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

Sir:

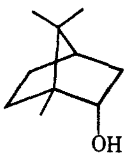
Assessment of reliability of hypotheses regarding the results of crystallographic studies by the use of significance tests on the crystallographic R factor has been described by Hamilton.[1,2] We report here several examples of this significance testing as applied to the interpretation of the lanthanide-induced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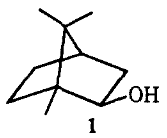

1

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.

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

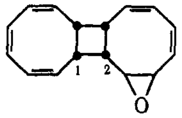

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Table I. Models Tested for Cyclooctatetraene Dimer Epoxide

	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).

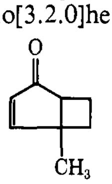
(4) In each of these examples equal weights were given to all shifts (*i.e.*, w_i = 1 for all i).

(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).

is in as good agreement with the data as is the syn isomer. The number of degrees of freedom is 16 − 4 = 12. The minimum value of the *R*-factor ratio to reject the hypothesis at the 0.5% level is 1.407; the experimental ratio, 0.341/0.092 = 2.89, is large enough to strongly reject the hypothesis. We conclude that the epoxide ring is in the syn arrangement, but we cannot distinguish the planar from the nonplanar cyclobutane model at the 0.5% level.

In one of our early attempts to fit the LIS spectrum of 5-methylbicyclo[3.2.0]hept-3-en-2-one (**4**) the methyl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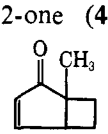
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group was misplaced at carbon 1 (**5**), giving a best fit of $R = 0.129$. When the methyl group was correctly placed at carbon 5, the R value obtained was 0.080. The 1-methyl possibility can be rejected in favor of the 5-methyl isomer at about the 5% level, using the significance tests.

It should be noted that this method of significance testing gives a *statistical* measure of the confidence at which certain hypotheses can be rejected. Any other information which can be brought to bear on the problem must also be used. For example, our confidence in rejecting the 1-methyl isomer in the choice between structures **4** and **5** is further heightened by the observation that the improbably short O–Eu distance of 2.0 Å was necessary to obtain the 0.129 fit. We must also note that the method does not test whether the model accepted is the *best possible* model—only whether it is preferred, at a determined confidence level, over another proposed model. For instance, even though the planar syn model of **3** is preferred at the 2.5% level, it is still possible that a statistically better fit would be obtained with another conformation of the syn structure or with some mixture of several conformations.

The foregoing applications are illustrative of the power of the method and suggest several obvious extensions. It is already clear to us that monofunctional compounds need not have a complete set of proton LIS data and that refinement of partial sets of data can be used to define local stereochemistry and to identify unassigned resonances. Polyfunctional molecules are also amenable to this general treatment. The technique will prove useful in conformational analysis of flexible molecules, since the *R*-factor ratio test assesses the validity of several conformational models. Finally, appropriate statistical treatment provides an approach to the frequently perplexing question about whether the LIS method perturbs conformational equilibria. These problems are presently under investigation.

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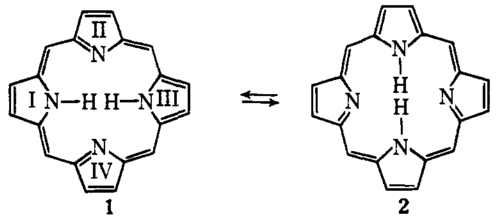
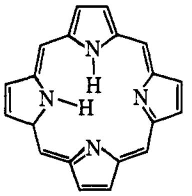
Nitrogen–Hydrogen Tautomerism in Porphyrins and Chlorins

Sir:

The question of N–H tautomerism in porphyrins has been under active consideration ever since the first definition of the basic porphyrin structure by Fisher and Küster. A variety of spectroscopic[1] and chemical[2] methods have been used in attempts to decide as to what is the most appropriate way to represent the porphyrin structure. Currently structures **1** and **2** are most favored, being in rapid equilibrium, although structure **3** is still used by some authors.[3]



1 **2**



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We wish to report here the results of low-temperature proton magnetic resonance (pmr) studies which indicate that we have frozen out the tautomerism between **1** and **2**. Furthermore, it appears that a similar facile isomerism takes place in chlorins, a possibility not previously considered to our knowledge.

Figure 1 presents the pmr spectra of the β-pyrrole hydrogens of α,β,γ,δ-tetraphenylporphin (TPP) at several temperatures.[4] At 30° a single fairly sharp peak is obtained (δ 8.72) for the eight β-pyrrole protons, suggesting a C_4 axis for the molecule, rather than the C_2 axis indicated by **1**. As the temperature is lowered the peak broadens, reaching a coalescence point at −40° and giving two distinct peaks at −80° (δ 8.61, 8.90). The peak at δ 8.90 is assigned to the protons on pyrrole rings I and III in **1** and the peak at δ 8.61 is assigned to the protons on rings II and IV in **1**. The broadening of the δ 8.90 peak is due to long-range coupling between an N–H proton and the β-pyrrole protons. Figure 2 shows the result of a double resonance experiment, at −80°, where the N–H protons (δ −3.33) were irradiated while observing the β-pyr-

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[1] (a) G. D. Dorough and K. T. Shen, *J. Amer. Chem. Soc.*, **72**, 3939 (1950); (b) C. Vestling and J. Downing, *ibid.*, **61**, 3511 (1939); (c) E. D. Becker, R. B. Bradley, and C. J. Watson, *ibid.*, **83**, 3743 (1961); (d) E. B. Fleischer, *Accounts Chem. Res.*, **3**, 105 (1970).

[2] (a) S. Aronoff and M. Calvin, *J. Org. Chem.*, **8**, 205 (1943); (b) J. G. Erdman and A. H. Corwin, *J. Amer. Chem. Soc.*, **68**, 1885 (1946).

[3] (a) R. E. Dickerson, H. B. Gray, and G. P. Haight, "Chemical Principles," W. A. Benjamin, New York, N. Y., 1970, p 414; (b) B. E. Douglass and D. H. McDaniel, "Concepts and Models of Inorganic Chemistry," Blaisdell Publishing Co., Waltham, Mass., 1965, p 337.

[4] The pmr spectra were obtained at 100 MHz on a 0.005 *M* solution in a 12-mm sample tube using CS_2 as a solvent. The temperature was calibrated using CH_3OH. Chemical shifts are reported in parts per million using the δ scale relative to TMS internal standard (TMS = δ 0). At this dilution the chemical shift of the porphyrin protons showed no temperature dependence.