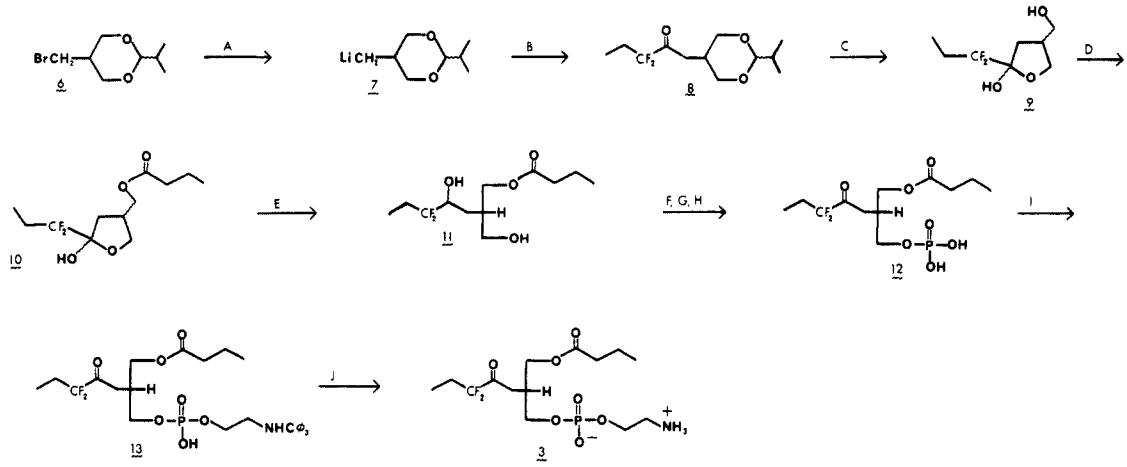
Scheme I[a]



[a] (A) 2.0 equiv of *t*-BuLi, Et_2O, −78 °C; (B) $CH_3CH_2CF_2CO_2CH_3$, −78 to 25 °C; (C) 1 N HCl, dioxane, 25 °C; (D) 1 equiv of butyryl chloride, 1.0 equiv of pyridine, $CHCl_3$, 0 °C; (E) $NaBH_4$, CH_3CH_2OH, 25 °C; (F) 3.0 equiv of $ClPO(OPh)_2$, pyridine, 25 °C; (G) 12.0 equiv of CrO_3·(pyridine)$_2$, CH_2Cl_2, 25 °C; (H) PtO_2, 1 atm of H_2, CH_3OH, 25 °C; (I) trityl-$NHCH_2CH_2OH$, triisopropylbenzenesulfonyl chloride, $CHCl_3$, 25 °C; (J) 25% CF_3CO_2H in CH_2Cl_2, 0 °C.

erties. Thus the interaction of **3** with the enzyme will be interpretable in terms of the standard treatment of enzyme inhibition kinetics in homogenous systems.

The preparation of **3** in racemic form is outlined in Scheme I. Bromide **6**[8] was converted to organolithium **7** and treated with methyl 2,2-difluorobutyrate to give difluoromethylene ketone **8** (35%). Cleavage of **8** under acidic conditions gave cyclic hemiketal **9** (100%). Compound **9** was selectively acylated with butyrl chloride to give **10** (90%) followed by hydride reduction to diol **11** (66%). Selective phosphorylation of the more nucleophilic, nonfluorinated alcohol with diphenyl chlorophosphate followed by oxidation of the fluorinated alcohol with CrO_3·(pyridine)$_2$ and finally hydrogenolysis of the phosphate triester gave **12** in 75% overall yield. Phosphatidic acid analogue **12** was condensed with *N*-tritylethanolamine in the presence of triisopropylbenzenesulfonyl chloride to **13** (45%). Detritylation of **13** with trifluoroacetic acid gave product **3** (90%).

Phosphatidylethanolamine analogue **3** was tested as an inhibitor of snake venom phospholipase A_2. Enzymatic activity toward the monomerically dispersed substrate 1,2-dibutyryl-*sn*-glycero-3-phosphatidylcholine[5] was measured by titration of the liberated butyric acid in a pH state.[9] Compound **3** was found to be a simple competitive inhibitor of phospholipase A_2 with a K_i = 50 μM. It is significant that **3** binds approximately 300-fold tighter than the analogous substrate (1,2-dibutyryl-*sn*-glycero-3-phosphatidylethanolamine, K_m = 14 mM). Furthermore, **3** is bound considerably tighter than amides **1** or carbamates **2**. The ability of **3** to act as a tight-binding inhibitor may be due to its unique ability to form tetrahedral species. The mixture of difluoromethylene alcohols **14**, prepared by borohydride reduction of **13** followed by detritylation, was also inhibitory (K_i = 200 μM). Although somewhat less inhibitory than **3**, **14** is significantly more potent than **1** and **2**. Taken together, these results suggest that **3** and **14** are mimics of a tetrahedral species formed by the attack of water onto the carbonyl group of a phospholipid substrate.

