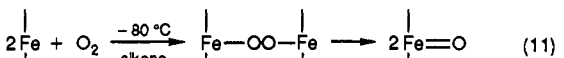
Table I. Products of Oxidation of Norbornene and Cyclohexene by Various Oxidation Systems[a]

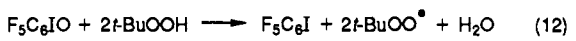
oxidant	intermediate	norbornene exo/endo[b]	cyclohexene epox/alc[c]	cyclohexene epox/(alc + ketone)
PFIB, TDCPPFeCl	$Fe^+{=}O$	13 (15%)[d]	27 (50%)[d]	20
O_2, TPPFe[e]	Fe=O	(0%)[d]	(0%)[d]	(0%)[d]
PFIB, *t*-BuOOH	*t*-BuOO•	>58[f] (4%)[d]	2.9 (3%)[d]	1.4
H_2O_2, TDCPPFeCl		18 (25%)[d]	26 (50%)[d]	15
t-BuOOH, TDCPPFeCl		15 (16%)[d]	13[g] (16%)	11

[a] The reaction solution contained 1 M alkene, 0.2 M in oxidant, and 0.001 M catalyst in a solvent containing 59.3% CH_2Cl_2, 39.3% methanol, and 1.4% water by volume except for the reaction with O_2. TDCPPFeCl: iron(III) tetrakis(2,6-dichlorophenyl)porphyrin chloride. [b] The ratio of *exo*-2,3-epoxynorbornane to *endo*-2,3-epoxynorbornane. The epoxides were identified by GC-mass spectroscopy. [c] The ratio of epoxycyclohexane to 2-cyclohexene-1-ol. [d] Total yields of all products, based upon oxidant are given in parentheses. All yields are corrected for response factors and compared to an internal standard with use of gas liquid chromatography. Yields were not optimized. Much higher yields have been obtained in all the catalyzed reactions by varying conditions. Cyclohexenone always accompanied cyclohexanol. [e] The oxo compound was generated at −80 °C in toluene as solvent in the presence of 3×10^{-3} M iron(II) tetraphenylporphyrin, 1 M alkene, or in phosphine and warmed to room temperature as described by Balch et al.[2] [f] Using peroxy radicals produced in other ways exo/endo ratios were ~1000. [g] Both the yield of epoxide, which disappears with time, and the epoxide/alcohol ratio are very sensitive to traces of oxygen under these conditions. It is difficult to prevent at least some autoxidation of cyclohexene since O_2 is evolved in the side reactions 3 and 4. Without freeze/thaw degassing, lower epoxide/alcohol ratios are observed.

with hydrogen peroxide or *tert*-butyl hydroperoxide. The methods used are reaction 8,[1] reaction 11,[2] and reaction 12.[5]



(11)



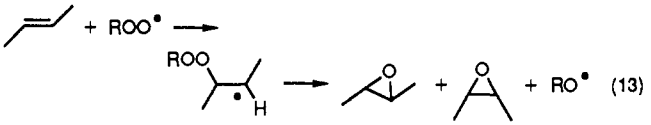
(12)

We first repeated the preparation of Fe=O described by Balch et al.[2] and obtained his results, both with respect to phosphine oxidation and the failure to obtain epoxide from alkenes. Reaction 12 has been shown both by Milas et al.[15] and in our laboratories to afford *t*-BuOO• radicals.

Except for the formation of the oxo species, which were produced exactly as described by Balch et al., all of the oxidations were carried out at room temperature in the same solvent.

The results are given in Table I. The conclusions are clear. The reactions of hydrogen peroxide or *tert*-butylhydroperoxide with TDCPPFeCl produce essentially the same exo/endo epoxynorbornane ratios and epoxycyclohexane/cyclohexenol ratios as does the reaction with PFIB. The oxo species affords no epoxide, and the *tert*-butyl peroxy radical gives essentially *no* endo epoxide. This, we propose, is evidence that at least that portion of the reaction which produces oxidized products proceeds by *heterolytic cleavage* of hydrogen peroxide or *tert*-butyl hydroperoxide.

