The structures of acetate and propionate of 6,7-dimethyl-9-hydroxymethylisoalloxazine (see formulas) are compatible with all these data for compounds I and II. Hydroxymethylflavin and its derivatives have not been described before.

The formation of I and II from FMF under our conditions would involve a step in which one carbon is lost, although the mechanism is likely to differ from that of the anaerobic photodegradation in which formaldehyde was demonstrated.[5]

Acknowledgments. Thanks are due to Dr. L. Dolejš and Dr. M. Buděšínský (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague) for the mass and nmr spectra and their interpretation, to Dr. J. Křížala for gas chromatography and the ir spectrum, and to Mrs. M. Škrancová and Miss V. Lankašová for technical assistance.

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Received September 24, 1971

Interpretation of the Pseudocontact Model for Nuclear Magnetic Resonance Shift Reagents. I. The Agreement Factor, R

Sir:

Controlled alteration of nmr spectra by addition of chelates of lanthanide ions has been a source of increasing excitement since Hinckley's initial report.[1] The phenomenon responsible for the signal dispersion has been discussed in terms of a contact (spin delocalized) shift, a pseudocontact (anisotropic) shift, or a combination of these two effects.[2,3] Many of the recent applications have been qualitative. In addition, graphical[3] and analytical[4,5] methods have been used to treat experimental data in a more quantitative sense. We are engaged in a systematic exploration of the applicability of the McConnell–Robertson[6] version of the pseudocontact interaction to the complete interpretation of the lanthanide-induced proton chemical shifts (LIS's)[7] in rigid oxygenated bicyclic molecules. Our approach, while similar to that employed by other workers,[4,5] offers so many advantages that we present this preliminary account.

For ease in calculation, a molecule is described with respect to an internal Cartesian coordinate system with oxygen at the origin (Figure 1). The lanthanide, L, is then moved incrementally over the surface of a sphere of radius d, the assumed L–O distance. The location of L on the sphere is described in terms of the two angles, ρ (the colatitude, measured from the positive Z axis) and ϕ (the azimuth, measured counterclockwise from the X–Z plane), as shown in Figure 1. At each lanthanide position, the variable term

$$(3 \cos^2 \theta_i - 1)/r_i^3$$

in the pseudocontact equation[6] is evaluated for all i protons. This set of numbers is then scaled by least squares against the relative observed shifts $(\Delta H/H)_{oi}$ to yield a set of calculated shifts $(\Delta H/H)_{ci}$. In order to assess the correspondence between the observed and calculated values, an agreement factor, R, is evaluated as

$$R = \left[\frac{\sum_i((\Delta H/H)_{oi} - (\Delta H/H)_{ci})^2 w_i}{\sum_i(\Delta H/H)_{oi}^2 w_i}\right]^{1/2}$$

We have used equal weighting factors, w_i, for the results in Table II. Hamilton has provided a comprehensive discussion of agreement factors of this form and their statistical interpretation.[8] The dependence of R on ρ and ϕ for a given distance, d, is conveniently displayed using contour lines on a map projection such as the Sanson–Flamsteed sinusoidal equal area projection.[9] Orientation of *endo*-norborn-5-en-2-ol as in Figure 2[10] and the resulting agreement factor plots of Figure 3 illustrate the method.

Table I shows minimum agreement values obtained

Table I. Minimum Agreement Factors Obtained for Oxygenated Hydrocarbons

Compound	R
cis-4-*tert*-Butylcyclohexanol[a]	0.043
trans-4-*tert*-Butylcyclohexanol[a]	0.081
Norcamphor	0.074
endo-Norborn-5-en-2-ol	0.034
Borneol[a,b]	0.081
Isoborneol[a,b]	0.050
Bicyclo[3.2.0]hept-3-en-2-one[c]	0.060
5-Methylbicyclo[3.2.0]hept-3-en-2-one[c]	0.080
Andamantan-2-ol[d]	0.032
Cyclooctatetraene dimer epoxide[e]	0.092

[a] P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5734 (1970). [b] This work. [c] Compound obtained from Professor R. L. Cargill, University of South Carolina. [d] G. H. Wahl and M. R. Peterson, *Chem. Commun.*, 1167 (1970). [e] M. R. Willcott, J. F. M. Oth, J. Thio, G. Plincke, and G. Schröder, *Tetrahedron Lett.*, 1579 (1971).

