ing oxygen and thereby induce homolysis of the peroxide bond in the molecule attacked forming methyl benzoate. The resulting acetoxy radical could then decarboxylate, and continue an induced chain spiraling along the screw axis.

Such an intermolecular mechanism was excluded by photolysis of 200 mg of approximately equimolar solid solutions of ABP-d_8[12,13] in unlabeled ABP prepared by removing ether from a fluid solution of the two. After photolysis to 13% conversion at −65 to −70° and titration of the remaining ABP, methyl benzoate and toluene were extracted into pentane and analyzed by vpc–mass spectrometry. The molecular ions of toluene from one run consisted of 50.6% d_0, 3.5% d_7, and 44.5% d_8 with 1.4% of d_3 and d_5 cross-products. Those of methyl benzoate from another run consisted of 50.7% d_0, 3.5% d_7, and 45.3% d_8 with no more than 0.5% of d_3 and d_5 cross-products. Thus, 97% of the toluene and greater than 99% of the methyl benzoate were formed intramolecularly showing that the crystalline matrix suppresses the induced chain mechanisms prevalent in the melt.

The lattice could exert a more subtle influence by favoring attack of the methyl radical on one of the two oxygens of the geminate benzoyloxy radical. Within a molecule in crystalline ABP the methyl carbon is slightly closer to the acyl oxygen of the benzoyloxy group (3.68 Å) than to the carbonyl oxygen (4.13 Å), but it is difficult to predict which benzoyloxy oxygen should be more accessible to the methyl radical formed by acetoxy radical decarboxylation in the crystalline matrix. It is also difficult to assess the likelihood of oxygen equilibration by rotation of the carboxy group or of the whole benzoyloxy radical about its major axis. ABP-$^{18}O_2$ with 97 atom % ^{18}O in the peroxidic positions[14] was prepared by autoxidation[12b] under $^{18}O_2$. An 84-mg sample of this solid was photolyzed to 8% conversion and the product methyl benzoate analyzed as above. The molecular ion showed 98.3% $^{16}O^{18}O$ and 1.7% $^{16}O^{16}O$.[15] The ratio[15] of unlabeled (*m/e* 105) to ^{18}O-labeled (*m/e* 107) benzoyl fragments is 1.65, demonstrating a preference for coupling of the methyl radical with the formerly peroxidic oxygen (acyl coupling) over its coupling with the previously carbonyl oxygen (carbonyl coupling). The mass spectrum of methyl benzoate from a photolysis to completion of 21 mg of ABP-$^{18}O_2$ in 0.5 ml of absolute ethanol (0°) showed an *m/e* 105/107 ratio[15] of 1.01 indicating random coupling in fluid solution. The solid-state preference for acyl coupling is not limited to the crystal photolysis, since complete photolysis of 20 mg of ABP-$^{18}O_2$ in 0.5 ml of ethanol as a glass at 77°K gave an acyl/carbonyl coupling ratio[15] of 2.62.

