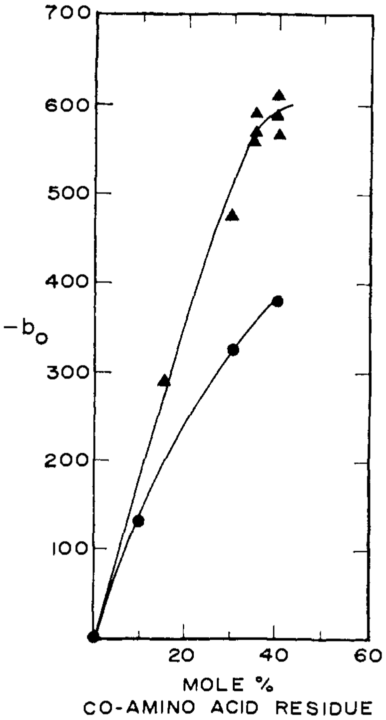

Fig. 2.—The dependence of b_0 (calculated from the Moffitt equation[5] over the wave length range 313 to 578 mμ using λ_0 = 212 mμ) upon amino acid composition for a series of high molecular weight copolypeptides derived from γ-N-[2-morpholinylethyl]-α,L-glutamamide and L-alanine, ●-●-●; poly-γ-N-[2-morpholinylethyl]-α,L-glutamamide and L-methionine, ▲-▲-▲. The solvent in all cases was water containing 0.2 *M* NaCl. All measurements were performed at 25 ± 1° except those on the copolypeptides containing 30, 35 and 40% L-methionine with which compounds the measurements were made at 5 ± 1°.

cent. alanine or methionine were water-soluble. Some rotatory dispersion data of the water-soluble copolypeptides are shown in Fig. 2, where b_0 is plotted as a function of mole per cent. coamino acid residue content. The solubility of the copolypeptides containing 30 or more per cent. L-methionine is increased at lower temperatures so the dispersion measurements on these compounds were made at 5°. The data reveal that, by incorporation of ~40% L-methionine in I, it is possible to obtain an essentially completely helical water-soluble synthetic polypeptide.

Other experiment were performed with the copolypeptide containing 30 mole percent L-alanine which, in 0.2 *M* NaCl, had a b_0 value of −331 indicating slightly more than 50% helix content. When this copolypeptide's rotatory dispersion was measured in 8 *M* urea or in 8.6 *M* lithium bromide solution, the b_0 value was found to be zero.

From the experiments and the data reported here we can conclude the following. The helical form of poly-γ-N-[2-morpholinylethyl]-α,L-glutamamide is not stable in water solution but this polypeptide may be converted to a helical conformation by changing to less polar solvents such as methanol or dioxane. In certain water soluble polypeptides L-methionine residues are more effective in promoting helix formation in aqueous solution than L-alanine residues. Also, it is clear that polypeptide helix formation in water solution depends not only on the formation of intramolecular peptide hydrogen bonds but also is aided by "hydrophobic bond" and dispersion force stabilizations[11] provided by residues such as methionine and alanine. Further, these new water-soluble polypeptides having high helix contents, can be transformed by reagents known to denature proteins into completely random conformations.

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[11] For a discussion see W. Kauzmann in "Advances in Protein Chemistry," Vol. 14, C. B. Anfinsen, Jr., M. L. Anson, K. Bailey and J. T. Edsall, Editors, Academic Press, Inc., New York, N. Y., 1959.

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FLUORINE N.M.R. SPECTROSCOPY. XII. PROOF OF OPPOSITE SIGNS FOR THE "DIRECT" CARBON-13 COUPLING CONSTANTS TO HYDROGEN AND TO FLUORINE

Sir:

The use of "spin decoupling" (double resonance)[1] for the determination of relative signs of coupling constants in fluorocarbon derivatives[2-4] and in proton compounds[5,6] has become important as an experimental check on theoretical predictions. The work of Lauterbur and Kurland[6] was particularly significant, since it was a test of the proposal[7] that "direct" couplings of C^{13} to protons have the same sign as vicinal proton–proton couplings.