Table II. Comparison of Observed and Calculated Lanthanide-Induced Chemical Shifts for the System Eu(DPM)$_3$-*endo*-Norborn-5-en-2-ol

Proton	Obsd	Calcd	Proton	Obsd	Calcd
1	11.0	9.9	5	6.0	6.1
2	22.8	23.0	6	8.0	7.9
3-endo	15.2	15.2	7-syn	5.7	6.3
3-exo	10.2	10.1	7-anti	4.8	4.4
4	5.3	5.7			

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[1] C. C. Hinckley, *J. Amer. Chem. Soc.*, **91**, 5160 (1969).

[2] J. K. M. Sanders and D. H. Williams, *Tetrahedron Lett.*, 2813 (1971).

[3] C. C. Hinckley, M. R. Klotz, and F. Patil, *J. Amer. Chem. Soc.*, **93**, 2417 (1971).

[4] (a) The first account of computer simulation of a LIS proton spectrum is that for borneol–Pr(DPM)$_3$: F. A. Hart, J. Briggs, G. H. Frost, G. P. Moss, and M. L. Staniforth, *Chem. Commun.*, 1506 (1970). (b) Computer simulation of the ^{13}C LIS spectrum of isoborneol–Pr(DPM)$_3$ is also recorded: G. P. Moss, J. Briggs, F. A. Hart, and E. N. Randall, *ibid.*, 364 (1971).

[5] Another computer simulation of Eu(DPM)$_3$ induced proton chemical shifts for three compounds has been reported by S. Farid, A. Ateya, and M. Maggio, *ibid.*, 1285 (1971).

[6] H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).

[7] The descriptor LIS has been introduced into the literature by B. L. Shapiro, J. R. Hlubucek, G. R. Sullivan, and L. F. Johnson, *J. Amer. Chem. Soc.*, **93**, 3281 (1971).

[8] W. C. Hamilton, "Statistics in Physical Science," Ronald Press, New York, N. Y., 1964, pp 157–162.

[9] J. A. Steers, "An Introduction to the Study of Map Projections," University of London Press, London, 1965.

[10] C. K. Johnson, ORTEP, ORNL-3794 (Revised), Oak Ridge National Laboratory, Oak Ridge, Tenn., 1960.

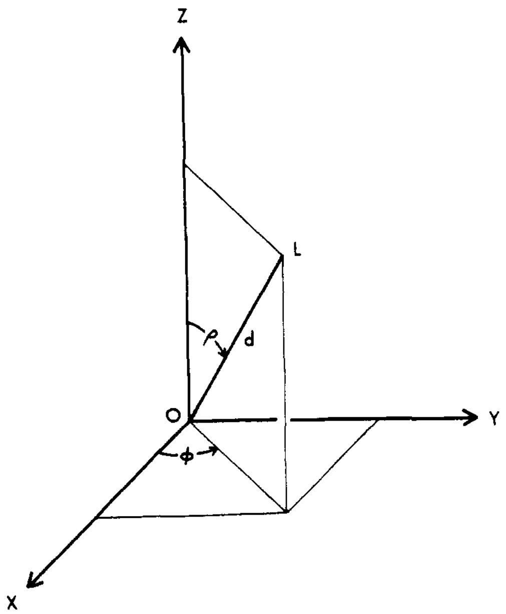

Figure 1. The coordinate system.

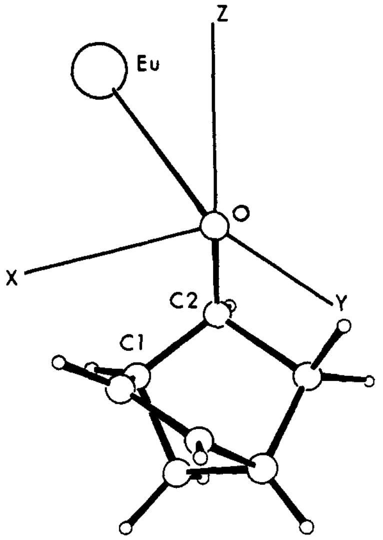

Figure 2. *endo*-Norbornenol as described with respect to the internal coordinate system. Oxygen is at the origin, the O–C-2 bond is placed along the negative *Z* axis, and atom C1 is placed in the *X*–*Z* plane with the positive *X* coordinate.

for a variety of substrates. Each substrate is a molecule of known stereochemistry for which proton nmr assignments were available. Table II gives the observed and calculated $(\Delta H/H)_i$ values for *endo*-norbornenol.

