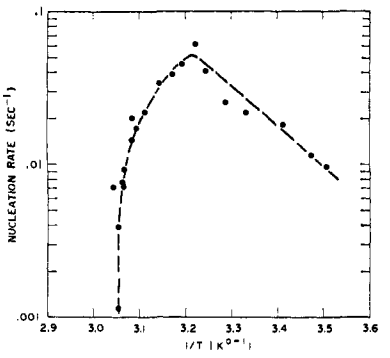


Figure 1. Plot of ln (nucleation rate) *vs.* $1/T$ for cholesteryl nonanoate.

where N is the nucleation rate, E_d is the free energy of activation for transport across the liquid crystal–nucleus interface, σ_e and σ_u represent interfacial energies for cylindrical nuclei, ΔH is the enthalpy of fusion, T_m is the melting point, T is the bath temperature, $\Delta T = T_m - T$, and α represents the proportion by which the homogeneous critical free energy is reduced by secondary nucleation processes. Equation 1 describes the nucleation data. For temperatures below the rate maximum the standard linear least-squares fit of ln (rate/T) *vs.* $1/T$ yielded $E_d = 9.3 \pm 1.0$ kcal/mol for cholesteryl nonanoate.[5] For temperatures above the rate maximum, the interfacial energies were obtained (Table I) from the derived slopes,[6] T_m, and ΔH. The interfacial energies for cholesteryl nonanoate and caproate are an order of magnitude lower than the lowest reported values[7] for other monomeric organic materials. (In order for these energies to approach normal values, α would have to be less than 10^{-3}.) This suggests that on a molecular level the mesophase is more like the solid than the isotropic phase, in accord with the postulate of short-range ordering in the liquid crystalline state.[2] (Short-range ordering should affect nucleation, as it is a rearrangement of molecules and not of domains.)

Table I. Enthalpies of Fusion and Interfacial Energies of Cholesteryl Nonanoate–Caproate Mixtures

Material[a]	ΔH,[b] kcal/mol	$\sigma = (\sigma_u^2\sigma_e)^{1/3}$,[c] erg/cm²
Cholesteryl nonanoate (N)	5.29 (±0.22)	2.15
Cholesteryl caproate (C)	6.62 (±0.10)	0.871
89.3% N–10.7% C	4.31 (±0.11)	1.49
73.4% N–26.6% C	4.46 (±0.61)	0.336
47.9% N–52.1% C	4.38 (±0.17)	0.327
23.5% N–76.5% C	3.55 (±0.15)	0.444
9.27% N–90.7% C	4.50 (±0.20)	1.04

[a] Mole percentages. [b] Standard deviation given in parentheses. [c] ±35%.

For the mixtures, the interfacial energies exhibit a minimum which is lower than that of either pure component. This must result from a favorable cooperative interaction of the components in both the mesophase and the crystal phase. Also, of particular interest is the rapid decrease in ΔH upon addition of a few mole per cent of one component to the other (Table I). These data again imply a strong interaction between the components. The nature of the interaction is still under investigation.

We will report the correlation of nucleation rate, interfacial energies, and phase diagrams with structure in subsequent publications.

Acknowledgment. We wish to thank Drs. J. O'Reilly, W. Gunther, and J. Becker for their helpful discussions.

John M. Pochan,* Harry W. Gibson
Research Laboratories, Xerox Corporation
Rochester, New York 14603
Received October 22, 1970

(5) Owing to crystallization, we were unable to obtain viscosity data in the supercooled liquid crystal state for correlation with rate below maximum.

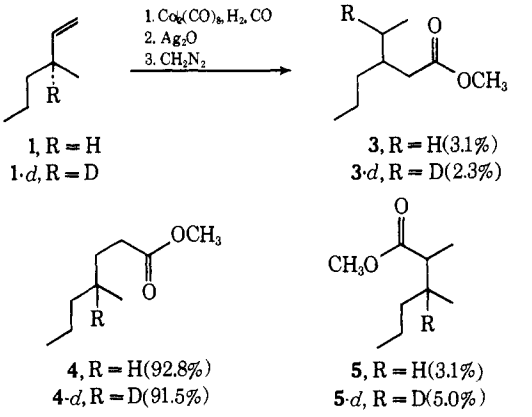
(6) A linear least-squares fit of ln (rate/T) *vs.* $T_m^2/T(\Delta T)^2$ was used, assuming $\alpha = 1$.

