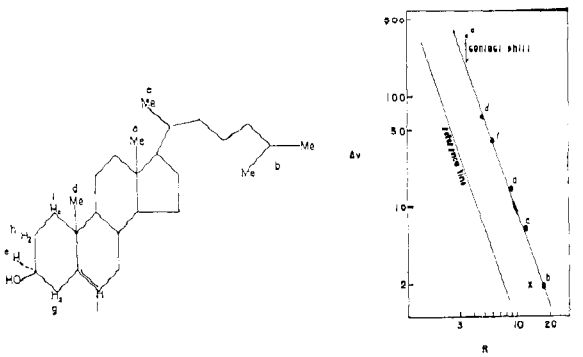

Figure 1. A logarithmic plot of europium-induced paramagnetic shifts, $-\Delta\nu$, of the assigned resonances of cholesterol *vs.* the europium–hydrogen distance (R) reported in ref 1. The point indicated by an X is that originally suggested for the side-chain methyl group, b. This plot indicates that there is little coiling of the side chain in carbon tetrachloride solution.

tion. The molecular conformations of these models were in all other respects the same as testosterone.

Table I[a]

Distance, Å	Proton i	j	k	l
		T		
R_A	3.5	7.0	3.25	10.0
R_B	9.5	5.5	11.5	3.4
		MT		
R_A	3.3	6.5	3.0	9.5
R_B	9.5	5.5	12.0	3.4

[a] Distances in ångströms from the metal ion coordinated at the hydroxyl group (R_A) and the carbonyl groups (R_B) to the protons with assigned resonances for testosterone (T) and 17α-methyltestosterone (MT).

Table II[a]

	R_A, Å i	j	k	l
T	4.7	6.5	3.3	9.5
MT	4.7	7.0	3.3	8.5

[a] Distances in ångströms from the metal ion coordinated at the hydroxyl group (R_A) to the protons with assigned resonances taken from models of the α-hydroxyl epimers of testosterone (T) and methyltestosterone (MT).

Results and Discussion

More than one coordination site complicates the interpretation of the observed paramagnetic shifts which are now sums of contributions due to magnetic interaction from metal association at each site. Equilibrium constants for the various coordinating groups are different and unknown, with the result that the different pseudocontact contributions (eq 1) from each coordination site have different proportionality constants, K. These complications make straightforward plots of shift, $\Delta\nu$, *vs.* $1/R^3$ ineffective as aids in data analysis. Logarithmic plots offer an important simplification. The logarithm of eq 1 is

$$\log \Delta\nu_i = -3 \log R_i + \log K \qquad (2)$$

Plots of log $\Delta\nu$ *vs.* log R should be linear with slopes of -3 for any single coordination site regardless of the

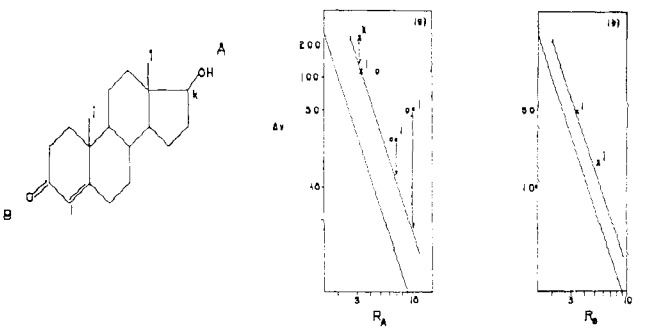

Figure 2. Logarithmic plots of observed paramagnetic shifts for a solution of testosterone 0.046 M in europium chelate.

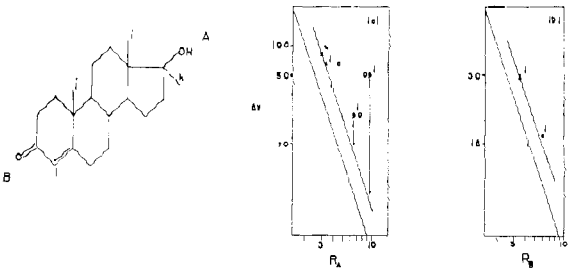

Figure 3. Logarithmic plots of observed paramagnetic shifts for a solution of 17α-methyltestosterone 0.046 M in europium chelate.

equilibrium constant for the metal association at that site.