It is seen from the uniformly good agreement factors that the pseudocontact shift model provides a useful approach to describing lanthanide-induced proton nmr shifts. We find for every molecule noted in Table I that the lanthanide position corresponding to the best agreement factor is stereochemically sensible. For example, the best fit for norbornenol is at $d = 3.2$ Å, $\rho = 50°$, $\phi = 290°$, as shown in Figure 2. Both of these conclusions are in complete agreement with those drawn by Farid, *et al.*[5] However, we further note that, in general, the fit is quite insensitive to the value assumed for the lanthanide–oxygen distance, and the map projections reveal a single smooth minimum in *R* for all distances between 2.5 and 3.5 Å. Agreement factors do not differ noticeably within a small range of uncertainty in specifying atomic coordinates.[10a]

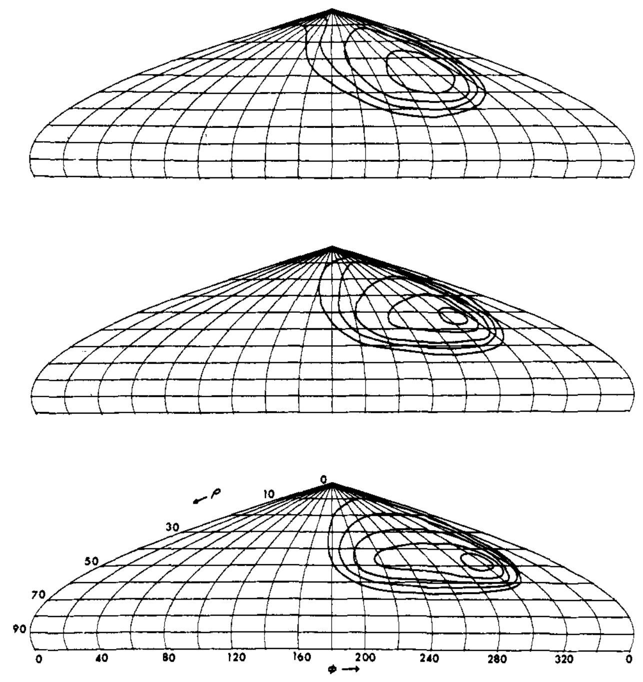

Figure 3. Plots of the agreement factor *R* for *endo*-norbornenol. Assumed O–Eu distances are, top to bottom, 2.9, 3.1, and 3.3 Å. In each case, contours are at intervals of 0.02 in *R*, with the outer contour at $R = 0.12$.

We have not yet realized all of the advantages of this approach, but the following ones have already been of considerable utility to us. (1) The geographer's plot provides a visual description of the agreement factor, permitting rapid appraisal of the effects of altering the computational parameters. (2) Internal coordinates determined from molecular models (*e.g.*, Dreiding) can be used without jeopardizing the method. Errors of *ca.* 0.1 Å cause changes of less than 0.01 in *R*. (3) Since calculated values are scaled to observed, we only require relative induced chemical shifts.[11] (4) The method is rapid. When data of reasonable precision are used directly, the nmr measurements, calculations, and interpretation require about 2 man-hr. (5) The insensitivity of *R* to most variables except structure and signal assignment make it a useful assessing function. (6) *R* is a statistically useful function, as indicated in the accompanying paper.

(10a) Note Added in Proof. The value θ_i, defined as the angle between an assumed symmetry axis of the lanthanide chelate and the vector from lanthanide to proton, is the angle χ employed by McConnell and Robertson.[6] Many restrictions are placed on the structure of the lanthanide–substrate complex by our utilization of θ_i. We justify these simplifications in the present case by the close agreement between experimental and calculated pseudocontact shifts.

(11) During these studies we have employed a variety of different methods for obtaining LIS values. Among these are the Δ_{Eu} value at 1:1 mole ratio, the slope of the line obtained from plotting the lanthanide–substrate ratio *vs.* frequency, and pairwise utilization of frequencies obtained during a dilution experiment. All of these give similar relative chemical shifts and virtually coincident *R* factors.

Acknowledgment. We acknowledge with gratitude the financial assistance provided by the Robert A. Welch Foundation (Grants E-183 and F-233).

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Received December 3, 1971

Interpretation of the Pseudocontact Model for Nuclear Magnetic Resonance Shift Reagents. II. Significance Testing on the Agreement Factor R

Sir:

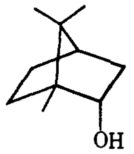
Assessment of reliability of hypotheses regarding the results of crystallographic studies by the use of significance tests on the crystallographic R factor has been described by Hamilton.[1,2] We report here several examples of this significance testing as applied to the interpretation of the lanthanide-induced proton nmr shifts (LIS).