(7) D. G. Thomas and L. A. K. Staveley, *J. Chem. Soc.*, 4569 (1952).

Hydroformylation of 3-Methyl-1-hexene-3-d_1. Evidence Against Direct Formylation of a Methyl Group in the "Oxo" Reaction

Sir:

The hydroformylation of (+)-(*S*)-3-methyl-1-hexene (**1**) under conditions which minimize olefin isomerization[1] has been reported to give 3.1% (*R*)-3-ethylhexanal (**2**) in addition to the normal hydroformylation products 4-methylheptanal and 3,4-dimethylhexanal.[2] Conversion of **2** to the corresponding methyl ester **3** of known absolute rotation indicated that **2** had been formed with 70% net retention of configuration. Consequently, 2-ethyl-1-pentene was excluded as an intermediate in the formation of **2**.



Pino, *et al.*, proposed that **2** arose *via* direct insertion of cobalt into a carbon–hydrogen bond of the methyl group of **1** followed by hydroformylation.[2] The related insertions of transition metals into the ortho carbon–hydrogen bonds of arylphosphines, arylphosphites, benzylamines, and azobenzenes[3] and into the methyl groups of methylphosphines[4] and *o*-tolylphosphines[5] are now well documented. However, this proposed insertion of cobalt into the methyl group of

(1) P. Pino, S. Pucci, and F. Piacenti, *Chem. Ind. (London)*, 294 (1963).

(2) F. Piacenti, S. Pucci, M. Bianchi, and P. Pino, *J. Amer. Chem. Soc.*, **90**, 6847 (1968).

(3) G. W. Parshall, *Accounts Chem. Res.*, **3**, 139 (1970), and references therein.

(4) J. Chatt and J. M. Davidson, *J. Chem. Soc.*, 843 (1965).

(5) M. A. Bennett and P. A. Longstaff, *J. Amer. Chem. Soc.*, **91**, 6226 (1969); R. Mason and A. D. C. Towl, *J. Chem. Soc. A*, 1601 (1970).

1 would constitute the first example of an insertion into an unactivated alkyl–hydrogen bond.[6]

There are two alternate explanations for the retained stereochemistry of **2** which Pino did not exclude. Retention could have resulted either from isomerization of an alkylcobalt intermediate by successive elimination and addition of cobalt hydride[7] or from isomerization of an intermediate cobalt–olefin complex *via* 1,3-hydrogen shifts.[8] In both cases optical activity would be retained because of the asymmetry of the organocobalt intermediates.

To distinguish between these mechanisms we have examined the hydroformylation of 3-methyl-1-hexene-*3*-d_1 (**1**-*d*). Direct insertion into the methyl group would leave the label unshifted, while isomerization *via* an addition–elimination process would lead to migration of deuterium to the methylene carbon of the ethyl group in **2** and isomerization *via* 1,3-hydrogen shifts in an olefin complex would lead to deuterium in the methyl group of the ethyl side chain of **2**.

Here we report that hydroformylation of **1**-*d* followed by oxidation and esterification gives methyl 3-ethylhexanoate-*1*-d_1 (**3**-*d*). This product must arise from a 1,2-deuterium shift in the formation of **2** and can be accommodated only by an isomerization of an asymmetric cobalt–olefin complex *via* additions and eliminations of a cobalt hydride. These additions and eliminations must be fast relative to loss of olefin from the asymmetric cobalt–olefin complex to obtain the high degree of retention of optical activity found by Pino and the high retention of deuterium in **2**.