Figure 1 is a logarithmic plot of previously published data[1] of the paramagnetic shifts taken from pmr spectra of solutions of cholesterol and Eu(DPM)$_3$·2py in carbon tetrachloride, and is presented here as a clear-cut example. The reference line having a slope of -3 is useful in interpreting these graphs and is conveniently plotted on three-cycle log paper using the two points (log 2, log 100) and (log 4.3, log 10). The expected contact shift in the resonance of the proton vicinal to the hydroxyl group is readily separable from the pseudocontact contribution. In aromatic systems, where unpaired spin density is transferred through π system electrons, contact shifts may make important contributions to observed shifts of resonances for protons throughout the molecule. In aliphatic systems, where spin density is transferred through σ bonds, contact-shift contributions are rapidly attenuated and affect resonances of only those protons nearest to the coordination site.

The pmr spectra of testosterone and 17α-methyltestosterone exhibit four assignable resonances due to protons labeled in the structure diagrams of Figures 2 and 3. The 18- and 19-methyl groups are labeled i and j, respectively, and the vinyl protons at the 4 positions near the 3-carbonyl groups are labeled l for both molecules. The 17 proton vicinal to the hydroxyl group is labeled k in testosterone. In 17α-methyltestosterone the protons of the 17-methyl are also labeled k. Association between these molecules and the europium chelate will occur at both the hydroxyl and carbonyl groups and the induced paramagnetic shifts will be the result of magnetic interactions from both sites. Pmr spectra are recorded and the positions of the resonances plotted (Figure 4) for several metal concentrations in order to avoid confusion in the

assignments of the shifted resonances. Paramagnetic shifts are then the difference in field position of the shifted resonances and the position recorded from the spectrum of the pure substrate. All shifts of substrate resonances using $Eu(DPM)_3 \cdot 2py$ as the shift reagent are downfield and therefore negative (Table III).

Table III[a]

	Δν, cps							
	T				MT			
[Eu]	i	j	k	l	i	j	k	l
0.018	45.2	10.8	91.2	19.1				
0.030	77.1	18.5	156.1	33.9	39.6	5.3	43.4	25.0
0.039	97.4	23.8	196.9	43.8	57.3	16.8	71.1	42.3
0.046	111.4	27.4	225.6	51.3	66.8	19.8	82.8	50.5

[a] Paramagnetic shifts, $-\Delta\nu$, observed at various $Eu(DPM)_3 \cdot 2py$ concentrations for the assignable resonances of testosterone (T) and 17α-methyltestosterone (MT).

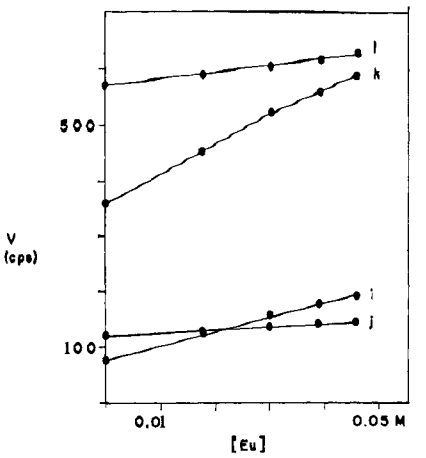

Figure 4. The field position in cycles per second downfield from the tetramethylsilane resonance is plotted for each of the assigned resonances of testosterone as a function of europium chelate concentration.

Figures 2a and 3a are logarithmic plots of paramagnetic shifts (plotted as X's) induced in the assigned resonances of the substrate pmr spectra *vs.* the distance of the proton from europium ion in association with the 17-hydroxyl group, A. Protons k and i are closest to the hydroxyl coordination site and the induced shifts measured for these resonances are due almost entirely to metal–hydroxyl group association.

