revolutions per second, it is clear that an accurate measurement of the instantaneous speed is essential at very low field strengths. In this equation, $\bar{v}$ is the partial specific volume of the virus, ρ the density of the solution, f the activity coefficient at concentration c in g./100 ml., x the distance in centimeters of a position in the solution from the center of rotation, T the absolute temperature and R the molar gas constant.

The magnetically suspended ultracentrifuge[2] fulfills the requirement for very low speeds without rotor hunting. Accordingly, a detailed study of several viruses has been initiated. Results with southern bean mosaic virus (SBMV) and with bushy stunt virus (BSV) are here presented to establish applicability of this method.

Sedimentation was carried out in 3 mm. columns of solution by the two-speed method for rapid attainment of equilibrium.[3] For SBMV, the initial speed was held at 15.0 rps. for 24 hours before decelerating to 10.5 rps. Equilibrium was attained within 5 hours after cutback in speed; the experiment was allowed to continue longer, but no change in the Jamin interference fringe pattern was observed. A plot of the logarithm of the concentration *versus* the square of the distance from the center of rotation was linear to the bottom of the cell. Using a value of $\bar{v}$ = 0.696 ml./g.,[4] ρ = 1.0049 g./ml. for the virus in 0.075 *M* KCl–0.005 *M* phosphate (K), *p*H 7.0 (total ionic strength = 0.10) and an initial virus concentration, c_0, of 0.35% from an assumed specific refractive increment of 0.0019 ml./0.01 g. at 546 mμ, the apparent molecular weight of this preparation at 20.00 ± 0.01° is 6.3 × 10^6.

Linear ln c *vs.* x^2 plots at this temperature also were obtained with BSV in the same solvent. The apparent molecular weight, both at c_0 = 0.12% and 0.32% was 7.8 × 10^6 where $\bar{v}$ was assumed to be 0.739 ml./g.[5] In these experiments, the initial speed was held at 20.0 rps. for 22 hours for the lower concentration before decelerating to 13.9 rps., whereas for the higher concentration the same initial speed was held for 21 hours before decelerating to 12.4 rps.

The absence of upward curvature in the ln c *vs.* x^2 plots attests to the purity of the samples.[6] Also, the schlieren patterns by velocity sedimentation in the Spinco Model E ultracentrifuge gave no indication of polydispersity. For the purpose of identification, the sedimentation coefficient corrected to that in water at 20° of SBMV (0.35%) was 117 × 10^{-13} sec. and that of BSV (0.12%) was 136 × 10^{-13} sec.

These data indicate that the magnetically suspended ultracentrifuge is very well suited for further thermodynamic studies on viruses and large polymers.

It might be noted that for rotor speeds much below 10 rps. where the ratio of the gravitational to the centrifugal field no longer is small the centrifuge cell can be so hinged in the rotor that the flat surfaces of the cell windows automatically are approximately parallel to the resultant of the two fields in the cell.

We are indebted to Dr. Russell L. Steere of the U. S. Department of Agriculture, Beltsville, Maryland, for these samples of purified virus and to Mr. T. E. Dorrier for special assistance. This work was supported by a Grant (A-3118) from the U. S. Public Health Service; H. G. K. is an American Machine and Foundry Fellow, and R. F. B. is a postdoctoral fellow of the U. S. Public Health Service.

DEPARTMENT OF PHYSICS AND
DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE
UNIVERSITY OF VIRGINIA
CHARLOTTESVILLE, VIRGINIA

P. E. HEXNER
D. W. KUPKE
H. G. KIM
F. N. WEBER, JR.
R. F. BUNTING
J. W. BEAMS

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COMMENTS ON THE SIGNS OF PROTON COUPLING CONSTANTS

Sir:

A number of recent papers[1] concerned with the spin–spin coupling of non-bonded protons have contained experimental results that appear to be inconsistent with certain quantitative aspects of the approximate valence-bond theory.[2] The essential point is that in the compounds studied the relative signs of the geminal (1,1) and vicinal (1,2) proton coupling constants were determined to be of opposite sign,[3] while the simple fragment-model theory indicates that the constants should be of like sign if reasonable values for the bond angles are assumed.