The deuterated olefin **1**-*d* (85% d_1 by mass spectrometry) was prepared by reaction of 2-methylpentenal-*2*-d_1[9] with methylenetriphenylphosphorane in tetrahydrofuran at −25°. The hydroformylation of **1**-*d* was conducted under conditions similar to those used by Pino.[2] A solution of 3.0 g of **1**-*d* in 15 ml of benzene containing 64 mg of $Co_2(CO)_8$ was heated to 110° for 24 hr under 100 atm of H_2 and 100 atm of CO pressure in a glass-lined bomb. The reaction mixture was bulb-to-bulb distilled and the mixture of aldehydes was immediately oxidized with silver(I) oxide, the resulting acids were converted to the corresponding methyl esters by treatment with diazomethane in ether. Gas chromatographic analysis (10 ft × 0.25 in. 17% Carbowax 20M, Chromosorb P, 125°) showed that the mixture of esters consisted of 2.3% **3**, 91.5% **4**, 5.0% **5**, and 1.2% of two unidentified methyl esters. Pure samples of the esters were obtained by preparative gas chromatography (10 ft × 0.37 in. 25% Carbowax 20M, Chromosorb W, 120°) and were identified by spectral comparison with authentic samples of undeuterated compounds prepared by unambiguous routes.[12]

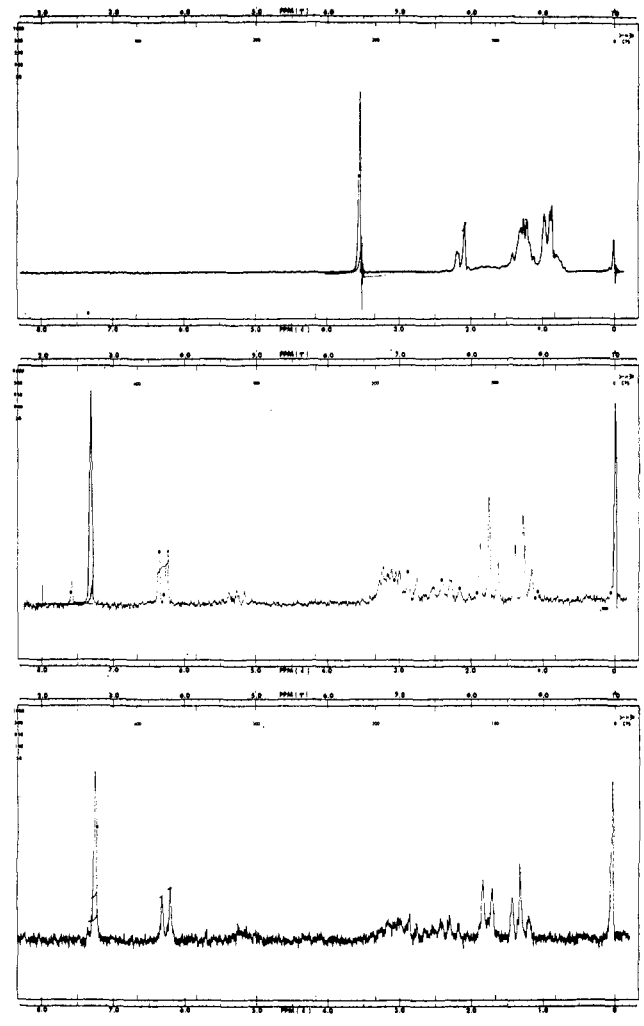


Figure 1. Nmr spectra at 60 MHz of methyl 3-ethylhexanoate in CCl_4: top, normal spectrum of undeuterated ester; center, spectrum of undeuterated ester in the presence of 30 mol % Eu(DPM)$_3$; bottom, spectrum of deuterated ester **3**-*d* in the presence of 30 mol% Eu(DPM)$_3$.

The location of deuterium in methyl 3-ethylhexanoate (**3**-*d*) (84% d_1 by mass spectrometry) was determined from its nmr spectrum in the presence of tris(dipivaloylmethanato)europium(III), Eu(DPM)$_3$, a reagent which induces pseudocontact chemical shifts in molecules capable of coordination with europium[16] (see Figure 1). In the nmr spectrum of undeuterated **3** taken in the presence of 30 mol % Eu(DPM)$_3$ all absorptions but those due to two methylene groups are cleanly separated and first order; the tertiary hydrogen atom appears as a multiplet at δ 5.2 and the methyl protons of the ethyl group are a sharp triplet at 1.7. In a similar Eu(DPM)$_3$-shifted spectrum of the deuterated ester **3**-*d*, the presence of a tertiary proton is clearly indicated by the multiplet at δ 5.2; the presence of a

(6) The insertion of platinum into an *n*-propyl group of *trans*-$PtCl_2[P(tert\text{-}Bu)_2(n\text{-}Pr)]_2$ was recently claimed: A. J. Cheney, B. E. Mann, B. L. Shaw, and R. M. Slade, *Chem. Commun.*, 1205 (1970).

