lent to k_{-2} in Scheme I) and a dual path for acyl enzyme aminolysis (k_{-2} and k_{-2}') will result in a pH dependence for the partitioning of a formylphenylalanyl enzyme between water and formylhydrazine; at high pH there should be relatively less aminolysis than at low pH. Stated quantitatively, from eq 2–4 at high pH

$$\frac{\text{obsd } k_{\text{amine}}/(\text{amine})}{\text{obsd } k_{\text{H}_2\text{O}}} = \frac{k_{-1}k_{-2}}{(k_{-1} + k_2)k_{\text{H}_2\text{O}}} \tag{5}$$

and at low pH

$$\frac{\text{obsd } k_{\text{amine}}/(\text{amine})}{\text{obsd } k_{\text{H}_2\text{O}}} = \frac{k_{-1}k_{-2}'K_a''}{k_{\text{H}_2\text{O}}k_2'K_a'''} \tag{6}$$

The ratio of the partitioning of the acyl enzyme between amine and water at high and low pH is equal to eq 5/eq 6; this equals $k_2/(k_{-1} + k_2)$. The proposed change in rate-determining step[2] requires that $k_{-1} > k_2$, so that Scheme I predicts an increased yield of amide at lower pH. The results given in Table I are not consistent with Scheme I; acyl enzyme partitioning between water and formylhydrazine is invariant over the range pH 7.5–4.5. Scheme I does not apparently describe the enzyme-catalyzed hydrolysis of formylphenylalanine formylhydrazide.

Table I. Partitioning of a Formylphenylalanylchymotrypsin Intermediate between Water and Formylhydrazine as a Function of pH[a]

pH	[Amine],[b] M	[Enzyme], M	% amide formed[c]	$k_{\text{amine}}/k_{\text{H}_2\text{O}}$[d]
7.5	0.0505	1×10^{-7}	5.76	1.21
7.5	0.101	1×10^{-7}	10.5	1.16
6.0	0.0505	4×10^{-6}	5.56	1.17
6.0	0.101	4×10^{-6}	10.7	1.19
4.5	0.0493	1×10^{-4}	5.44	1.17
4.5	0.0986	1×10^{-4}	10.2	1.17

[a] Reactions were run at 25° in 0.1 M KCl with an initial concentration of N-formyl-^{14}C-phenylalanine methyl ester (2.18×10^6 cpm/μmol) of 0.7 mM. The pH was controlled with a pH stat and reactions were followed to completion as indicated by a cessation of proton release. [b] Concentration of the amine free base. [c] The yield of amide was determined by an isotope dilution technique. [d] Equal to (% amide)/(% carboxylic acid) (concentration of the amine free base).

Barry Zeeberg, Michael Caswell, Michael Caplow*
Department of Biochemistry, University of North Carolina
Chapel Hill, North Carolina 27514

Received November 22, 1972

Determination of the Average Singlet–Triplet Splitting in Biradicals by Measurement of the Magnetic Field Dependence of CIDNP[1]

Sir:

We have previously reported the CIDNP spectra of alkenal products resulting from photochemical α cleavage of alicyclic ketones.[2] We now report measurements of the average singlet–triplet splitting in biradicals resulting from α cleavage, based on the magnetic field dependence of the CIDNP aldehyde signal in the alkenal products. To our knowledge, this is the first determination of the average isotropic exchange interaction in highly reactive biradical species.

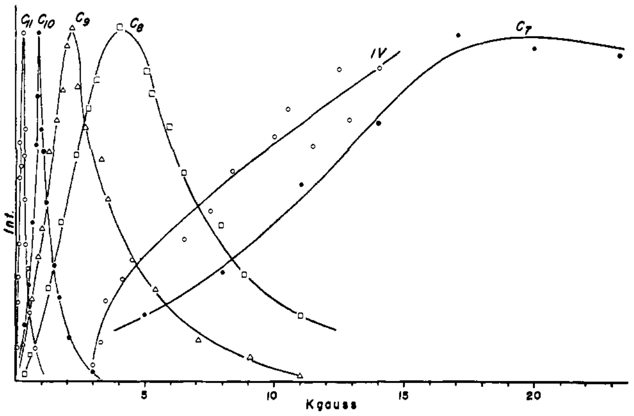
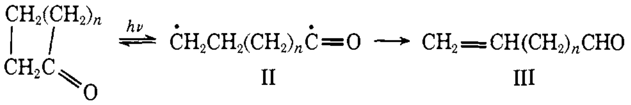


Figure 1. Intensities of aldehyde proton emission signals of IIIa–e (C_7–C_{11}) and of aldehyde derived from IV, as function of magnetic field. The intensities are in arbitrary units and not normalized among the different compounds.