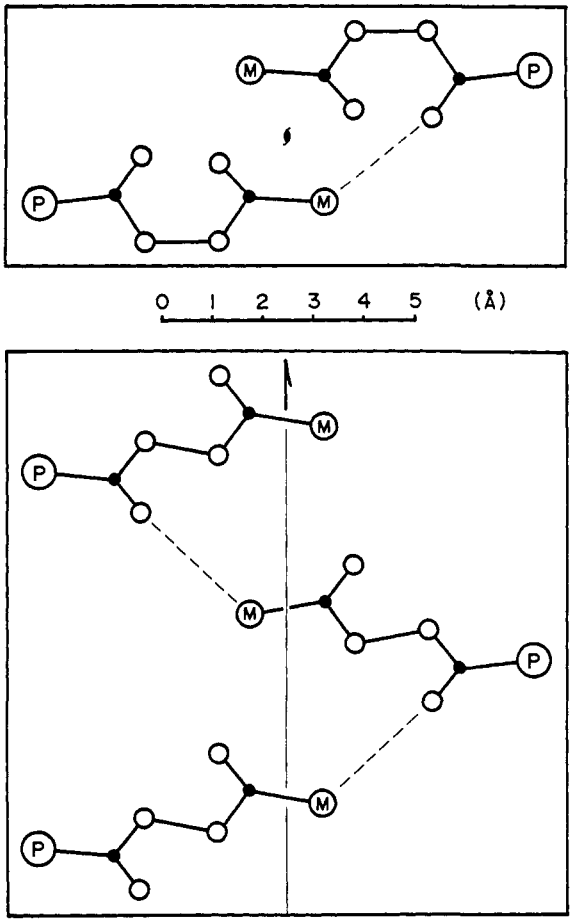

Figure 1. Top and side views of the crystal packing of ABP. Lower frame shows a family of molecules related by the indicated screw axis projected on (10$\bar{2}$) with [010] vertical. Upper frame shows molecules of the lower frame projected on (010) with (10$\bar{2}$) horizontal. Oxygens are denoted by open circles, carbonyl carbons by filled circles, methyl carbons by M, and C-1 of the phenyl rings by P. Dashed lines show the 3.45-Å contacts that might have led to induced methyl benzoate formation.

(12) (a) Prepared by autoxidation[12b] of benzaldehyde-d_6[12c] in acetic acid-d_6;[12d] (b) C. Walling and E. A. McElhill, *J. Amer. Chem. Soc.*, 73, 2927 (1951); (c) prepared by Ce(IV) oxidation of toluene-d_8, L. Syper, *Tetrahedron Lett.*, 4493 (1966); (d) A. Murray, III, and D. L. Williams, Ed., "Organic Synthesis with Isotopes," Part II, Interscience, New York, N. Y., 1958, p 1290.

(13) Mass spectral analysis of the methyl benzoate parent peaks showed 93% d_8–7% d_7, but the stronger benzoyl fragment showed 91%–9% d_5, implying that the methyl position was probably completely labeled. We thank Dr. Walter McMurray and Mr. Peter Arnesen for these measurements. The parent peaks of toluene gave 93% d_8–7% d_7.

(14) Mass spectral analysis of O_2 from permanganate oxidation of perchloric acid hydrolysate following treatment with sodium methylate; a modification of the procedure of J. C. Martin and S. A. Dombchik, *Advan. Chem. Ser.*, No. 75, 269 (1968). We thank Professor Martin for sending us a copy of the revised procedure.

(15) In light of the results in fluid ethanol below, we believe that the discrepancy between these numbers and those from peroxide oxygen analysis may be due to a small amount of scrambling during hydrolysis.[14] The 105/107 ratios reported below have been corrected for 1.7% $^{16}O^{16}O$ benzoate but not for any scrambling in the starting material before photolysis. Photolytic scrambling is negligible.

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Nuclear Magnetic Resonance Studies of ^{13}CO Binding to Various Heme Globins

Sir:

Whether, and to what extent, the various subunits of hemoglobin interact differently with ligands (such as oxygen or carbon monoxide) remains one of the unanswered questions in the hemoglobin saga. These possible differences may manifest themselves in the kinetic or thermodynamic affinities for ligands or in the environments experienced by ligands when bound to various subunits.