The differences in kinetic results among different laboratories[3,7] and previous failures to obtain epoxides from hydrogen peroxide or hydroperoxides[16] are now becoming clear. The catalase reaction 3 prevents epoxidation and/or leads to loss of stereochemistry.



(13)

Additionally, the consequent buildup of alkylperoxy and alkoxy radicals which occurs only with hydroperoxides leads to reactions of these radicals with the products used to detect rates[3,17] and significant errors can result.[3,9,17]

Apparently, more electronegative oxenes are less selective toward reaction 3 with hydroperoxides and allow higher yields of epoxides from hydrogen peroxide. All of the reactions of the oxidants RIO, RCO_3H, ROOH, and H_2O_2 with TDCPPFeCl in hydroxylic solvents are now understandable as heterolytic processes like those which occur in peroxidase and catalase. The importance of these conclusions is that we are encouraged to explore methods of obtaining high yield, stereo- and regiospecific catalytic epoxidation using hydrogen peroxide and hydroperoxides. These studies will be reported elsewhere.

Acknowledgment. Support for this work by the National Science Foundation (Grant CHE87-21364) is gratefully acknowledged. Helpful discussion with and the preparation of the catalyst by Dr. Shinji Tsuchiya are also appreciated.

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(18) Hydroperoxides in contrast to peracids or hydrogen peroxide are particularly prone to a variety of free radical chain processes. Since either Fe=O or $Fe^+{=}O$ can initiate such processes, it becomes difficult to establish the mechanism of the initial reaction under conditions where these chain reactions (evidenced by RO• cleavage, O_2 evolution, peroxy radical addition, etc.) are present. We suggest that this is responsible for the suggestion[7] that peracids react by heterolytic cleavage and hydroperoxides by homolytic cleavage. In fact, our studies along with those of Groves et al.[19] and Balch et al.[20] indicate that the change from heterolytic to homolytic cleavage occurs with both peracids and hydroperoxides at a point where general acid catalysis becomes ineffective. Thus in basic media or in the absence of hydroxylic solvents homolytic cleavage is to be expected. In hydroxylic solvents all the reactions appear to be heterolytic with the catalysts we have used.
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Evidence for a Double-Minimum Potential for Intramolecular Hydrogen Bonds of Aqueous Hydrogen Maleate and Hydrogen Phthalate Anions

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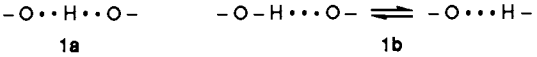
In the study of hydrogen bonds one of the fundamental questions is whether the potential energy for motion of the hydrogen has a single minimum (**1a**) or two minima (**1b**). If single, the hydrogen is fixed between the two donor atoms. If there are two minima, the hydrogen will be found closer to one donor than to the other, even if those donors are identical. There are then two forms in rapid equilibrium (**1b**). Both situations have been observed by a variety of experimental methods.[1] One direct method for distinguishing them is the method of isotopic perturbation of degenerate equilibrium.[2] Saunders and Handler[3] have applied



1a **1b**

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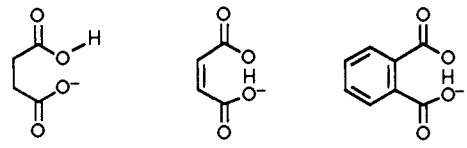
this to the enol of 2,4-pentanedione-1-d_n and confirmed that this shows a double-minimum potential. We now report that not only hydrogen succinate (**2**) but also hydrogen maleate (**3**) and hydrogen phthalate (**4**) anions show a double-minimum potential, despite opposite results from previous methods.[4]



2 **3** **4**

The method depends on the observations that isotopic substitution decreases acidity[5] and that the carbonyl ^{13}C NMR chemical shift varies sufficiently from carboxylic acid to carboxylate anion.[6] For a mono-^{18}O-substituted dicarboxylic acid, with K_1 and K_2 the first and second acidity constants of the –COOH group and with K_1' and K_2' the acidity constants for the $-C(^{16}O,^{18}O)H$ group, it can be shown that Δ, the isotope shift of the ^{18}O-labeled carbonyl, relative to the ^{16}O carbonyl, is given by eq 1, where Δ_0 is