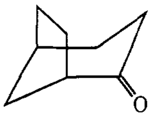
Irradiation of ketones Ia–e and IV in chloroform solution yielded the corresponding alkenals. Each irradiation was carried out for 50 sec on a 0.140 M sample of ketone in chloroform placed between the pole pieces of a Varian 12-in. magnet at the desired field strength. The sample was then transferred quickly to the probe of an HA-100 spectrometer, and the aldehyde CIDNP signal was immediately recorded on a CAT. For each ketone, the relative integrated intensity of aldehyde CIDNP signal is plotted as a function at the field strength at which the sample was irradiated and is displayed in the curves of Figure 1.



$$\dot{C}H_2CH_2(CH_2)_n\dot{C}{=}O \longrightarrow CH_2{=}CH(CH_2)_nCHO$$

I II III

a, $n = 4$; b, $n = 5$; c, $n = 6$; d, $n = 7$; e, $n = 8$



IV

These curves show two striking features. As the length of the biradical increases, the maxima move to lower energies, and the curve widths decrease dramatically. The first of these phenomena is straightforward to interpret.

For simplicity we first consider a fictitious, totally rigid biradical of the type II with a singlet–triplet splitting $2J$, corresponding to an effective exchange Hamiltonian $-J(^1/_2 + 2\mathbf{S}_1 \cdot \mathbf{S}_2)$. Moreover, the principal hyperfine-induced singlet–triplet mixing will occur between the T_- and S states.[2] By varying the magnetic field H_0 in which the biradical is created, one can shift the T_- level (with energy $g\beta H_0$ below the T_0 level) to become essentially degenerate with the S level. From

---

[1] Supported by grants from the Petroleum Research Fund (3965 C-4), administered by the American Chemical Society, and the National Science Foundation (GP 18719X).

[2] G. L. Closs and C. E. Doubleday, *J. Amer. Chem. Soc.*, **94**, 9248 (1972).

time-dependent perturbation theory[3] it follows that the evolution of the singlet character from the initial zero-order triplet spin state will be proportional to $(H_{ST}/\Delta E)^2$, where H_{ST} is the nuclear spin dependent matrix element of the hyperfine Hamiltonian and ΔE is the difference between the energy of the zero-order singlet and triplet states. When intersystem crossing caused by this mechanism is in competition with that caused by spin–orbit coupling, it is apparent that the most intense CIDNP spectra will be those in which the biradical is created in a magnetic field such that $g\beta H_0 = 2J$, implying a maximum in the intensity *vs.* field curve at the point where the Zeeman energy equals the singlet–triplet splitting.

A real biradical of type II is constantly changing its conformation, giving a distribution of singlet–triplet splittings over its lifetime. Thus, a field-dependence plot should show a maximum at that field for which $g\beta H_0 = \langle 2J \rangle_{av}$, where $\langle 2J \rangle$ is the weighted average of the singlet–triplet splitting over all conformations. Table I lists these averages for the biradicals of type II included in this study.

Table I

Ketone	Curve max, cm^{-1}	Width at half-height, cm^{-1}
IV	>1.31[a]	>2.0[b]
Ia	1.87 ± 0.28	~2.3[b] ± 0.5
Ib	0.374 ± 0.037	0.527 ± 0.03
Ic	0.196 ± 0.009	0.257 ± 0.02
Id	0.0854 ± 0.003	0.0448 ± 0.003
Ie	0.0261 ± 0.005	0.0304 ± 0.005

[a] Due to limitation of experimentally available magnitude of H_0 the maximum could not be determined. [b] Estimated, assuming a symmetrical curve.

The fact that J decreases as the length of the biradical increases is consistent with three exchange mechanisms: (1) indirect exchange through the σ bonds, (2) exchange *via* the solvent molecules, (3) direct exchange through space.[7] Some evidence on the exchange mechanism can be obtained by comparison of our work with that of Glarum and Marshall, who used esr to study the carbonate diester of 2,2,6,6-tetramethyl-4-piperidonyl-1-oxyl. This stable, relatively rigid biradical has localized radical centers separated by 15 Å and ten σ bonds. In chloroform solution at 40°, $2J = 0.0028$ cm^{-1} (30 G).[8] In IIe, the radical centers are separated by 13 Å in the fully extended conformation and are also ten σ bonds apart, yet J is an order of magnitude larger. This discrepancy cannot be explained by mechanism 1 for IIe. Mechanism 1 is probably also unimportant for IIa–d, since these biradicals should spend very little time in the fully extended conformation.