We have studied the binding of ^{13}C enriched carbon

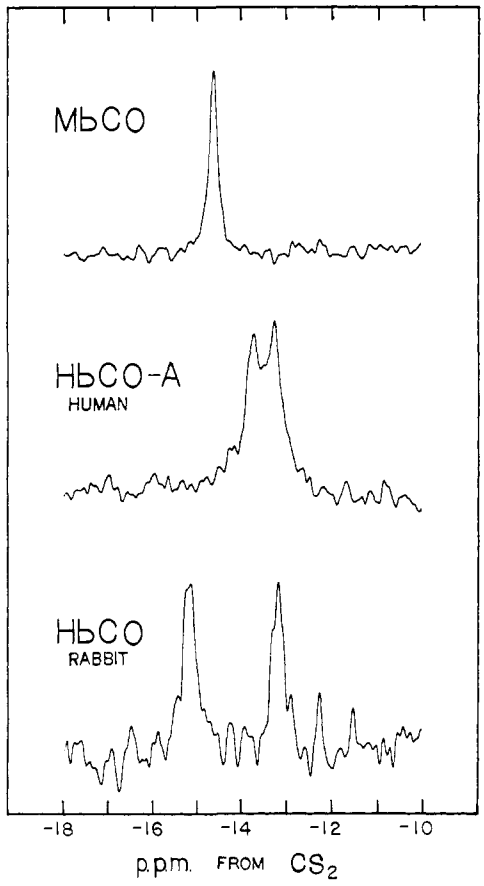

Figure 1. Representative spectra of the carbon monoxide resonances from sperm whale carboxymyoglobin (MbCO), human adult hemoglobin (HbCO-A), and rabbit hemoglobin at 25.15 MHz. Protein concentrations ranged from 2 to 5 m*M* in 0.1 *M* NaCl. Enrichment in ^{13}CO was 90%.

monoxide to a variety of heme globins, specifically sperm whale myoglobin, adult human heme globin, fetal human hemoglobin, mouse hemoglobin, and rabbit hemoglobin. The technique involved observing, by a Fourier transform method using a Varian Associates XL-100-15 spectrometer, the ^{13}C resonances of carbon monoxide bound to the proteins listed. Representative spectra are shown in Figure 1. All carboxyhemoglobins studied showed two distinct resonances of equal intensity originating from carbon monoxide bound either to the α or β subunits. Data on the chemical shifts for the ^{13}CO resonances of the proteins studied are collected in Table I. (Another report[1] has appeared on ^{13}CO bound to sperm whale myoglobin and human hemoglobin though these earlier data differ considerably from the shifts we have consistently observed.) The results clearly indicate significant differences between the magnetic environments experienced by carbon monoxide bound to α or β subunits. Whether these differences are due to changes in the bond between the iron and carbon monoxide or to other subtler interactions between the ligand and other groups around the heme pocket is presently unknown. Recent work[2] with a spin-labeled analog of ATP has led to the conclusion that oxygen binding to hemoglobin may be quantitatively described by a two-state model such as that of Monod, Wyman, and Changeux,[3] with the additional condition that the α and β subunits are nonequivalent.

Table I. Chemical Shifts of Heme-Bound ^{13}CO[a]

	δ_1	δ_2	pH
MbCO	−14.89		6.79–7.49
(sperm whale)	−14.61		5.42
HbCO-A (human)	−13.74	−13.26	6.35–7.90
HbCO-F (human)	−13.74	−13.30	6.80
HbCO (mouse)	−13.58	−13.14	6.21
HbCO (rabbit)	−15.16	−13.18	6.94–7.38

[a] Ppm from CS_2, ±0.04.

The pH dependencies of the ^{13}CO resonances showed that the MbCO resonance was independent over the pH range 6.79–7.49, though at pH 5.42 an upfield shift of 0.28 ppm was observed. The resonances of adult human HbCO were independent of pH over the range 6.35–7.90 as were the resonances of rabbit HbCO over the range 6.94–7.38. These results are somewhat unexpected as Shulman[4] reports that the heme proton chemical shifts of cyanoferrihemoglobin and several related "mixed state" hemoglobins are markedly sensitive to pH changes in the region near pH 7, although the resonances of these hydrogens, being strongly shifted by unpaired electron density on the carbons to which they are attached, may be much more susceptible to very small changes in protein conformation than the ^{13}C resonances in the carboxy heme globins. Also, the addition of a 2:1 molar excess of 2,3-diphosphoglycerate at pH 7.0 did not affect the chemical shifts of rabbit HbCO.

Interestingly, no significant difference is observed between the shifts of the ^{13}CO resonances when bound to fetal or adult human hemoglobin despite 39 amino acid substitutions[5] in human γ chains with respect to human β chains, *i.e.*, ^{13}CO bound either to a human β or γ subunit apparently experiences essentially the same environment. Conversely, the sequence differences between rabbit and human hemoglobin exert a marked influence on the environment for bound CO.