Although a detailed comparison of theory and experiment is complicated by a number of uncertain factors (*e.g.*, effect of substituents, lack of knowledge of bond angles) the most important problem is that only *relative* signs are provided by the available data, in contrast to the absolute signs obtained from the calculations. Thus, the observed difference in relative signs for the geminal and vicinal couplings does not show which theoretical results are in disagreement with experiment. In this Communication, we wish to suggest a method for obtaining an indication of the absolute sign of a proton coupling constant

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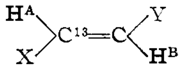
[1] R. R. Fraser, R. V. Lemieux and J. D. Stevens, *J. Am. Chem. Soc.*, **83**, 3901 (1961) (dioxolanes); C. A. Reilly and J. D. Swalen, *J. Chem. Phys.*, **35**, 1522 (1961) (epichlorohydrin); F. Kaplan and J. D. Roberts, *J. Am. Chem. Soc.*, **83**, 4668 (1961) (diethyl sulfite); R. Freeman, K. McLauchlan, K. Pachler and J. Musher, private communication (2,3-dibromopropionic acid); S. Manatt, private communication (alkyl ethers); H. S. Gutowsky, private communication (bridge in 2,2-metacyclophane).

[2] (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) H. Gutowsky, M. Karplus and D. M. Grant, *ibid.*, **31**, 1278 (1959); (c) S. Alexander, *ibid.*, **34**, 106 (1961).

[3] The opposite relative sign is in agreement with that found for the coupling between an atom X (X = Sn, P, Hg, etc.) and geminal *versus* vicinal protons. See, for example, P. T. Narasimhan and M. Rogers, *J. Chem. Phys.*, **31**, 1430 (1959); for X = F, the constants apparently have been shown to be of the same sign by S. L. Stafford and J. D. Baldeschwieler, *J. Am. Chem. Soc.*, **83**, 4473 (1961).

and to present some comments on what might be significant approximations in the theory.

It is known that the absolute signs of coupling constants in molecules can be determined, in constants in molecules can be determined by appropriate methods, such as molecular beam resonance techniques.[4] However, the difficulty of making direct measurements for many molecules of chemical interest suggests consideration of a somewhat indirect approach. The theoretical results for the sign of the spon-coupling constant between directly-bonded atoms are considerably more straightforward than those for nonbonded species. From the detailed calculations[5] for H_2 and the more approximate treatments of the contact interaction in complex systems (*e.g.*, C–H, B–H)[6] it is very probable that the sign of the coupling constant is *positive* for covalently bonded atoms. (Here the positive sign is taken to signify that an antiparallel nuclear spin orientation corresponds to a more stable state than the parallel orientation.) This result suggests that an "absolute" sign determination could be made by a measurement of the sign of a non-bonded proton-proton coupling constant relative to the coupling constant between a proton and a directly-bonded atom. By the means of the double-irradiation technique[7] that was first applied to relative sign determinations by Evans and Maher[8] and has been effectively exploited more recently by Freeman and Whiffen,[9] Manatt,[10] and others, the required experiment should be feasible. For the sake of illustration, we discuss here the simplified example of the first-order spectrum of a substituted ethylene



where X and Y are non-magnetic nuclei. In such a system, experimental data[11] indicate that the approximate coupling constants are $J(C^{13}, H^A) \cong 160$ cps., $|J(C^{13}, H^B)| \cong 2.5$ cps., and $|J(H^A, H^B)| \cong 20$ cps. The C^{13} spectrum consists of two $J(C^{13}, H^B)$ doublets separated by 160 cps., due to $J(C^{13}, H^A)$. Correspondingly, the H^B spectrum consists of the two $J(C^{13}, H^B)$ doublets separated by the 20 cps. splitting of $J(H^A, H^B)$. If one irradiates at the appropriate frequency to collapse the up-field doublet in the C^{13} spectrum, the up-field doublet in the H^B spectrum will be affected if $J(H^A, H^B)$ and $J(C^{13}, H^A)$ are of the same sign, and the down-field doublet will be affected if $J(H^A, H^B)$ and $J(C^{13}, H^A)$ are of opposite sign [independent of the sign of $J(C^{13}, H^B)$]. Thus, one obtains an "absolute" sign determination of $J(H^A, H^B)$. A corresponding experiment can be done to obtain the sign of the geminal hydrogen coupling by use of a system such as $HDC^{13}X_2$ with X an appropriate substituent (*e.g.*, CN). By looking at the D resonance while irradiating the C^{13} resonance so as to affect one or the other of the $J(D, C^{13})$ doublets, the sign of $J(H,D)$ relative to $J(D, C^{13})$ could be determined. Thus, the "absolute" sign of $J(H,D)$ and, consequently, of $J(H,H)$ for geminal hydrogens would become available.

