a consequence of this, they will be neglected. Therefore, the δ values for the nitro, formyl and cyanophenoxides are estimated to be +7.7, 4.8 and 5.2 cal/(deg mol), respectively.

These results clearly indicate that the origin of the $\Delta\bar{S}_i°$(aq) for the proton transfer reaction, eq 2, is almost exclusively due to the difference in entropy of hydration of the anions. This is in agreement with the interpretation of Hepler for the variations found for the entropies of ionization of isomeric substituted phenols. It has been stated in a previous publication that, based upon the corresponding $\Delta\bar{V}_i°$ differences and the partial molar volumes of the species involved in the equilibria, the solvation contribution to differences in acidity of isomeric phenols may have their primary origin in solvation differences in the un-ionized forms.[4a] It now appears that $\Delta\bar{S}_i°$ and $\Delta\bar{V}_i°$ measure different aspects of the solvation phenomenon and that the relationship between these parameters suggested by simple electrostatic[10] theory and from one of the Maxwell relationships[12] deserves reconsideration.

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(12) L. G. Hepler, *J. Phys. Chem.*, **67**, 496 (1963).

C. L. Liotta*
School of Chemistry, Georgia Institute of Technology
Atlanta, Georgia 30332

H. P. Hopkins, Jr.,* P. T. Kasudia
Department of Chemistry, Georgia State University
Atlanta, Georgia 30303

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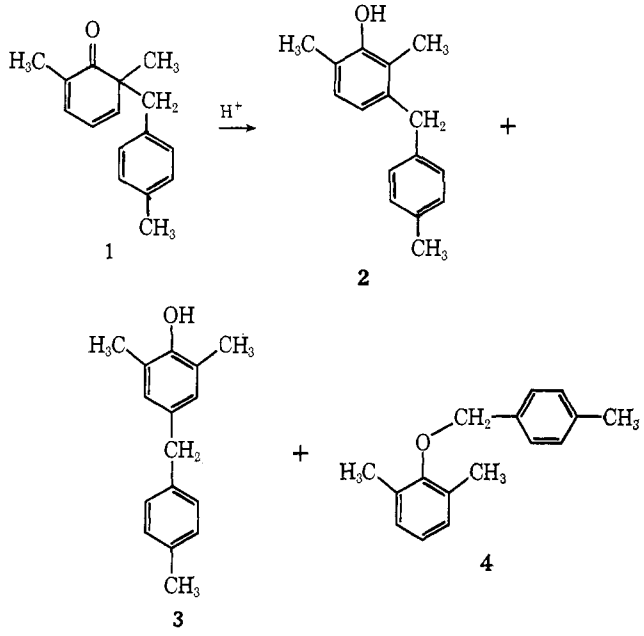
[1,3]Sigmatropic Shifts of Carbon–Carbon Bonds in Acid Catalyzed Rearrangements of Cyclohexadienones[1]

Sir:

Suprafacial [1,3]sigmatropic shifts of carbon–carbon bonds are classified as "forbidden" reactions.[2,3] The known examples of [1,3] rearrangements, whether they proceed by symmetry allowed antarafacial paths[4,5] or by other mechanisms,[6,7] proceed at temperatures high enough to provide most or all of the energy necessary for homolytic cleavage of the migrating bonds.[4-8] Distinguishing between concerted and diradical processes is therefore difficult, and attempts to establish the rearrangement mechanisms must rely on estimates of the degree of stereospecificity to be expected of homolytic dissociation–recombination processes.

I have observed that addition of small amounts of sulfuric acid to solutions of cyclohexadienone **1** in methanol or acetic acid results in rapid disappearance of **1** to give the [1,3] rearrangement products **3** and **4**, in addition to the [1,2] rearrangement product **2**. The solvolysis products, 2,6-dimethylphenol (2,6-DMP) and 4-methylbenzyl methyl ether or 4-methylbenzyl acetate, are also obtained. Phenols **2** and **3** are obtained as an inseparable mixture, whose composition may be determined by vpc[9] or (preferably) by nmr[10] analysis. The structures



of these phenols were proved by their independent synthesis and by comparison of the spectra and vpc retention times of appropriate synthetic mixtures with those of the mixture of **2** and **3** obtained from the rearrangement of **1**. Product yields are listed in Table I.