Studies of the spin–lattice relaxation times (T_1) of the ^{13}CO resonances using the technique of progressive saturation yield values of $T_1 \sim 0.3$ sec for both resonances in rabbit HbCO at pH 7.0 and 3 m*M* concentration.

Exposure of rabbit HbCO to oxygen results in a significantly faster diminution in the low-field resonance (−15.16 ppm from CS_2) than of the high-field resonance. This indicates a disparity between the relative affinities for carbon monoxide *vs.* oxygen by the different subunits. Based on kinetic studies using stopped-flow techniques, Gibson[6] has concluded that carbon monoxide replaces oxygen faster in the β than in the α subunits of a variety of hemoglobins. If these kinetic re-

(1) F. Conti and M. Paci, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **17**, 149 (1971).

(2) R. T. Ogata and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.*, **69**, 335 (1972).

(3) J. Monod, J. Wyman, and J.-P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

(4) S. Ogawa and R. G. Shulman, *Biochem. Biophys. Res. Commun.*, **42**, 9 (1971).

(5) W. A. Schroeder, J. R. Shelton, J. B. Shelton, J. Cormick, and R. T. Jones, *Biochemistry*, **2**, 992 (1963).

(6) J. S. Olson, M. E. Andersen, and Q. H. Gibson, *J. Biol. Chem.* **246**, 5919 (1971).

sults also reflect the relative thermodynamic affinities (*i.e.*, β subunits prefer carbon monoxide to oxygen as ligands by a larger margin than do α subunits), we should expect carbon monoxide to be replaced by oxygen more readily from α than from β subunits. Accordingly, if Gibson's kinetic results do reflect the differential thermodynamic affinities for oxygen *vs.* carbon monoxide of α and β subunits, we tentatively conclude that the resonance at lower field (which is the one more readily removed by oxygen) represents ^{13}CO bound to α subunits of rabbit hemoglobin.

In summary, this work indicates that significant differences exist in the nature of the environment experienced by carbon monoxide when bound to α or β subunits of a variety of hemoglobins. These differences do not appear to be significantly affected over the range pH 6.5–7.5 and, at least in the case of rabbit HbCO, are not affected by 2,3-diphosphoglycerate. Moreover, the ease of displacement of CO by O_2 differs markedly for CO bound to the α or β subunits of rabbit hemoglobin.

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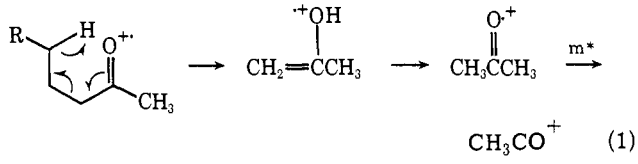
Reketonization of a McLafferty Product Ion Studied by Ion–Molecule Reactivity

Sir:

The nature of the McLafferty product of a ketone molecular ion has been inferred to be of enolic structure at its time of formation.[1] Recently it has been determined that for at least some aliphatic ketones the metastable McLafferty product decomposing by loss of methyl scrambles in such a way as to suggest reketonization of the enolic form (eq 1).[2] In the case of an aryl alkyl

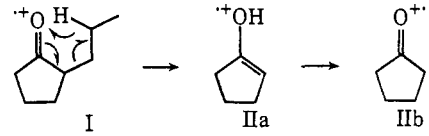


(1)

ketone, the ion kinetic energy spectrum indicates that the metastable McLafferty product which loses methyl is not reketonized.[3] A distinction may therefore be made between the behavior of purely aliphatic ketones and that of aryl alkyl ketones during loss of methyl from the metastable McLafferty product.[2c]