The examples and method[12] outlined above are given only to focus attention on the general nature of the measurements that should be done. Depending on the compounds available and the ingenuity of the experimenter, a variety of alternatives will easily suggest themselves. Ideally, the $J(C^{13}, H)$ *vs.* $J(H,H)$ sign would be determined in hydrocarbon systems with few or no strongly perturbing substituents and known valence angles for the protons involved. Since the most detailed geminal coupling calculations were done for methane, a study of $C^{13}H_nD_{4-n}$ would be of great interest. Also, it would be useful to have signs relative to the directly-bonded coupling for the geminal and vicinal constants in the same molecule.

Until the appropriate measurements have been carried out, no absolute comparison between experiment and theory is possible. However, the presently available results show that, while the theory is qualitatively correct, it may yield values for the coupling constants that are quantitatively in error. Although there are a large number of approximations in the valence-bond method, the inadequacy being considered here can probably be traced to the sensitive dependence of the sign and magnitude of the geminal constants on the differences between a number of effective exchange integrals. As is evident from Eq. (7) of ref. 2(b) for the geminal coupling,[13] a cancellation of terms occurs and rather minute changes in the contributing integrals can significantly alter the result. Certain cancellations also are found in the vicinal coupling (particularly for the dihedral angles close to 90°), which may be expected therefore to deviate somewhat from Eq. (11) of ref. 2(a).

A comparison of the two sets of fragment-model calculations (ref. 2a and 2b) indicates that the geminal coupling treatment is more likely to yield significantly inaccurate results, such as the wrong sign when applied to the compounds referred to in Reference 1. An additional fact to be considered is that in some compounds that have been studied (*e.g.*, ethylene, some vinyl groups), the geminal and vicinal couplings have the same (probably positive) sign. These results seem to indicate that a number of factors (*e.g.*, nature of substituents,

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(11) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Company, New York, N. Y., 1959; R. Truscott and N. Sheppard, private communication.

(12) Instead of the double-resonance technique, the more "old-fashioned" method for determining relative signs by consideration of the higher order spectrum could of course be used in appropriate systems.

(13) The cancellation involved in geminal couplings probably is responsible for the fact that $|J^{\text{gem}}_{\text{HX}}| < |J^{\text{vic}}_{\text{HX}}|$ for the results mentioned in ref. 3.

lack of orbital following, types of neighboring bonds), other than those included in the simple model, are important in determining the extent of the deviation from perfect pairing that occurs in a given system. Confirmation of these suggestions will have to await experimental measurements corresponding to those outlined here, as well as applications of the theory to more refined molecular wave functions.

DEPARTMENT OF CHEMISTRY
AND WATSON LABORATORY
COLUMBIA UNIVERSITY
NEW YORK 27, N. Y.
MARTIN KARPLUS

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ORGANOSULFUR DERIVATIVES OF METAL CARBONYLS. I. THE ISOLATION OF TWO ISOMERIC PRODUCTS IN THE REACTION OF TRIIRON DODECACARBONYL WITH DIMETHYL DISULFIDE

Sir:

Compounds of the general formula $[Fe(CO)_3SR]_2$ have been isolated in the reaction of triiron dodecacarbonyl with dialkyl sulfides, alkyl mercaptans, and dialkyl disulfides.[1-3] I wish to report the separation of the methyl derivative into two isomeric compounds.