(7) A similar mechanism was advanced to account for the 2.9% 3-methylhexenal present in the hydroformylation mixture of 4-methyl-1-pentene when only 1% olefin isomerization was observed: A. J. Chalk and J. F. Herrod, *Advan. Organometal. Chem.*, **6**, 119 (1968).

(8) See T. A. Manuel (*J. Org. Chem.*, **27**, 3941 (1962)) for a discussion of similar mechanisms.

(9) 2-Methylpentenal-*2*-d_1 was obtained by deuterolysis[10] of the isomeric mixture of enol acetates[11] of 2-methylpentenal.

(10) J. Hine, J. G. Houston, J. H. Jensen, and J. Mulders, *J. Amer. Chem. Soc.*, **87**, 5050 (1965).

(11) H. O. House and V. Kramar, *J. Org. Chem.*, **28**, 3362 (1963).

(12) **3** was prepared by CH_2N_2 esterification of the corresponding acid obtained by the procedure of Lavene, *et al.*[13] **4** was prepared by CH_2N_2 esterification of 4-methylheptanoic acid obtained by Huang-Minlon reduction of ethyl 4-methyl-5-ketoheptanoate.[14] **5** was prepared by CH_2N_2 esterification of the diastereomeric mixture of 2,3-dimethylhexanoic acids.[15]

(13) P. A. Lavene and R. E. Marker, *J. Biol. Chem.*, **91**, 687 (1931).

(14) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Amer. Chem. Soc.*, **85**, 207 (1963).

(15) M. S. Kondakowa and M. M. Katznelson, *Dokl. Akad. Nauk SSSR*, **18**, 271 (1938); *Chem. Zentralbl.*, **109**, 4444 (1938).

(16) J. K. M. Sanders and D. H. Williams, *Chem. Commun.*, 422 (1970).

deuterium atom on the methylene carbon of the side chain is indicated by the collapse of the methyl triplet to a doublet at 1.7 and by a decrease in the integral for the broad multiplet at 3.1. Thus, a deuterium atom shifted from the tertiary carbon to the methylene carbon of the ethyl side chain in the course of formation of **2**.

The $Eu(DPM)_3$-shifted nmr spectra of the normal hydroformylation products **4**-*d* and the diastereomeric mixture **5**-*d* both contained singlets for the 4-methyl and the 3-methyl groups, respectively, and demonstrated that these products were formed without shift of deuterium.

Charles P. Casey,* Clifford R. Cyr
Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706
Received December 17, 1970

Inactivation and Reactivation of Hemocyanin by Radiolytic OH and e_{aq}^-

Sir:

When exposed to ionizing radiation the biochemical function of enzymes and proteins can be impaired or destroyed. In this communication we demonstrate that ionizing radiation can also restore the biochemical function of a previously radiation-damaged macromolecule.

In experiments with the oxygen-carrying copper protein, hemocyanin, we find that in neutral, *oxygen-free media*, radiolytically produced OH eliminates the oxygen-carrying capacity by oxidizing the protein-bound copper to the cupric state, while the primary reducing species e_{aq}^- fully restores the oxygen-carrying capacity by reducing cupric copper to the cuprous state. Similarly, the secondary radical[1,2] CO_2^-, produced when formate is used as an OH scavenger, is capable of restoring inactivated hemocyanin. The radiation doses employed (0–110 krads) do not cause any significant changes or damage to the protein moiety. It has been demonstrated previously[3,4] that the elimination and restoration of oxygen-carrying capacity of hemocyanins by irradiation in *oxygenated* media was solely due to H_2O_2 produced during irradiation. It has long been known that H_2O_2 and other oxidizing–reducing agents modify the oxygen-carrying capacity of hemocyanin.[5,6]

We also find that the reduction of Cu(II) to Cu(I) parallels the restoration of the oxygen-carrying capacity of hemocyanin derived from one species of Mollusca (*Busycon*, the channeled whelk). However, no restoration of oxygen-carrying capacity is effected in hemocyanin obtained from a species of Arthropoda (*Limulus*, the horseshoe or king crab), despite the fact that complete reduction of Cu(II) to Cu(I) occurs following irradiation in the presence of suitable scavengers. These observations, indicative of a difference in the nature of the binding of the copper in the hemocyanins from the two different species, appear to be borne out by certain distinctive features in their respective circular dichroism (CD) spectra as described below.