$$\Delta = \Delta_0 + (\delta_- - \delta_H)K(K-1)(1 + K - \{(n-1)^2[(1+K)^2 - 4rK] + 4rK\}^{1/2})/[(1+K)^2 - 4rK] \quad (1)$$

an intrinsic shift (downfield positive), δ_H and δ_- are the chemical shifts of –COOH and $-CO_2^-$, respectively, n is the number of carboxylate equivalents ($0 < n < 2$), $K = K_1/K_1' = K_2/K_2'$, and $r = K_2/K_1 = 4K_{a2}/K_{a1}$, with K_{a1} and K_{a2} the observed acidity constants of the dicarboxylic acid.[7] For maleic and phthalic acids eq 1 is only approximate, since the chemical shift of the monoanions (**3** and **4**) is not simply the average of δ_H and δ_- but is affected by the internal hydrogen bond. This Δ shows an extremum at $n = 1$, so that if there exists an equilibrium between tautomers of the monoanion, it will be manifested in the observed isotope shift, even though the tautomers equilibrate too rapidly for their independent signals to be seen.

The ^{18}O-labeled dicarboxylic acids were prepared by adding 0.1 mmol of recrystallized maleic anhydride or sublimed succinic anhydride to 20 μL of 98% $H_2{}^{18}O$ (Icon) and warming at 40 °C for several hours. Sublimed phthalic anhydride in tetrahydrofuran was reacted similarly and lyophilized. Formic acid was labeled at 40 °C in 50% $H_2{}^{18}O$. These conditions produced up to 20% of doubly labeled acids. Each resulting solution or solid was dissolved in 0.5 mL of H_2O containing 10%–20% D_2O for spectrometer lock. Approximately 10 μmol of Na_2EDTA was added to chelate traces of paramagnetic metal ions that broaden carboxylate resonances. Dioxane was used as an internal standard (δ = 67.6 ppm).

Spectra were obtained on a QE-300 FT-NMR spectrometer operating at 7.05 T (75 MHz ^{13}C). The temperature was regulated at 26 °C. Typically 8K data points (real + imaginary) were collected and zero-filled to 8K with use of a 45° pulse, a spectral width of ±256 Hz, 100–500 transients, and 0.2 Hz line broadening. Peak assignments were made by adding an authentic sample of ^{16}O acid. The solutions were titrated with 5-μL aliquots of 4.9 M KOH, and ^{13}C NMR spectra were obtained after each addition. For maleic and phthalic acids, which are more acidic and appreciably dissociated in water, the final sample was reacidified with excess 12 M HCl.

As the acid is titrated, the carbonyl peaks (and ipso peak of phthalic acid) shift downfield. The limiting chemical shifts δ_H and δ_- of carboxylic acid and carboxylate, respectively, are listed in Table I. These values are in adequate agreement with those previously reported.[6] The extent of neutralization was then taken from the observed shift, rather than from the stoichiometry of KOH added.

Table I. Isotope Shifts, Chemical Shifts, and Isotope Effects on Acidity Constants of Carboxylic Acids

	$-\Delta_0$ per ^{18}O, ppb	δ_H, ppm	δ_-, ppm	K per ^{18}O
formic acid	25	166.9	172.0	1.0108 ± 0.0005
succinic acid	27	178.2	183.4	1.0060 ± 0.0001[a]
maleic acid	26	170.8	176.3	1.0044 ± 0.0001[b]
phthalic acid				
carbonyl	26	172.9	178.5	1.00926 ± 0.00009
ipso	≤5	133.0	138.7	1.0201 ± 0.0002
meta	<4	132.3	129.7	1.0128 ± 0.0003
ortho	<4	129.8	128.2	1.0121 ± 0.0008

[a] 1.0060 ± 0.0001 from di-^{18}O acid. [b] 1.0043 ± 0.0002 from di-^{18}O acid.

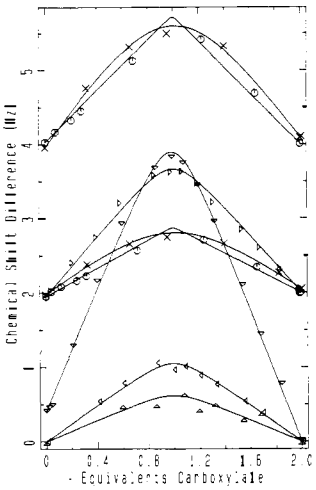


Figure 1. Observed isotope shift vs extent of neutralization: succinic acid (×), maleic acid (○), phthalic acid: carbonyl (△), ipso (▽), meta (△), and ortho (△).

