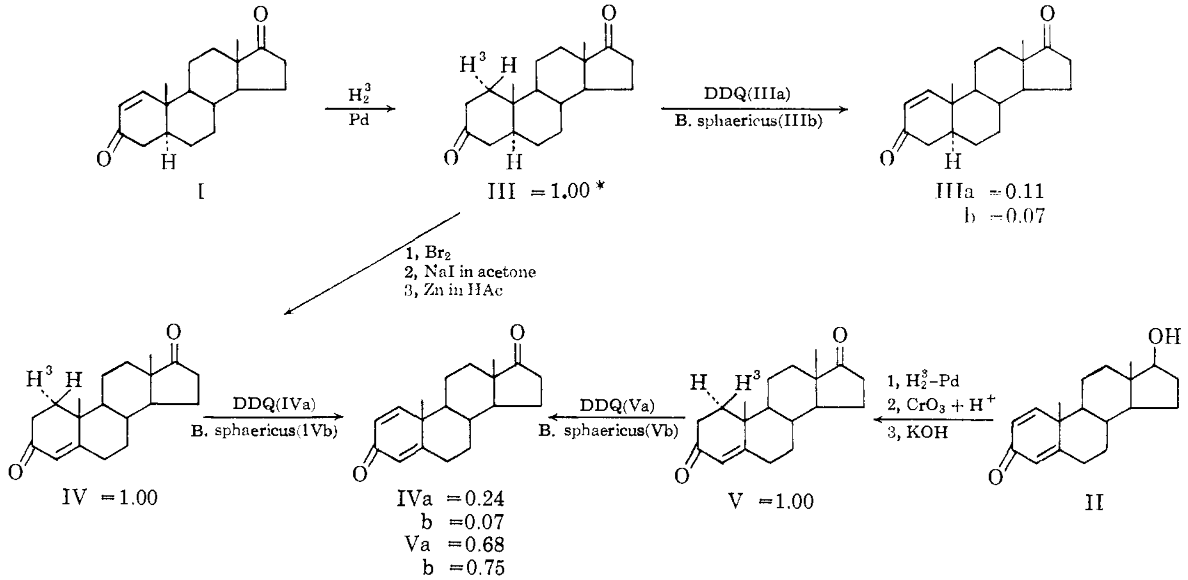

* Numbers refer to the relative specific activities of reactants and products.

from the mixture by paper chromatography, oxidized to the diketone using the Jones method[9] and equilibrated with base to give V. Compound V was oxidized both with DDQ to give compound VA (68% retention of tritium) and with *B. sphaericus* to give compound VB (75% retention of tritium). The remaining tritium was shown to be at C-1 by oxidizing another sample of compound V to androsta-1,4,6-triene-3,17-dione using chloranil[10] and then DDQ.[5] When this compound was treated with acid it rearranged to 1-methylestrone acetate which was inactive (specific activity<0.01%). In this reaction, the methyl group originally at C-10 displaces the remaining hydrogen at C-1 (dienone-phenol rearrangement[11]).

Because the oxidation with *B. sphaericus* is essentially specific for the elimination of the 1α-hydrogen, this reaction shows that the previous reduction of androst-1-ene-3,17-dione with tritium to the saturated compound gives 93% 1α-tritium and 7% beta and the reduction of the $\Delta^{1,4}$ compound gives 25% 1α-tritium and 75% beta. That androsta-1,4-diene-3,17-dione is reduced preferentially from the beta side probably is due to the planarity of ring A resulting from the dieneone structure. A consideration of Dreiding models shows that the planar ring A is tilted downward away from the beta angular methyl group at C-10 and toward the alpha axial hydrogen at C-9. Thus approach of catalyst may be hindered more by the C-9 hydrogen on the alpha face than by the methyl group on the beta face.

Reductions of $\Delta^{1,4}$ compounds further substituted on the beta face, *e.g.*, Δ^1-cortisol in which there is a hydroxyl group at C-11 indicates that here the alpha face may be attacked preferentially.[12] Thus for the reduction of $\Delta^{1,4}$ compounds it is difficult, *a priori*, to predict on which side of the molecule reduction will occur to the greater extent. Accessibility to either face can be changed by slightly modifying the molecule. A study of the effect of various groupings on the steric course of the reduction is being carried out.

Acknowledgment.—This work was supported in part by U. S. Public Health Service grants A-2672, A-3419 and Training Grant CRTY-5001.

[9] A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2554 (1953).

[10] E. J. Agnello and G. D. Laubach, *J. Am. Chem. Soc.*, **82**, 4293 (1960).

[11] Ref. 1, p. 328.