The evidence outlined below suffices to eliminate most of the possible mechanisms for formation of the [1,3] rearrangement products **3** and **4**. (a) All of the reaction products, either individually or in combination, are stable for prolonged periods under the conditions of the rearrangements. (b) Addition of 10- to 20-fold molar excess of 2,6-DMP to solutions of **1** in small volumes of methanol or acetic acid (although not in more dilute solutions) resulted in an increase in the yield of **3**. (See lines 4–6 in the table.) However, the ratio of **2** to **4**, or **2** to 4-methylbenzyl acetate, did not significantly change when excess 2,6-DMP was added to the rearrangement mixtures. Rearrangement in the more nucleophilic solvent methanol, although it gave cleavage products as the major components of the reaction mixture, actually gave somewhat higher ratios of **3** and **4** to **2** than did rearrangement in acetic acid. Finally, rearrangement in an acetic acid–ethanethiol

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(2) R. B. Woodward and R. Hoffmann, "The Conservation of Orbital Symmetry," Academic Press, New York, N. Y., 1970.

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(4) (a) J. A. Berson and G. L. Nelson, *J. Amer. Chem. Soc.*, **89**, 5503 (1967); **92**, 1096 (1970); (b) W. R. Roth and A. Friedrich, *Tetrahedron Lett.*, 2607 (1969); (c) S. Masamune, S. Takada, N. Nakatsuka, R. Vukov, and E. N. Cain, *J. Amer. Chem. Soc.*, **91**, 4422 (1969).

(5) J. A. Berson, *Accounts Chem. Res.*, **5**, 406 (1972).

(6) See J. S. Swenton and A. Wexler, *J. Amer. Chem. Soc.*, **93**, 3066 (1971), for references to [1,3] shifts in rearrangements of vinylcyclopropanes.

(7) (a) G. S. Hammond and C. D. De Boer, *J. Amer. Chem. Soc.*, **86**, 899 (1964); (b) J. A. Berson and R. W. Holder, *ibid.*, **95**, 2037 (1973); (c) R. C. Cookson and J. E. Kemp, *Chem. Commun.*, 385 (1971).

(8) For arguments that the activation energy for a concerted rearrangement may *exceed* the dissociation energy of the migrating bond, see J. A. Berson, T. Miyashi, and G. Jones, II, *J. Amer. Chem. Soc.*, **96**, 3468 (1974).

(9) Partially overlapping peaks for **2** and **3** are obtained by vpc on a 5 ft × 1/8 in., 1.5% OV-101 column.

(10) The ratio of **2** to **3** in a mixture is best determined from relative areas of the singlets in the nmr spectrum of the mixture at δ 3.92 and at 3.80, due to the methylene groups in **2** and **3**, respectively.

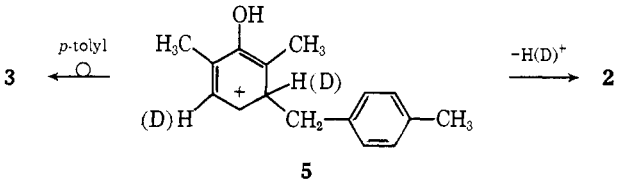
Table I

No.	Solvent	[1], M	[H_2SO_4], M	Other reagents (M)	Product composition[a] mole ratio: [CH_2OX][b]/[CH_3] / [2]	[2]/[3]	[4]/[2]	[2]/[EtSPh]	n[c]
1	HOAc	8.9×10^{-2}	4×10^{-2}		0.36 ± 0.03	1.41 ± 0.03	0.076 ± 0.006		4
2	CH_3OH	8.9×10^{-2}	10^{-1}		1.59 ± 0.07	1.26 ± 0.03	0.092 ± 0.004		4
3	HOAc	1.5×10^{-2}	4×10^{-2}		0.33 ± 0.03	1.43 ± 0.02	0.073 ± 0.01		2
4	HOAc	2.0×10^{-2}	4×10^{-2}	2,6-DMP (0.3)	0.34	1.40	0.074		1
5	HOAc	8.9×10^{-2}	4×10^{-2}	2,6-DMP (0.66)	0.37	1.26	0.080		1
6	HOAc	8.9×10^{-2}	4×10^{-2}	2,6-DMP (1.4)	0.35	1.06	0.071		1
7	HOAc	8.9×10^{-2}	3.5×10^{-2}	EtSH (2.7)	0.31 ± 0.01	1.48 ± 0.04	0.079 ± 0.00	1.69 ± 0.03	2