The full interpretation of the variation in width of the field-dependence curves must include a consideration of the random fluctuations in J due to changes in conformation, the relative densities of states for the transitions involved, the relative contributions of hyperfine and spin–orbit coupling, and the lifetime of the states. This last consideration, *i.e.*, lifetime broadening, provides by itself at least a qualitative understanding. If the reasonable assumption is made that the singlet state of the biradical has a very short lifetime inversely related to the length of the biradical (10^{-10}–10^{-12} sec), then the uncertainty broadening of the singlet energy qualitatively reproduces the observed variation in width. A more quantitative interpretation will rest on a consideration of all the effects involved and is in progress.

G. L. Closs,* C. E. Doubleday
Department of Chemistry, The University of Chicago
Chicago, Illinois 60637

Received January 27, 1973

---

[3] Perturbation theory is used here for brevity. The well known[4–6] full solution requires the solution of the time-dependent Schroedinger equation for all states, but the qualitative results are the same.

(4) G. L. Closs and A. D. Trifunac, *J. Amer. Chem. Soc.*, **92**, 2183 (1970).

(5) J. I. Morris, R. C. Morrison, D. W. Smith, and J. F. Garst, *ibid.*, **94**, 2406 (1972).

(6) R. Kaptein and J. A. de Hollander, *ibid.*, **94**, 6269 (1972).

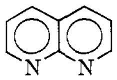
(7) C. Herring in "Magnetism," Vol. IIB, G. T. Rado and H. Suhl, Ed., Academic Press, New York, N. Y., 1966, pp 1–181.

(8) S. H. Glarum and J. H. Marshall, *J. Chem. Phys.*, **47**, 1374 (1967); *cf.* R. Briere, R. M. Dupeyre, H. Lemaire, C. Morat, A. Rassat, and P. Rey, *Bull. Soc. Chim. Fr.*, 3290 (1965).

A Binuclear Complex of 1.5 Valent Nickel

Sir:

In the last years the coordinating properties of 1,8-naphthyridine (napy) have been intensively studied.[1]



We have found that from the reaction of anhydrous nickel(II) halides (1 mmol) with napy (2 mmol) and sodium tetraphenylborate (1 mmol) in 1-butanol (60 ml) a pale green compound (I) with the general formula $[Ni(napy)_2X]B(C_6H_5)_4$ (X = halogen) is formed. This product is insoluble in common organic solvents. The magnetic moments are 3.34 and 3.36 BM for the chloro- and bromo complexes, respectively, clearly corresponding to nickel(II) compounds.

If the reaction solution is heated to boiling without separating the initial green product, the solution slowly darkens and eventually shining black crystals begin to separate from the greenish black solution. Recrystallization of the black product (II) from acetone effects separation from any remaining green product (I). Product II has the formula $[Ni_2(napy)_4X_2]B(C_6H_5)_4$ (X = halogen, NCS, NO_3) and contains therefore nickel in the formal oxidation state 1.5. Analogous complexes $[Ni_2(napy)_4X_2]PF_6$ (III) are formed when $NaPF_6 + NaBH_4$ is used in ethanol instead of $NaB(C_6H_5)_4$. In this case the greenish black color appears immediately and no green intermediate product is observed.

The magnetic moments per nickel atom in both II and III vary from 2.91 to 3.07 BM, but are in the range 4.19–4.34 BM for the dimeric complexes. These values are consistent with the presence of three unpaired electrons in the dimer. The dependence of $1/\chi$ on temperature is linear in the range 88–293°K for the bromo derivative with a θ value of −3°K.

---

(1) D. G. Hendricker, *Inorg. Chem.*, **8**, 2328 (1969); T. E. Reed and D. G. Hendricker, *Inorg. Chim. Acta*, **4**, 471 (1970); D. G. Hendricker and R. L. Bodner, *Inorg. Chem.*, **9**, 273 (1970); R. L. Bodner and D. G. Hendricker, *ibid.*, **12**, 33 (1973); D. G. Hendricker and R. J. Foster, *ibid.*, **12**, 349 (1973).