[12] Inspection of a Dreiding model shows severe non-bonded interaction between the 11β-hydroxyl and the 10β-methyl group which may force the latter to bend closer over ring A, thereby preventing reduction of the C-1,2 double bond from the beta face.

[13] Post-doctorate trainee in The Training Program for Steroid Biochemistry.

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS.

HARRY J. BRODIE[13]
MIKA HAYANO
MARCEL GUT

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SIGNS OF PROTON COUPLING CONSTANTS

Sir:

The recent findings[1] of opposite relative signs of J_{vic-HH} and J_{gem-HH} in typical ethane derivatives are in disagreement with the theoretical predictions[2] that both constants should be positive. Since the experimental and calculated coupling constants are in numerical agreement, it is not immediately obvious where the error lies.

The calculations, particularly of J_{gem}, involve small differences between large terms, and therefore may not be reliable. On the other hand, calculations[3] of magnitudes and signs of the larger coupling constants of directly bonded nuclei (*e.g.*, C^{13}-H) should be more secure. It was therefore of interest

[1] R. R. Fraser, R. U. Lemieux and J. D. Stevens, *J. Am. Chem. Soc.*, **83**, 3901 (1961); F. Kaplan and J. D. Roberts, *ibid.*, **83**, 4666 (1961); F. A. L. Anet, *ibid.*, **84**, 1053 (1962); A. McLauchlan and D. H. Whiffen, *Proc. Chem. Soc.*, 144 (1962); C. A. Reilly and J. D. Swalen, *J. Chem. Phys.*, **35**, 1522 (1961).

[2] M. Karplus and D. H. Anderson, *ibid.*, **30**, 6 (1959); H. S. Gutowsky, M. Karplus and D. M. Grant, *ibid.*, **31**, 1278 (1959); M. Karplus, *ibid.*, **30**, 11 (1959).

[3] M. Karplus and D. M. Grant, *Proc. Nat. Acad. Sci.*, **45**, 1269 (1959); N. Muller and D. E. Pritchard, *J. Chem. Phys.*, **31**, 768, 1471 (1959); J. N. Shoolery, *ibid.*, **31**, 1427 (1959); N. Muller, *ibid.*, **36**, 359 (1962).

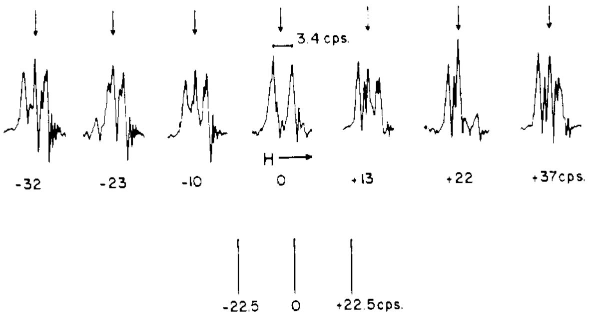

Fig. 1.—Top: spectra of the C^{13} satellite of the methylene protons (60 Mc./sec.) of CH$_2$DOH under conditions of rapid exchange of the hydroxyl protons and with double-irradiation at about 15.1 Mc./sec. The frequency of the second radio-frequency field relative to the resonance frequency of the center C^{13} resonance is shown under each spectrum. The arrows correspond to the center of the satellite in the unperturbed spectrum. Bottom: diagrammatic representation of the center triplet of the C^{13} spectrum of CH$_2$DOH at constant field.

to determine the relative signs of $J_{\mathrm{C^{13}H}}$ and $J_{gem-\mathrm{HH}}$ (or $J_{vic-\mathrm{HH}}$). This,[4] in effect, would allow an assignment of absolute signs to the experimentally determined J_{vic}, J_{gem} and other proton–proton coupling constants.

By using a modified spin-decoupling technique, we have now found that $J_{\mathrm{C^{13}H}}$ and $J_{gem-\mathrm{HD}}$ (and hence $J_{gem-\mathrm{HH}}$) are of opposite signs in CH$_2$DOH. If the proton spectrum is to be observed, the double-irradiation method[5] for the determination of relative signs would involve, in the present case (or in simpler systems, *e.g.*, RRC^{13} DH), "decoupling" the C^{13} for a particular deuterium spin state. This is not possible, because of the relative magnitudes of the coupling constants (*e.g.*, $J_{\mathrm{C^{13}-H}}$,140.6; $J_{\mathrm{C^{13}-D}}$, 22.5; J_{HD}, 1.7 cps. for CH$_2$DOH). However, the desired result can be achieved if the C^{13} is merely perturbed for a particular deuterium spin state, but not for others. This can be done by irradiating the C^{13} with a relatively weak magnetic field H'' ($\gamma_{\mathrm{C^{13}}}\mathrm{H}''/2\pi << J_{\mathrm{C^{13}-D}}$) at a frequency corresponding to a single C^{13} transition. The effect on the proton spectrum then can be calculated from the double-irradiation theory of Bloom and Shoolery.[6]