[a] Total molar yield, >95% in all runs. [b] In line 2, X is CH_3. In other lines, X is $COCH_3$. [c] Number of runs averaged.

mixture, although it yielded appreciable amounts of ethyl 4-methylbenzyl sulfide, gave **2**, **3**, **4**, and 4-methylbenzyl acetate in almost precisely the same ratios as did rearrangement in acetic acid. These results can best be explained as demonstrating SN2 attack of acetic acid, 2,6-DMP, or ethanethiol at the benzylic methylene group of protonated **1**. Clearly, under these conditions, no significant formation of an interceptible 4-methylbenzyl carbenium ion occurs. (c) The ratios of products from reaction of **1** with acid in either acetic acid or methanol are independent of the concentration of **1** in solution. (*E.g.*, compare lines 1 and 3 in the table.) This fact, as well as the observation reported above that addition of 2,6-DMP does not affect the ratio of **2** to **4** or of **2** to **3** in dilute solution, rules out the possibility that any significant amounts of **3** or **4** are formed by reaction of protonated **1** with the small quantities of 2,6-DMP produced by solvolysis of **1**. (d) The rate of disappearance of **1** in acetic acid is first order in **1**. This fact, as well as the observation that the ratios of yields of products are independent of the concentration of **1**, rules out the possibility that formation of **3** or **4** occurs *via* bimolecular migration of benzyl groups between two molecules of **1**.[11] (e) Rearrangement of **1**-d_2, bearing 0.86 ± 0.005 atoms of deuterium at C-3 and C-5, gave **2** and **3** in the ratio 1.38 ± 0.02, while the ratio in methanol was 1.30 ± 0.05. These ratios are identical, within experimental error, with those obtained from rearrangement of **1**.

If **3** were obtained *via* a sequence of two "normal" [1,2] shifts of the 4-methylbenzyl group, the ratio of **2** to **3** in the product would depend upon the relative rates of rearrangement and aromatization of carbenium ion **5**. Since aromatization of cyclohexadienyl carbenium ions (obtained either from rearrangment of a cyclohexadienone[12] or from protonation of aromatic molecules)[13] proceeds with deuterium isotope effects



5

of *ca.* 4–5, exclusive formation of **3** *via* **5**-d_2 should give **2** and **3** in about a ratio of 0.4 (allowing for residual hydrogen at C-3 and C-5), rather than the *ca.* 1.3 ratio actually observed. Thus, **3** cannot be formed by a series of simple Wagner–Meerwein shifts.[14] (f) The rate of disappearance of **1** (in 2×10^{-3} M solution in acetic acid) is unaffected by the presence of 5×10^{-4} M benzoyl peroxide or 10^{-3} M thiophenol. Taken together with the observation that no change in the ratios of products is caused by the presence of 2.7 M ethanethiol, these observations effectively eliminate possible radical chain mechanisms for formation of **3** and **4**. (g) Dissociation of **1** into phenoxy and benzyl radicals should require an activation energy of >40 kcal/mol, in agreement with the observations that dienone **1** is stable (in dilute solution to prevent dimerization) for months at room temperature and for a minimum of several hours in decalin at 150°. Increasing the polarity of the bond between the benzyl group and the cyclohexadienone ring by protonating the carbonyl should actually increase its dissociation energy.[15,16] Nonetheless, reaction is complete (more than ten half-lives) in 4×10^{-2} M sulfuric acid in acetic acid solution after 10 min at room temperature. A free radical dissociation–recombination mechanism therefore cannot account for formation of **3** or **4**.