Protons of the 18-methyl groups, i, are separated from the metal ions coordinated at the hydroxyl groups by several bonds which attenuate the contact interactions so that the paramagnetic shifts measured are essentially all pseudocontact. In Figures 2a and 3a the straight lines drawn through point i, parallel to the reference line, represent the pseudocontact shifts induced by coordination of the europium chelate at the hydroxyl groups as a function of distance, R_A. A contact-shift contribution to the paramagnetic shift of the proton k in testosterone, which is vicinal to the hydroxyl group, was found as in the case of the similar resonance of cholesterol. Little or no contact shift is observed for the 17-methyl group, k, of 17α-methyltestosterone. Evidently the carbon–carbon σ bond between the 17-carbon and the attached methyl group in 17α-methyltestosterone efficiently attenuates the spin density transfer necessary for contact shift. The resonances of protons j and l are affected substantially by association at both coordination sites. The difference between the measured shifts plotted in Figures 2a and 3a and the extrapolated pseudocontact contribution from association at the hydroxyl group yields the paramagnetic shift for the j and l protons due to coordination of the metal complex at the carbonyl group. Figures 2b and 3b are logarithmic plots of this shift *vs.* the distance, R_B, of the protons j and l from the metal ion coordinated at the carbonyl group, B. The fact that the corrected shifts of the j and l resonances do not fall on the same line having a slope of −3 in Figures 2b and 3b may be attributed to the cumulative errors inherent in a graphical analysis and to a small-contact-shift contribution to the observed shift of the vinyl proton l near the carbonyl group. Within experimental error, paramagnetic shifts of all the resonances may be described as substantially the result of pseudocontact interactions induced by metal coordination at the two sites A and B. Contact shifts contribute to the observed paramagnetic shifts of protons near the coordination sites but are rapidly attenuated by the σ bonds of aliphatic molecules.

Several factors contribute to the value of graphical analysis of the above systems. The coordinating groups are attached to the rigid frameworks of the steroidal ring structure and rotation is limited. This has the effect of reducing the uncertainty in distance measurements. Graphical presentation of the data allows the effect of uncertainties to be visualized as well as the interrelations of the various plotted points. This last feature is of special importance when alternative conformations are possible.

In order to examine the feasibility of using pseudocontact shift data as a tool in problems of molecular conformation, the paramagnetic shifts obtained were plotted *vs.* the distances, R_A, from the metal ion coordinated at the hydroxyl group measured from a model of the α-hydroxy epimers. These points are indicated in Figures 2a and 3a by open circles with the exception of the points for protons k which are essentially coincident with those already plotted. Comparison of these points with the reference line shows that for these incorrect structures no decomposition of the shifts into pseudocontact contributions is possible without the unwarranted assumption of a very large contact-shift contribution to the 18-methyl resonance, i.

Relative associative capacities of the coordinating groups with the europium complex may be approximated by ratios of induced pseudocontact shifts at equivalent distances from the metal coordinated at the sites. Using the shifts observed for the solutions which are 0.046 *M* in the europium chelates, metal association at the hydroxyl group induces −61 and −35-cps shifts at 4.0 Å for testosterone (T) and 17α-methyltestosterone (MT), respectively, as read from the graphs in Figures 2a and 3a. Association at the carbonyl groups of both compounds induces a −29-cps shift at a distance of 4.0 Å as read from the graphs of Figures 2b and 3b. These numbers indicate that if the association for the hydroxyl group of testosterone is defined as unity, $K_{AT} = 1$, then the corresponding association

constant for 17α-methyltestosterone is 0.57, *i.e.*, K_{AMT} = 0.57. The carbonyl groups of both compounds have association constants of 0.47, *i.e.*, $K_{BT} = K_{BMT} = 0.47$.

The graphical reduction of the data presented in this report is susceptible to computerization, but such efforts should probably be approached with caution. Difficulties include the amplified effect of uncertainties for small distances (3–4 Å) and problems associated with the estimation of contact-shift contributions to protons near coordination sites. Angle functions assumed constant in this study may be important in other systems.[10,11] Operations that can be performed visually in a graphical reduction of data require careful programming in a computer analysis.

Acknowledgments. Discussions with Dr. R. E. Beyler were extremely helpful. The Varian HA-100 nmr spectrometer was purchased with the aid of a grant from the National Science Foundation.

(10) George H. Wahl, private communication. Angle factors appear to be important in shifts induced in the pmr spectrum of 2-adamantanol.

(11) Demarco, Elzey, Lewis, and Wenkert have studied europium-induced shifts in alcohols[12] and steroids.[13] Their findings also indicate some angular dependence in observed shifts.

(12) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5734 (1970).

(13) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, **92**, 5737 (1970).