The predictions of signs and magnitudes of couplings involving fluorine, though less certain than those for protons, nevertheless represent a further development of the theory. It is of prime importance to test the supposition that the "direct" couplings, $J(C^{13}F)$ and $J(C^{13}H)$, have the same sign. This has now been done for the particularly simple case of $CHCl_2F$, dichlorofluoromethane.

Experimental.—The basic n.m.r. spectrometer and techniques were as previously described.[8,9] For the double resonance work a Model SD-60 Spin Decoupler[10] was employed. It was equipped with modules designed for strong irradiation of fluorine nuclei at 37.65 mc./sec., while observing protons at 40.000 mc./sec. (symbolized as $H\{F\}$ decoupling[1]) and for $F\{H\}$ decoupling with irradiation at 42.50 mc./sec. With this equipment some ten watts of radiofrequency power is available to the probe; however, far less than this is required since the F–H coupling constant in $CHCl_2F$ is

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[1] J. D. Baldeschwieler, *Chem. Revs.*, **63**, in press (1963).

[2] D. F. Evans, *Mol. Phys.*, **5**, 183 (1962).

[3] S. L. Manatt and D. D. Elleman, *J. Am. Chem. Soc.*, **84**, 1305 (1962).

[4] D. D. Elleman and S. L. Manatt, *J. Chem. Phys.*, **36**, 1945 (1962).

[5] S. L. Manatt and D. D. Elleman, *J. Am. Chem. Soc.*, **84**, 1579 (1962).

[6] P. C. Lauterbur and R. J. Kurland, *ibid.*, **84**, 3405 (1962).

[7] M. Karplus, *ibid.*, **84**, 2458 (1962).

[8] G. Filipovich and G. V. D. Tiers, *J. Phys. Chem.*, **63**, 761 (1959).

[9] G. V. D. Tiers, *J. Chem. Phys.*, **35**, 2263 (1962).

[10] Manufactured by the Nuclear Magnetic Resonance Specialties Co., Inc., Box 145, Greensburg Road, New Kensington, Pa.

only 53 c./sec., for the collapse of which H_2 = 20 milligauss is entirely adequate.

For the exact measurement of changes in irradiation frequency, an EC-60 Counter Adapter,[10] which produces an easily-countable beat frequency by means of a standard crystal, was employed. A BC-221-J frequency meter (Zenith Radio Corp.) was used to establish the direction of increase.

Dichlorofluoromethane was obtained from the Matheson Co.; its doublet fluorine resonance, centered at $+80.881\ \phi^*$, ± 0.003[8] (5 vol. % in CCl_3F, 0.5% Me_4Si, 24.5°) had $J(HF)$ = 53.65 ± 0.16 c./sec. In the proton spectrum of this solution the doublet center is at 2.587 τ, ± 0.002. The neat liquid, in a 4 mm. i.d. thin-walled tube, was required for the study of the C^{13} compound at natural abundance. The fluorine isotope shift[9] due to C^{13}, $\Delta\phi(C^{13}F)-(C^{12}F)$, was found to be +0.156 ± 0.003 p.p.m., and that for H, +0.004 ± 0.001_5 p.p.m.; while $J(C^{13}F)$ = 293.8 ± 0.2 c./sec., and $J(C^{13}H)$ = 220.0_0 ± 0.1_3 c./sec.

Though three dissimilar nuclei are employed here, the spin decoupling procedure and the interpretation as to relative signs are essentially the same as in the case previously described.[6] Upon irradiation of the low-field (high frequency) C^{13} satellite doublet in the proton spectrum, the high-field C^{13} doublet in the fluorine spectrum drew together. When the irradiation frequency was lowered by about 512 c./sec., the low-field C^{13}–F doublet narrowed instead. Had the coupling constants been of like sign, it would have been necessary to *raise* the irradiation frequency by about 75 c./sec. to achieve this result. Analogous observations were made for H{F} decoupling, further confirming the assignment of unlike signs for $J(C^{13}F)$ and $J(C^{13}H)$.

I thank Emmett B. Aus for the careful n.m.r. spectral work reported here.

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NON-ADDITIVE SUBSTITUENT EFFECTS ON C^{13}-PROTON SPIN-SPIN COUPLINGS[1]

Sir:

It has been reported[2] that C^{13}-proton couplings in the nuclear magnetic resonance spectra of substituted methanes conform to "a simple, but precise, additivity relation." In the belief that a further experimental test of this generalization was desirable, we have measured coupling constants for a number of compounds not previously studied. The results, presented in Table I together with coupling constants calculated using zeta-values,[2] show that the additivity relation is not generally valid.