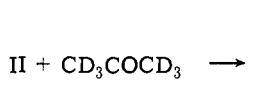
Previous studies of aliphatic ketones by ion cyclotron resonance (icr), however, have not led to observation of reketonization of a large fraction of the McLafferty product ions, for the keto and enol forms remain distinguishable by their ion-molecule chemistry.[4] We now report on a McLafferty rearrangement whose product, monitored by its ion-molecule reactivity, is found to change structure as the time scale of the icr experiment is increased. Standard[4] reactions for distinguishing keto and enol structures of ions were used to follow this transformation. It is, to our knowledge, *the first reported example of structural change followed by ion-molecule reactions.*

The mass and icr spectra of 2-propylcyclopentanone (I) contain an ion of *m/e* 84 (II) resulting from the loss of propylene in a McLafferty rearrangement (eq 2).

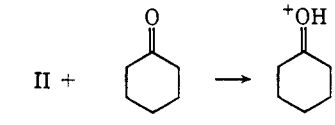


I IIa IIb (2)

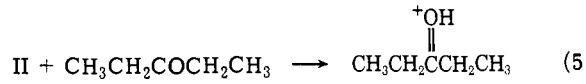
Under conventional conditions for operation (5×10^{-6} Torr for I, 5×10^{-6} Torr for the other ketone, total calculated transit time[5] (τ_T) *ca.* 4×10^{-4} sec to *ca.* 1×10^{-3} sec, ionizing voltage 12–30 V) the product behaves like the expected enol, as the following observed reactions typical of enolic structure[4] suggest (eq 3–5). Double resonance experiments confirm



(3)



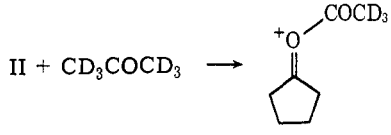
(4)



(5)

these assignments.

When the residence time of the ions is increased by adjustment[5] of the drift voltages (τ_T *ca.* 5×10^{-3} sec to *ca.* 1.6×10^{-1} sec) and all other conditions are maintained the same, eq 3–5 can no longer be detected in the analyzer. In their place, new reactions appear.



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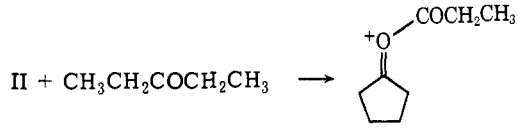

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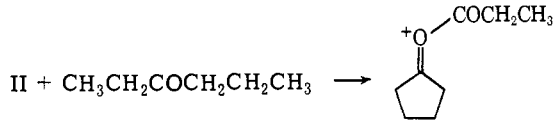

(8)

These are analogous to the reactions shown to be indicative of the keto form,[4] and in fact are also observed

(1) (a) J. A. Gilpin and F. W. McLafferty, *Anal. Chem.*, **29**, 990 (1957); (b) P. P. Manning, *J. Amer. Chem. Soc.*, **79**, 5151 (1957); (c) S. Meyerson and J. D. McCollum, *Advan. Anal. Chem. Instrum.*, **2**, 179 (1963).

(2) (a) D. J. McAdoo, F. W. McLafferty, and J. S. Smith, *J. Amer. Chem. Soc.*, **92**, 6343 (1970); (b) F. W. McLafferty, D. J. McAdoo, J. S. Smith, and R. Kornfeld, *ibid.*, **93**, 3720 (1971); (c) see, however, S. Meyerson and E. K. Fields, *Org. Mass Spectrom.*, **2**, 1309 (1969).

(3) J. H. Beynon, R. M. Caprioli, and T. W. Shannon, *ibid.*, **5**, 967 (1971).

(4) (a) J. Diekman, J. K. MacLeod, C. Djerassi, and J. D. Baldeschwieler, *J. Amer. Chem. Soc.*, **91**, 2069 (1969); (b) G. Eadon, J. Diekman, and C. Djerassi, *ibid.*, **91**, 3986 (1969); (c) G. Eadon, J. Diekman, and C. Djerassi, *ibid.*, **92**, 6205 (1970).

(5) T. B. McMahon and J. L. Beauchamp, *Rev. Sci. Instrum.*, **42**, 1362 (1971).