The spectra show three peaks in the carbonyl region, corresponding to $C^{16}O_2H$, $C(^{16}O,^{18}O)H$, and $C^{18}O_2H$. The carbons bearing the ^{18}O appear upfield ($\Delta < 0$), as is generally observed.[2b,c] At both ends of the titration (carboxylic acid and carboxylate) the same intrinsic isotope shift Δ_0 is observed, except perhaps for the ipso carbon of phthalic acid. Values are listed in Table I.

Figure 1 shows how the isotope shift varies with the extent of neutralization. The data for the dicarboxylic acids were fit to eq 1 by least squares. Separate determinations of K could be obtained for $-C(^{16}O,^{18}O)H$ and $-C^{18}O_2H$ acids and from all three aromatic carbons of phthalic acid. Values of the acidity constant ratio K are listed in Table I, along with the standard deviation. The value of K for formic acid agrees well with the 1.0117 (interpolated to 20% D_2O) obtained by Ellison and Robinson.[5a] The value for succinic acid is similar but not identical, since K is sensitive to vibrational frequencies. Such a value is expected, since hydrogen succinate anion (**2**) is probably not internally hydrogen-bonded in aqueous solution, so it certainly exists as two forms whose equilibrium can be perturbed by ^{18}O substitution.

The surprising result is that maleic and phthalic acids also show this perturbation. The values of K in Table I are only approximate, and the apparent variation with reporter nucleus arises because δ_H and δ_- are not exact models for the chemical shifts in the monoanions. However, the values of K are similar to those of the other two acids. Therefore even hydrogen maleate (**3**) and hydrogen phthalate (**4**) anions exist as two forms. If there were only one form, with a single-well potential, there would be no equilibrium to perturb, so that the isotope shift would be independent of the extent of neutralization and vanishingly small at the aromatic carbons of phthalic acid. Moreover, the isotope shifts for phthalic acid decrease with increasing temperature, so this phenomenon is due to an equilibrium. We therefore conclude that the protons in these hydrogen bonds are in a double-well potential.

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Hydrogen maleate (**3**) and hydrogen phthalate (**4**) anions are the paradigms of a symmetric hydrogen bond.[1] Yet previous studies[4] were carried out in crystal or in nonpolar solvents. This is the first study of hydrogen-bond structure in aqueous solution. The internal hydrogen bond of **3** and **4** is still present even in aqueous solution, as judged from the large difference between first and second pK_as.[7] We suggest that the asymmetry that we find is caused by the disordered aqueous environment,[8] and this possibility is currently under investigation.

Acknowledgment. This research was supported by National Science Foundation Grant CHE-8714451.

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Biosynthetic Mechanism of C–P Bond Formation. Isolation of Carboxyphosphonoenolpyruvate and Its Conversion to Phosphinopyruvate

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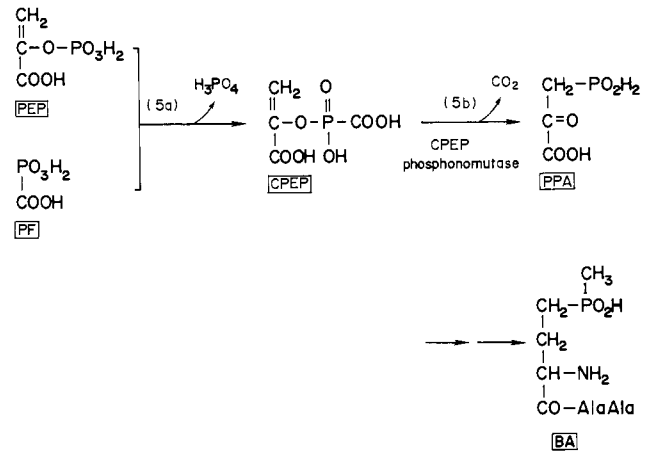
Since the first discovery of natural phosphonate, 2-aminoethylphosphonic acid,[1,2] the very unique C–P bond formation mechanism has attracted considerable interests in the past three decades; all the attempts, however, were so far unsuccessful to verify the enzymatic formation of the plausible intermediate phosphonopyruvate from phosphoenolpyruvate (PEP).