If the proton spectrum is observed by a frequency-sweep method, the affected lines (*e.g.*, the C^{13} satellites of CHCl$_3$) are symmetrically split by about $\gamma_{\mathrm{C^{13}}}H''/2\pi$, when the frequency of the C^{13} decoupling field is equal to either of the two C^{13} transitions. Alternatively, if the proton spectrum is observed by a field-sweep method, and the frequency of the C^{13} field is equal to either of the two C^{13} transitions when the magnetic field corresponds to the proton resonance of one of the C^{13} satellites, then that C^{13} satellite will be split[7] into a doublet. The other C^{13} satellite will not be affected if $\gamma_{\mathrm{C^{13}}}H''/2\pi << J_{\mathrm{C^{13}-H}}\,\gamma_{\mathrm{C^{13}}}/\gamma_{\mathrm{H}}$.

In the double-irradiation experiments on CH$_2$DOH the C^{13} perturbing field was such that $\gamma_{\mathrm{C^{13}}}H''/2\pi$ = 3–4 cps., and the high-field C^{13} satellite of the methylene protons was observed by the usual field-sweep method with the magnetic field increasing from left to right. The extent of the field-sweep (about 4 cps.) in observing the spectrum corresponds to a shift of the C^{13} resonance of only 1 cps., and thus does not lead to complications. Because the frequency of the perturbing field is constant to only ± 1 cps. in the present experiments, the lines of the doublet are broadened and have much less than their correct heights. It is, however, very easy to see which line of the original triplet is being perturbed (*i.e.*, split into a doublet), as this line disappears from the spectrum. The nine frequencies of the C^{13} resonance were located by such experiments and showed the correct spacings. Figure 1 shows the effect when the frequency of the decoupling field is in the neighborhood of the center triplet of the C^{13} resonance. The three lines of the proton spectrum, and likewise of the C^{13} spectrum, arise from the three possible (+1, 0, −1) deuterium spin states. When the frequency corresponds to the low-, middle- or high-frequency lines of the C^{13} triplet, it is the low-, middle-, or high-field lines, respectively, of the proton spectrum which are perturbed. Frequencies which are different from these by more than 10 cps. are without appreciable effect. Since a low-field line in a sweep-field spectrum corresponds to a high-frequency line in a frequency-sweep spectrum, and *vice versa*, the coupling constants $J_{\mathrm{C^{13}-H}}$ and $J_{gem-\mathrm{HD}}$ are of opposite signs. Since the magnetic moments of H and D have the same signs, $J_{\mathrm{C^{13}-H}}$ and $J_{gem-\mathrm{HH}}$ are also of opposite signs. If the positive sign of $J_{\mathrm{C^{13}-H}}$ is accepted, then $J_{gem-\mathrm{HH}}$ is negative in methanol, and probably in general, provided the carbon atom concerned is sp^3 hybridized. Thus, the calculated sign of the geminal coupling constant is in disagreement with experiment.

The spectra were taken on a Varian V-4302 spectrometer equipped with a "spin-decoupler" made by NMR Specialties. I wish to thank Dr. L. Leitch for a sample of deuteriated methanol.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OTTAWA
OTTAWA, ONT., CANADA
F. A. L. ANET

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(4) Dr. Martin Karplus (private communication) has reached similar conclusions and has suggested experiments making use of the normal[5] double-irradiation method for relative sign determination.

(5) D. F. Evans and J. P. Maher, *Proc. Chem. Soc.*, 208 (1961).

(6) A. L. Bloom and J. Shoolery, *Phys. Rev.*, **97**, 1261 (1955). See also R. Freeman and D. H. Whiffen, *Proc. Phys. Soc.*, **79**, 794 (1962); J. D. Baldeschwieler, *J. Chem. Phys.*, **36**, 152 (1962).

(7) *This has been confirmed experimentally.*

THE DIRECT MEASUREMENT OF THE RATE OF A HAPTEN-ANTIBODY REACTION

Sir:

We wish to report the direct measurement of the rate of the bimolecular reaction of a hapten (Hp) and its specific antibody (Ab). Such reactions generally are so rapid that in an earlier attempt to measure the rate by a spectrophotometric stopped-flow method, we found that half or more of the reaction was completed by the time measurements could be started.[1] Recourse recently has been had to equilibrium perturbation methods of measurement,[2] and while such methods

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(1) J. M. Sturtevant, L. Wofsy and S. J. Singer, *Science*, **134**, 1434 (1961).

(2) A. Froese, M. Eigen and A. Sehon, *Can. J. Chem.*, in press.