A mixture of 84 g. (0.167 mole) of triiron dodecacarbonyl, 150 ml. of dimethyl disulfide, and 1 liter of thiophene-free benzene was refluxed for 6 hr. under nitrogen with magnetic stirring. After cooling to room temperature, the reaction mixture was filtered giving a red filtrate and a black pyrophoric residue. Solvent was removed from the red filtrate at 30 mm. leaving about 28 g. (27% yield) of red crystals which may be purified either by recrystallization from pentane or by sublimation at 50° (0.1 mm.).

Samples of either recrystallized or resublimed $[Fe(CO)_3SCH_3]_2$ prepared in the above manner showed in the proton n.m.r. three methyl resonances at 2.13, 2.07 and 1.62 p.p.m.[4] of varying intensities suggesting that the product was a mixture of isomers. It was found possible to separate the product into two isomers by chromatography in pentane solution on a 2 × 110 cm. alumina column. This gave rise to two very distinct bands on the column, a large red band followed by a smaller orange band. Each band was eluted with pentane and the air-stable eluates were evaporated to dryness. From 3 g. of the original mixture about 2.4 g. of red crystals, m.p. 65–67.5°, hereafter designated as Isomer A, was isolated from the first red band and about 0.2 g. of orange crystals, m.p. 101.5–103.5°, hereafter designated as Isomer B, was isolated from the orange band.

Analyses showed Isomers A and B to have the same composition (Calcd. for $C_8H_6O_6S_2Fe_2$: C, 25.7; H, 1.6; S, 17.1; Fe, 29.9. Found on Isomer A: C, 25.4; H, 1.8; S, 17.2; Fe, 29.0. Found on Isomer B: C, 26.1; H, 1.9). The infrared spectra also were quite similar although not identical (Isomer A: 2085 (s), 2050 (vs), 2000 (vs), 1428 (sh), 1420 (w), 1310 (sh), 1303 (w), 1260 (vvw), 967 (w), 964 (m) and 710 (vw) cm.$^{-1}$; Isomer B: 2075 (s), 2040 (vs), 2000 (vs), 1995 (sh), 1430 (m), 1318 (m), 1260 (vw), 966 (w), 959 (w) and 703 (vw) cm.$^{-1}$). The proton magnetic resonance spectra provide information as to the nature of these isomers. Isomer A was found to exhibit two methyl peaks at 2.13 and 1.62 p.p.m.[4] in a 1:1 intensity ratio indicating each of the two methyl groups to be different. However, Isomer B was found to exhibit a single methyl peak at 2.07 p.p.m. indicating both methyl groups to be identical.

The absence of carbonyl bands in the 1700–1850 cm.$^{-1}$ region of the infrared spectrum indicates the absence of carbonyl bridging in each of the two isomers. In view of this and in view of the diamagnetism of each isomer as evidenced by the ability to obtain high resolution n.m.r. spectra, the most reasonable structure for each of the two isomers contains an iron–iron bond and two RS-bridges. The two isomers differ therefore only in the relative orientations of the methyl groups attached to the sulfur atoms. From the n.m.r. data it is apparent that the two methyl groups of Isomer A are in different positions but that the two methyl groups of Isomer B are in identical positions. However, on the basis of the presently available information on these compounds, the exact locations of the methyl groups and the nature of the iron-iron bonds in each of the isomers are still uncertain.

EXPLOSIVES DEPARTMENT
EXPERIMENTAL STATION LABORATORY
E. I. DU PONT DE NEMOURS & COMPANY
WILMINGTON, DELAWARE
R. B. KING[5]

RECEIVED MAY 2, 1962

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[5] Mellon Institute, Pittsburgh 13, Pennsylvania.

THE SYNTHESIS OF A HEPTADECAPEPTIDE POSSESSING ADRENOCORTICOTROPIC, MELANOTROPIC AND LIPOLYTIC ACTIVITIES

Sir:

Adrenocorticotropins (ACTH) isolated from pituitaries of various species[1,2,3,4] are polypeptides consisting of 39 amino acids. Since the first synthesis of a biologically active nonadecapeptide corresponding to the first 19 amino acid residues of the hormone was published,[5] two other laboratories[6,7,8] have described the synthesis of ACTH analogs with chain lengths of 19, 20, 23 and 24

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