We have described in the preceding paper[3] a straightforward method for analyzing the LIS spectrum, using the pseudocontact model for the induced shifts. We employ the minimum value of the agreement factor

$$R = \left[\frac{\sum_i \left(\left(\frac{\Delta H}{H}\right)_{oi} - \left(\frac{\Delta H}{H}\right)_{ci}\right)^2 w_i}{\sum_i \left(\frac{\Delta H}{H}\right)_{oi}^2 w_i}\right]^{1/2}$$

as a criterion for identifying the best fit of the lanthanide position. It may be noted that a minimum value of R corresponds to the *least-squares* best fit of a particular model, whether obtained by linear or nonlinear least-squares methods or by systematic variation of parameters, as in our present description. Thus, the hypothesis testing described by Hamilton can be applied here. For instance, several stereochemical models for the substrate in the LIS experiment (*e.g.*, endo or exo, cis or trans, chair or boat, etc.) can be compared to one another by the following procedure. Treatment of the observed LIS spectrum, using the coordinates for one of the several models, gives a best fit situation, characterized by a minimum R value. Statistical testing of one model against another is then accomplished by applying the R-factor ratio test to determine a confidence level at which one model may be rejected in favor of the other.[1,2] Rejection of a hypothesis at a given confidence level α % means that we risk rejecting a true hypothesis α % of the time.

Four examples illustrate the utility of this method, as applied to the LIS interpretation of three different stereochemical problems—structure, pmr signal assignment, and conformation.[4] Treatment of the pmr Eu(DPM)$_3$ spectrum of isoborneol (**1**) generates an R value of 0.050.[5] When the coordinates for borneol (**2**) are used in the treatment of the isoborneol spectrum, the minimum R value obtained is 0.445. Can we conclude that the borneol model is inconsistent with the spectrum? The one-dimensional hypothesis[1] may be formulated as: the borneol structure gives as good agreement with the data as does the isoborneol structure. The number of observations (measured proton shifts) is 11, while the number of parameters varied is 4 (three positional parameters for Eu and one scale factor between the observed and calculated LIS spectra). The number of degrees of freedom is thus $11 - 4 = 7$. An examination of a tabulation of the significance points of the R-factor ratio[1,2] shows that the minimum R-factor ratio necessary for rejection of this one-dimensional hypothesis at the 0.5% level is 1.822. The observed R-factor ratio is $0.445/0.050 = 8.90$. Hence we can reject the hypothesis at this level. Obviously the hypothesis could be rejected at a much lower level than 0.5%, but the smallest available tabulated value of α was used.



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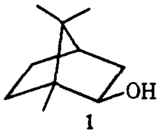
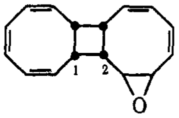


1

A similar analysis of the pmr Eu(DPM)$_3$ borneol spectrum gives minimum R values of 0.081[5] for the borneol coordinates and 0.351 for the isoborneol coordinates. The hypothesis to be tested is: the isoborneol structure is in as good agreement with the data as is the borneol structure. The observed R-factor ratio is $0.351/0.081 = 4.33$, clearly much in excess of the minimum value of 1.822 necessary to reject the hypothesis at the 0.5% level.



3

We can also apply this method to a case in which the spectrum for only one isomer is available, but in which the LIS spectrum alone permits assignment of stereochemistry—the cyclooctatetraene dimer epoxide **3** reported by Willcott, *et al.*[6] The stereochemical question here concerns the disposition of the epoxide ring (syn or anti with respect to the proton at carbon 2). Four models were tested, with the results shown in Table I. Let us make the hypothesis: the anti isomer

Table I. Models Tested for Cyclooctatetraene Dimer Epoxide

	R
(Epoxide ring — H-2) syn planar cyclobutane	0.092
(Epoxide ring — H-2) syn nonplanar cyclobutane	0.119
(Epoxide ring — H-2) anti planar cyclobutane	0.341
(Epoxide ring — H-2) anti nonplanar cyclobutane	0.342

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[1] W. C. Hamilton, "Statistics in Physical Science," Ronald Press, New York, N. Y., 1964, pp 157–162.

[2] W. C. Hamilton, *Acta Cyrstallogr.*, **18**, 502 (1965).

[3] M. R. Willcott, R. E. Lenkinski, and R. E. Davis, *J. Amer. Chem. Soc.*, **94**, 1742 (1972).

[4] In each of these examples equal weights were given to all shifts (*i.e.*, $w_i = 1$ for all i).

[5] The data used for this computation were obtained in our laboratory, but are essentially those reported by P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, **92**, 5734 (1970).

[6] M. R. Willcott, J. F. M. Oth, J. Thio, G. Plincke, and G. Schroder, *Tetrahedron Lett.*, 1579 (1971).