Since possible alternative mechanisms have been eliminated, it seems necessary to conclude that **3** and **4** are formed by concerted [1,3]sigmatropic shifts of the 4-methylbenzyl group to carbon and oxygen, respec-

(11) A bimolecular mechanism has been proposed to account for the acid catalyzed [1,3] shifts of benzyl groups in dihydroisoquinolines: J. Knabe, R. Dorr, S. F. Dyke, and R. G. Kinsman, *Tetrahedron Lett.*, 5373 (1972).

(12) B. Miller, *J. Amer. Chem. Soc.*, **87**, 5111 (1965).

(13) L. Melander and S. Olsson, *Acta Chem. Scand.*, 10, 879 (1956); S. Olsson, *Ark. Kemi*, **14**, 85 (1959); see also V. Gold, R. W. Lambert, and D. P. N. Satchell, *Chem. Ind. (London)*, 1312 (1959); V. Gold, R. W. Lambert, and D. P. N. Satchell, *J. Chem. Soc.*, 2461 (1960); A. J. Kresge and Y. Chiang, *J. Amer. Chem. Soc.*, 83, 2877 (1961); J. Schulze and F. A. Long, *ibid.*, **86**, 331 (1964).

(14) Since deuterium is present at C-3, an intervening degenerate [1,5] shift of the benzyl group [B. Miller, *J. Amer. Chem. Soc.*, **92**, 6252 (1970)] would not change the result of this experiment.

(15) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, Chapter 3.

(16) Protonated **1** thus differs markedly from ylides (as in, for instance, the Stevens, Meisenheimer, and Wittig rearrangements), whose dissociation into radicals would result in elimination of charge separation.

tively.[17] The very low temperature required for these acid-catalyzed migrations contrasts dramatically with those necessary for purely thermal [1,3]sigmatropic shifts. We are presently investigating the stereochemistry of these rearrangements.

Acknowledgments. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for a grant in support of this research.

(17) The term "concerted" is used here in an operational sense, implying the absence of intermediates, such as normal carbenium ions or radicals, which can be intercepted. The possibility of a shallow minimum in the reaction surface (a π-complex, for instance) is not excluded.

Bernard Miller

Department of Chemistry, University of Massachusetts Amherst, Massachusetts 01002

Received July 13, 1974

Carbon Magnetic Resonance Study of the Conformational Changes in Carp Muscle Calcium Binding Parvalbumin

Sir:

The subtle conformational changes associated with the release of a calcium ion from the parvalbumin protein of carp have been investigated by carbon-13 nuclear magnetic resonance spectroscopy. This communication discusses the structural changes which occur in that part of the protein molecule made up of aromatic residues as monitored by measurement of chemical shift changes as well as nuclear Overhauser enhancement (NOE) contributions to signal intensity and spin–lattice relaxation times (T_1). Parvalbumin function is usually discussed in terms of control processes affected by calcium ion concentration such as muscle contraction[1,2] and glycogenolysis.[3] These proteins are low molecular weight (12,000), calcium binding ($2Ca^{2+}$/protein) monomeric species with unusual amino acid composition (10% phenylalanine, 20% alanine) found in the white muscle of vertebrates.[1,4]

The natural abundance carbon-13 nuclear magnetic resonance spectrum of parvalbumin component 3 with two bound calcium ions is presented in Figure 1a.[5] Many resonances are resolved in this spectrum including an unusually downfield shifted carboxyl carbon (184.6 ppm), two electron shielded carbonyl carbons (168.9 and 170.9 ppm), the guanido carbon of the single arginine residue (158.5 ppm), as well as some individual resonances arising from the Cγ carbons of the ten phenylalanine residues (134–139 ppm) and the δ-methyl carbons from the five isoleucine residues (10–13 ppm). Individual resonances were identified as arising from single carbons by comparison of integrated intensities to a known one carbon signal (*i.e.*, Arg guanido) in a spectrum without NOE contributions to signal intensities.[6]