Recently Seidel,[3] Bowman,[4] Takada,[5] and our group[6] have reported the purification of PEP phosphomutase from *Tetrahymena* or from *Streptomyces hygroscopicus*. Although this enzyme is verified to be responsible for the C–P bond formation, only the reverse reaction, i.e., transformation of phosphonopyruvate to PEP, has been shown to occur due to the enzymatic reaction equilibrium which is far in favor of the formation of PEP.[3,4,6] We report herein another example of the C–P bond formation, i.e., the transformation of a newly isolated compound, carboxyphosphonoenolpyruvate (CPEP) to phosphinopyruvate, catalyzed by a new enzyme carboxyphosphonoenolpyruvate (CPEP) phosphonomutase.[7]

During the biosynthetic studies of a tripeptide herbicide bialaphos (BA),[8] which is produced by *Streptomyces hygroscopicus* SF-1293, we have shown that the first natural phosphinate, phosphinopyruvate (PPA),[9] was formed by condensation between PEP and phosphonoformate (PF)[10] as illustrated in Scheme I. Involvement of the latter substrate in this reaction named step 5 was clarified by its accumulation in a deficient mutant NP213 obtained by NTG treatment.[10] Consideration of this reaction mechanism strongly suggested the involvement of a hitherto unknown biosynthetic intermediate with a carboxylated phosphonate ester (CPEP in Scheme I). This plausible mechanism, however, remained unanswered due to the failure to obtain any blocked mutants, which will accumulate the suggested intermediate, by conventional methods.

Scheme I. The Proposed Mechanism of C–P Bond Formation (Step 5) in Bialaphos Biosynthesis[a]



[a] AlaAla = alanylalanine.

Very recently we have succeeded in preparation of a desirable new deficient mutant NP71[7] by the use of a new gene replacement technique, in vitro derived mutation.[11] Although this organism could not catalyze the step 5 reaction, it was complementary to NP213. Cosynthesis experiments between these two mutants suggested the accumulation of an unknown intermediate of BA biosynthesis in NP213 and the presence of a new enzyme catalyzing C–P bond formation in the cells of NP71. This intermediate was exhausted upon starvation.

The new enzyme named CPEP phosphonomutase was purified by conventional methods about 200-fold in four steps to give a homologous protein by SDS-PAGE to be a monomer of 32 000.[7] With the purified enzyme in hand, it became possible to isolate the substrate of CPEP phosphonomutase from the fermentation broth of the mutant NP213. CPEP was purified from the broth filtrate[12] (1.2 L) by treatments with active carbon, Dowex 1 (Cl^-), Diaion HP-20, DEAE Sephadex A-25 (Cl^-), and finally by Sephadex G-10 column chromatography to give a pure sample (1 mg). CPEP was obtained as white amorphous powder [mp 220 °C>; δ_P −1.65 ppm, IR (KBr) 1585, 1411 cm^{-1}. Anal. ($C_4H_2O_7PNa_3 \cdot 2H_2O$) H, Na; C calcd, 16.12; found 16.73, P calcd, 10.39; found, 9.90]. Its ^1H NMR spectrum (D_2O) showed only two broad singlets at 5.20 and 5.54 ppm and was very similar to that of PEP. The structural similarity between CPEP and PEP was also confirmed by the ^{13}C NMR signals of CPEP at 171.1 (C-1, COOH), 149.7 (C-2, O—C=), and 104.2 ppm (C-3, CH_2=). However, an additional resonance was observed at 177.4 ppm (C-4, P-COOH, doublet, J_{C-P} = 240.6 Hz). Thus, CPEP is determined to be a P-carboxylated derivative of PEP (Scheme I). The proposed structure was supported by the long range

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(12) Fractions under purification were incubated at 27 °C overnight with starved mycelium of NP213 and CPEP phosphonomutase, and then the amount of BA produced was determined by antimicrobial activity against *Bacillus subtilis*.

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