The accompanying figure is a plot of J_{CH} against n for the two series $CH_{(4-n)}X_n$ in which X is either F or OCH_3. Clearly, the points are not colinear as would be required by the additivity relation. If the data[3] for $CH_{(4-n)}Cl_n$ are similarly plotted, the deviations from colinearity, though smaller, also exceed the experimental error. J_{CH} is usually determined with an uncertainty not exceeding ±1 c.p.s. from proton spectra, although values derived from C^{13} spectra[4,5] may be subject to larger errors.

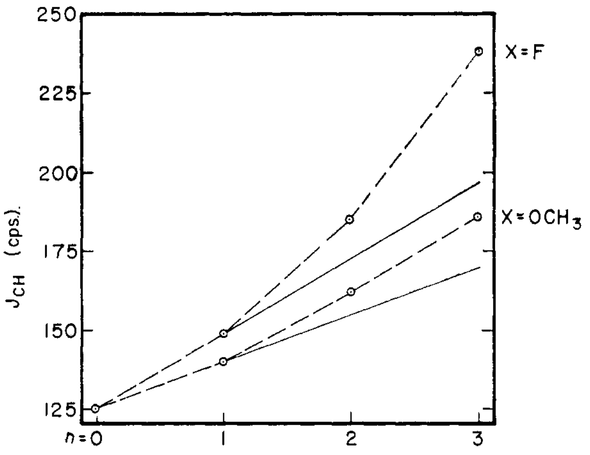

Fig. 1.—J_{CH} as a function of n for two series $CH_{(4-n)}X_n$. If the substituent effects were additive, the points for n = 2 and n = 3 should fall on the extrapolated lines through those for n = 0 and n = 1, shown as solid lines in the figure.

TABLE I

COMPARISON OF MEASURED COUPLING CONSTANTS AND PREDICTIONS BASED ON ZETA VALUES

Compound	J_{CH} (c.p.s.) Observed	J_{CH} (c.p.s.) Predicted	Difference
$C_6H_5\mathit{CH_2}F$	151[a]	150	+ 1
CH_2F_2	185[b]	173	+12
CHF_3	238	197	+41
$CHFCl_2$	220	203	+17
CHF_2Cl	231	200	+31
$CH_2(COOH)_2$	132[b]	136	− 4
$CH_2ClCOOH$	152	157	− 5
$CHCl_2COOH$	181	184	− 3
CH_3OCH_3	140	[140][c]	[0]
$\mathit{CH_2}(OCH_3)_2$	162	155	+ 7
$\mathit{CH}(OCH_3)_3$	186	170	+16

[a] D. T. Carr, Thesis, Purdue University, 1962. [b] Reference 8. [c] Used to evaluate zeta for the methoxy group as 56.6 c.p.s.

It is not clear how the inadequacy of the proposed additivity relation may affect the problem of interpreting these coupling constants. J_{CH} is believed[3,6–8] to depend primarily on the *s*-character of the carbon atomic orbital used in the C–H bond. It is not known whether this bonding parameter will vary linearly as additional substituents are introduced. Moreover, all theoretical discussions of the dependence of J_{CH} on hybridization have included a number of simplifying assumptions which may not be generally valid. In this connection, it is noteworthy that the largest deviations from the additivity relation occur when one or

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(1) Supported by a grant from the National Science Foundation.

(2) E. R. Malinowski, *J. Am. Chem. Soc.*, **83**, 4479 (1961).

(3) N. Muller and D. E. Pritchard, *J. Chem. Phys.*, **31**, 768, 1471 (1959).

(4) P. C. Lauterbur, *ibid.*, **26**, 217 (1957).

(5) H. Spiesecke and W. G. Schneider, *ibid.*, **35**, 726 (1961).

(6) J. N. Shoolery, *ibid.*, **31**, 1427 (1959).

(7) M. Karplus, *J. Phys. Chem.*, **64**, 1793 (1960).

(8) N. Muller, *J. Chem. Phys.*, **36**, 359 (1962).