The use of the difluoromethylene ketone unit as an isosteric replacement of ester linkages in phospholipids appears to be an effective strategy for the inhibition of lipolytic enzymes. The determination of the mode of binding of **3** to the enzyme (i.e., hydrate **4** or hemiketal **5**) will have important implications for the catalytic mechanism of enzymatic lipolysis. We hope to extend these results to the preparation of long-chain difluoromethylene ketone phospholipid analogues in order to study the inhibition in heterogenous, substrate/inhibitor aggregates.

Acknowledgment. The generous support of this research by the National Institutes of Health (HL-36235) and Merck and helpful discussions with Professor Paul Hopkins are gratefully acknowledged.

Supplementary Material Available: Experimental procedures and physical data for the preparation of all new compounds and enzyme kinetic data (7 pages). Ordering information is given on any current masthead page.

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(9) Enzyme assays were carried out under Argon at 37 °C in 1 mL of 10 mM $CaCl_2$ containing substrate (1-10 mM) and inhibitors (0.05-0.5 mM). Reactions were started by the addition of phospholipase A_2 (*Naja naja* venom, typically 25 units/mL). Reactions were maintained at pH 8.0 in a pH stat by the addition of 0.01 N NaOH.

Ring Opening of Cyclopropylidenes to Allenes: Reactions with Bifurcating Transition Regions, Free Internal Motions, Steric Hindrances, and Long-Range Dipolar Interactions

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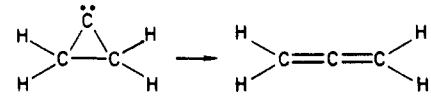
The mechanism of the ring opening of cyclopropylidene to allene,



the prototype bond fission of a cyclic carbene due to ring strain, has implications for many organic reactions. Substituted species only have been examined so far experimentally and these reactions are stereospecific.[1-6] Theoretical work has been limited to the

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unsubstituted species and earlier studies, semiempirical and ab initio, were handicapped by severe geometrical restrictions.[7-10] In two recent state of the art ab initio investigations the transition-state energy barrier has been determined by gradient techniques.[11-13] A full understanding of the reaction path requires a complete knowledge of the energy surface as a function of *three* relevant reaction coordinates, namely, the CCC ring opening angle ϕ and two angles δ_1,δ_2 describing the rotations of the two CH_2 groups.

We have mapped out this energy surface $E(\phi,\delta_1,\delta_2)$ in its entirety where, for each triple of (ϕ,δ_1,δ_2) values, the molecular geometry was optimized with respect to the remaining 12 internal coordinates. The rotation angles δ_1,δ_2 were defined as the dihedral angles between the CCC plane and the CH_2 planes. In order to account for the drastically changing binding patterns during the reaction a full optimized reaction space (FORS) wavefunction[14] was used, i.e., a superposition of the 20 configurations representing *all possible* couplings of the four reactive electrons in the four reactive molecular orbitals. (Conceptually, the bonding rearrangements are associated with changing valence interactions between two AO's on the central carbons and one AO on each of the outer carbons. The quantitative ab initio description of this picture is furnished by the orbital space spanned by the four reactive MCSCF MO's.) The remaining 18 electrons were paired off in three inner shells and seven σ-bond orbitals. All orbitals were completely MCSCF optimized. Preliminary calculations were made with an STO-3G basis. Refined calculations were made with an extended C(14s7p1d/3s2p1d), H(6s/2s) basis. The critical energies (reaction energy $\approx$ 65 kcal/mol, barrier $\approx$ 14 kcal/mol, allene isomerization $\approx$ 42 kcal/mol) were found to be in agreement with the best previous results.[11,13]