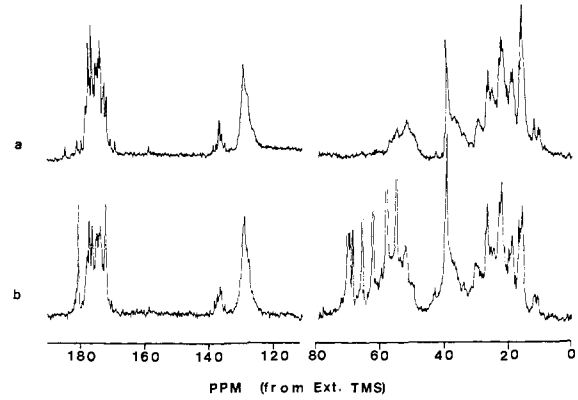


Figure 1. Proton decoupled natural abundance carbon-13 nuclear magnetic resonance spectra of parvalbumin component 3 (pH 7.8), obtained at 25.2 MHz with a Varian XL-100 spectrometer equipped with a Nicolet Technology Corp. pulse unit and data system. Free induction decays were collected following 90° rf pulses at 1.245-sec intervals. Data points (16K) were sampled, with an additional 16K points of zero added. The entire data table was multiplied by an exponential corresponding to 2.0 Hz line broadening followed by Fourier transformation: (a) parvalbumin (20 m*M*) with two bound calcium ions, 50,000 transients collected; (b) parvalbumin (15 m*M*) after the removal of the solvent accessible calcium by addition of EGTA. The additional large peaks in the spectrum are due to the added chelating agent. Transients (75,000) were collected.

The crystal structure[7] shows that the calcium ion which can be first removed from the protein by EGTA[8,9] addition is exposed to the solvent. It is coordinated through the carboxyl groups of aspartic acid residues 90, 92, and 94 and glutamic acid residue 101 as well as the carbonyl oxygen atom of lysine 96. Removal of this calcium gives rise to many spectroscopic changes (Figure 1b), most obviously the loss of the previously noted downfield carboxyl and the most upfield shifted carbonyl. The large changes in the chemical shift of the carboxyl carbon from 184.6 ppm to the overall carbonyl envelope is most likely due to the removal of the side chain of glu-81 from an internal salt bridge to Arg-75, a change previously postulated on mechanistic grounds.[7,10] This change represents a specific rearrangement of residues located more than 20 Å from the calcium binding site. The loss of the upfield carbonyl signal can tentatively be attributed to the removal of the carbonyl group of Lys-96 from the coordination sphere of the calcium ion. Such coordination through a carbonyl function to an alkaline earth metal is predicted to decrease the polarity of the carbon–oxygen bond, which increases the electronic shielding at the carbonyl carbon atom.[11] Additional structural changes, transmitted over large distances relative to the Ca^{2+} binding site, are reflected in the aliphatic region, most notably in the alanine methyl carbon region (15–18 ppm) and in the isoleucine δ-methyl carbon region.

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(2) J.-F. Pechere, J. Demaille, and J.-P. Capony, *Biochim. Biophys. Acta*, **236**, 391 (1971).
(3) J.-F. Perchere, *Biochimie*, **278D**, 2577 (1974).
(4) P. Lehky, H. E. Blum, E. A. Stein, and E. H. Fischer, *J. Biol. Chem.*, **249**, 4332 (1974).
(5) There are four major isotypes of the basic parvalbumin molecule in the carp (Cyprinus carpio), referred to as parvalbumin components 2, 3, and 5. Components 1 and 4 correspond to additional isotypes which are occasionally isolated in much lower yields.
(6) S. J. Opella, D. J. Nelson, and O. Jardezky, manuscript in preparation.
(7) R. H. Kretsinger and C. E. Nockolds, *J. Biol. Chem.*, **248**, 3313 (1973).
(8) EGTA = ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid.
(9) J. Parello, A. Cave, P. Puigdomenech, C. Maury, J.-P. Capony, and J.-F. Pechere, *Biochimie*, **56**, 61 (1974).
(10) R. H. Kretsinger in "Perspectives in Membrane Biology," S. Estrada and C. Gitler, Ed., Academic Press, New York, N. Y., 1974.
(11) G. E. Maciel, *J. Chem. Phys.*, **42**, 2746 (1965).