The hemocyanins employed in these experiments (obtained from the Marine Biological Laboratories, Woods Hole, Mass.) were usually used as naturally present in the hemolymph (serum) of the animals. However, before use, the hemocyanin hemolymph was conditioned[4] so as to remove extraneous clotting protein. The hemocyanin hemolymph was diluted with 0.05 *M*, pH 7.0, sodium dihydrogen phosphate buffer. The oxygen-carrying capacity was measured by the absorbance (A_0) at 345 nm and the activity of the hemocyanin was expressed as the per cent oxygen-carrying capacity, defined as the ratio of the A_0 of irradiated hemocyanin to that of a nonirradiated control sample. Deoxygenation of oxyhemocyanin was accomplished by passage of helium through the hemocyanin solution.[3,4] The total copper content of hemocyanin was determined by atomic absorption. The cuprous copper fraction in deoxyhemocyanin was measured by the 2,2′-biquinoline method.[7] The CD spectra were recorded with a Cary-60 spectropolarimeter on samples of hemocyanin isolated and purified by ultracentrifugation and dialysis. Irradiations were carried out at 25 ± 1° with a cobalt-60 γ source.

Hemocyanin from *Busycon* was first inactivated by irradiation in oxygenated media with 17 krads of cobalt-60 γ radiation followed by deoxygenation as described previously.[3,4] Immediately afterward, in an attempt to restore the oxygen-carrying capacity, the inactivated hemocyanin was irradiated with varying doses in oxygen-free media, in the presence and absence of 0.5 *M* sodium formate, an OH radical scavenger.[8] In the absence of formate, no restoration of the oxygen-carrying capacity was observed (Figure 1). However, in the presence of formate the oxygen-carrying capacity increased with increasing dose to the point that at 60 krads practically all of the original activity was restored. Concomitantly, the cuprous ion concentration increased in the same manner as the oxygen-carrying capacity (Figure 1). The restored or reactivated hemocyanin appeared to be identical with that of the nonirradiated hemocyanin, *e.g.*, the oxygenation–deoxygenation cycles, optical absorption spectra, and CD spectra.

Since the secondary CO_2^- radical is capable of reducing the cupric ion,[1] we investigated its restorative ability in the absence of e_{aq}^- by irradiating inactivated *Busycon* hemocyanin in the presence of both N_2O, an efficient e_{aq}^- scavenger, and sodium formate. We found that the CO_2^- radical did, indeed, reactivate *Busycon* hemocyanin. Finally, we investigated the restorative capability of e_{aq}^- in the absence of the CO_2^- radical by carrying out irradiations in the presence of 2-propanol. Again, we observed restoration of *Busycon* hemocyanin.

In related experiments with *Limulus* hemocyanin, we found that neither e_{aq}^- nor the CO_2^- radical restored the oxygen-carrying capacity. However, the reduction of Cu(II) to Cu(I) increased with increasing radiation

(1) D. M. Donaldson and N. Miller, *Radiat. Res.*, **9**, 487 (1958); N. Miller, *ibid.*, **9**, 633 (1958).
(2) A. Fojtik, G. Czapski, and A. Henglein, *J. Phys. Chem.*, 74, 3204 (1970).
(3) J. Schubert and E. R. White, *Science*, **155**, 1000 (1967).
(4) J. Schubert, E. R. White, and L. F. Becker, Jr., *Advan. Chem. Ser.*, **No. 81**, 480 (1968).
(5) G. Felsenfeld and M. P. Printz, *J. Amer. Chem. Soc.*, **81**, 6259 (1959).
(6) R. Lontie and R. Witters in "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1966, pp 455–463.
(7) G. Felsenfeld, *Arch. Biochem. Biophys.*, **87**, 247 (1960).
(8) E. J. Hart, J. K. Thomas, and S. Gordon, *Rad. Res. Suppl.*, **4**, 74 (1964).