An examination of the energy surface reveals the following features of the cyclopropylidene ring opening: (i) Singlet cyclopropylidene is stable at ϕ = 59.5° and about 9 kcal/mol lower than the triplet (stable at 65.5°). (ii) The CH_2 planes are perpendicular to the CCC plane (C_{2v}) and approximately remain so during the initial stages of the ring opening. (iii) Shortly before ϕ reaches 70° the CH_2 groups begin a disrotatory motion, preserving C_s symmetry. (iv) Around $\phi \approx 81°$, with a disrotatory inclination of about 35°, a conrotatory component admixes to the reaction path advance and C_s symmetry is lost. This component can be clockwise or counterclockwise and the reaction channel *bifurcates* correspondingly. The two exit channels are each other's mirror images (assuming the hydrogen atoms are distinguished by numbering) and lead to the two stereoisomeric products. (v) No inherent preference exists between the two branches. The reaction is therefore nonstereospecific and the deuterated molecule is predicted to exhibit only the small stereospecificity arising from the asymmetry of the *kinetic* energy tensor. (vi) The bifurcation occurs before the transition states [which are located around (ϕ,δ_1,δ_2) = (81°, 50°, 120°) and (81°, 60°, 130°)] and extremely close to it. The reaction has a *bifurcating transition region which lies within a few degrees and less than a kilocalories per* Mole *of being a mathematically exact bifurcating transition state.* The latter is generally believed to be a very rare occurrence. The described situation is the first instance of such a near coincidence being found on a reaction surface. (vii) The downhill path from the transition state to the product with ϕ opening to 180°, while conrotatory in a general way, leads across slopes of isoenergetic valleys such as shown along the lines $\delta_1 + \delta_2 = \pm 90°$ on the contour map of Figure 1 which represents $E(\delta_1,\delta_2)$ for ϕ = 100°. This means that, from about ϕ = 95° on, *the two CH_2 groups can rotate freely in a synchronized cogwheellike fashion.* Any stage of this free internal motion leads to staggered allene, because it degenerates into the rigid-body rotation of allene around its CCC axis when ϕ becomes 180°. Since this disrotatory cogwheel motion can mix unpredictably with the conrotatory downhill motion, *there does not exist a unique single reaction path from the transition state to the product.* (viii) The allene stereoisomerization requires a concerted twisting and bending, the transition state occurring at ϕ = 133°.

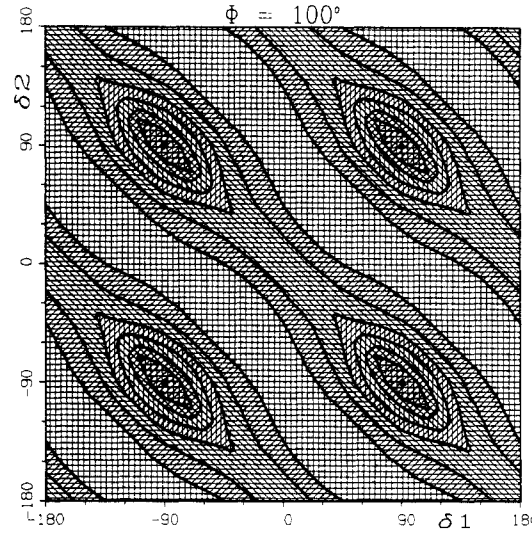

Figure 1. Increment between contours = 10 mhartree $\approx$ 6 kcal/mol. Lightest shading = lowest energy.

In view of the aforementioned results it seems likely that, also in the ring opening of σ substituted cyclopropylidenes, covalent interactions change nonstereospecifically and that the observed stereospecificities are caused by nonbonded interactions. Accordingly, we have determined energy surfaces for several such reactions by incorporating into the discussed cyclopropylidene calculations the two long-range interactions which have proven effective in the field of molecular mechanics; namely, van der Waals steric effects described by 6-9 Lennard-Jones potentials and dipolar electrostatic interactions. Standard literature values were used for the pertinent parameters.

The investigation of dimethylcyclopropylidene showed (i) that the cis isomer is nonstereospecific and has a lower barrier than the trans isomer and (ii) that the trans isomer is stereospecific in that, after the bifurcation, the preferred branch corresponds to that conrotatory motion which places a hydrogen rather than a methyl close to the CCC ring. Both results arise from the predominance of the steric effects and are in agreement with experiment.

A comparative investigation of 3-methyl- and 2-bromo-3-methylcyclopropylidene showed that both are stereospecific in the *same* direction and that the latter is more strongly so. *This result is counter to what would be found if the steric effects were dominant. It is caused by the dipolar attractions between the CBr bond and the trans CH bond.* A similar effect is to be expected when bromine replaces a para hydrogen on a phenyl substituent, as in the Jones–Krause experiment.[5] This prediction is in agreement with the experimental findings and it is thus not necessary to postulate that the covalent interactions are stereospecific.

A full account of these investigations will be given in a forthcoming publication.[15]

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Inhibition of Benzoylformate Decarboxylase by [*p*-(Bromomethyl)benzoyl]formate. Enzyme-Catalyzed Modification of Thiamine Pyrophosphate by Halide Elimination and Tautomerization

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Benzoylformate decarboxylase (benzoylformate carboxy-lyase, BFD; EC 4.1.1.7) from *Pseudomonas putida* catalyzes the decarboxylation of this *a*-keto acid to yield benzaldehyde and CO_2 (Scheme I).[1] The reaction requires thiamine pyrophosphate (TPP) as cofactor suggesting that the mechanism involves the formation of a covalent substrate–cofactor intermediate capable of stabilizing the carbanion generted by decarboxylation. Previous efforts by a number of groups have established this process for the thiaminepyrophosphate-dependent pyruvate decarboxylases.[3] During a study of a number of substituted benzoylformate analogues, we found that [*p*-(bromomethyl)benzoyl]formate (**1**)[4] was a remarkably potent inhibitor of BFD (K_i = 0.3 μM; for benzoylformate, K_m = 0.4 mM). In this report, we establish that the inhibition is due to an unusual enzyme-catalyzed processing of **1** resulting in decarboxylation, bromide ion elimination, and rearomatization by tautomerization.