Structure and Reactivity of Organic Ions in Gas-Phase Radiolysis. II. The Reactions of an Isopropyl Ion with Benzene and Toluene[1]

Setsuo Takamuku,* Kozo Iseda, and Hiroshi Sakurai

Contribution from the Radiation Laboratory, the Institute of Scientific and Industrial Research, Osaka University, Suita, Osaka 565, Japan. *Received June 22, 1970*

Abstract: The gas-phase reactions of the isopropyl ion with benzene and toluene have been investigated by the γ radiolysis of propane–benzene–O_2 and propane–toluene–O_2 mixtures at room temperature. The maximum G values of isopropylbenzene and isopropyltoluene observed at very low concentrations of the aromatics (less than 1%) were in fair agreement with each other (1.06) and decreased with the concentration of the aromatics or, more significantly, with the addition of ammonia. The isomer distribution of isopropyltoluene was nearly in statistical ratio (44.2% *o*-, 40.4% *m*-, and 15.4% *p*-isopropyltoluene) at low pressure of toluene while the formation of the meta isomer became dominant (~60%) with increasing toluene pressure. In the competitive isopropylation of benzene and toluene under a variety of reaction conditions, a low positional and low substrate (k_T/k_B = 0.6–0.9) selectivity and also a low kinetic isotope effect in the isopropylation of benzene-d_6 were observed. The reaction mechanism was discussed in connection with high-pressure mass spectra and with liquid-phase isopropylation.

There has been much interest recently in ion–molecule reactions of aromatic compounds studied by high-pressure mass spectrometry[2–8] or gas-phase radiolysis.[1,9–11] The mass spectral results suggested that attachment of ions to aromatic molecules plays an important role in the gas-phase radiolysis of aromatic compounds. Ions produced in the gas phase exist as free ions and may react with aromatic molecules in the absence of interaction with solvent and counterions which is generally observed in the condensed-phase ionic reactions. Therefore, it is of particular interest to clarify the difference between the gas-phase and the condensed-phase reaction in order to understand the reaction mechanism in ionic organic chemistry, especially in connection with the mechanism of Friedel–Crafts alkylation discussed recently by Brown,[12] Olah,[13] and Nakane.[14]

In a previous paper of this series,[1] it has been shown that a $C_7H_7^+$ ion produced by the gas-phase radiolysis of toluene, ethylbenzene, and *m*-xylene reacts with respective aromatic molecules forming benzylated products. Of interest was the result indicating that electrophilic substitution of alkylbenzenes by free ions in the gas phase is thermodynamically controlled and predominantly leads to the formation of meta isomers though alkyl groups are ortho–para directing in electrophilic aromatic substitution. Thus, it appeared desirable to investigate further the scope and mechanism of this reaction in the simpler systems. The present paper describes the results obtained in the study of the gas-phase reaction of the $C_3H_7^+$ ion,

(1) (a) Part I: Y. Yamamoto, S. Takamuku, and H. Sakurai, *J. Amer. Chem. Soc.*, **91**, 7192 (1969); (b) Y. Yamamoto, S. Takamuku, and H. Sakurai, *J. Phys. Chem.*, **74**, 3325 (1970).

(2) L. I. Bone and J. H. Futrell, *J. Chem. Phys.*, 47, 4366 (1967).

(3) V. Aquilanti, A. Giardini-Guidoni, and G. G. Volpi, *Trans. Faraday Soc.*, **64**, 3282 (1968).

(4) F. H. Field, *J. Amer. Chem. Soc.*, **89**, 5328 (1967).

(5) M. S. B. Munson and F. H. Field, *ibid.*, **89**, 1047 (1967).

(6) S. Wexler and R. P. Clow, *ibid.*, **90**, 3940 (1968).

(7) S. Wexler and L. G. Pobo, *ibid.*, **91**, 7233 (1969).

(8) F. H. Field, P. Hamlet, and W. F. Libby, *ibid.*, **89**, 6035 (1967).

(9) S. G. Lias and P. Ausloos, *J. Chem. Phys.*, 37, 877 (1962).

(10) L. I. Bone, L. W. Sieck, and J. H. Futrell, *ibid.*, **44**, 3667 (1966).

(11) F. Cacace and S. Caronna, *J. Amer. Chem. Soc.*, **89**, 6848 (1967).

(12) L. M. Stock and H. C. Brown, *Advan. Phys. Org. Chem.*, **1**, 45 (1963).

(13) (a) G. A. Olah and N. A. Overchuk, *J. Amer. Chem. Soc.*, **87**, 5786 (1965); (b) "Friedel–Crafts and Related Reactions," Vol. I–IV, G. A. Olah, Ed., Interscience, New York, N. Y., 1963–1965.

(14) R. Nakane, O. Kurihara, and A. Natsubori, *J. Amer. Chem. Soc.*, **91**, 4528 (1969).