Reaction of BFD (1 unit) and **1** (50 μM) afforded quantitative release of bromide ion (Figure 1).[5] In the presence of 1 mM TPP, bromide ion release under these conditions was complete in ~80 min.[6] Addition of 5 mM benzoylformate resulted in a transient inhibition of bromide ion release, clearly indicative of substrate protection of the enzyme. In the absence of excess TPP, however, a slow generation of bromide ion was observed which required >36 h to reach completion. Nonenzymatic, TPP-dependent generation of bromide ion was found to be negligible. The recovery of enzyme activity in the presence of 1 mM TPP as determined by the rate of benzaldehyde formation (Figure 1, inset) exhibited a time course similar to that for bromide ion release. In the absence of excess TPP no recovery of enzyme activity was found during the same time period.

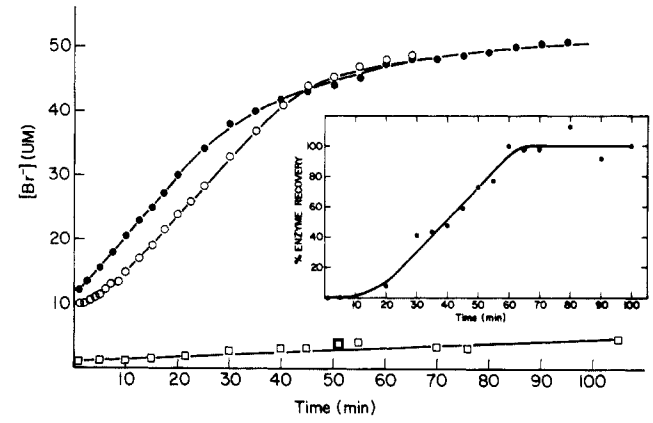
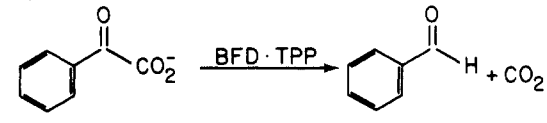
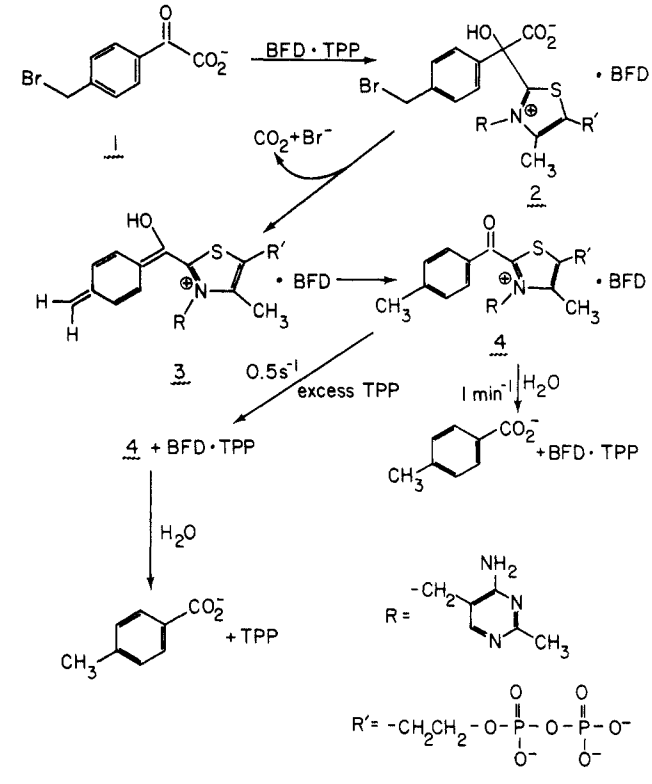


Figure 1. Analysis of BFD-catalyzed bromide ion release from (*p*-(bromomethyl)benzoyl]formate (**1**). Experimental procedure is described in ref 5. In addition to **1** (50 μM) and BFD (1 unit), reaction mixtures contained no added TPP (□), 1 mM TPP (●), and 1 mM TPP plus 5 mM benzoylformate (○). Background at time zero for TPP-containing reactions is due to residual chloride ion. (Inset) Time course of recovery of BFD activity in the presence of **1** (50 μM) and TPP (1 mM). Recovery was measured as the percent of maximal benzaldehyde formation from benzoylformate.[2]

Scheme I



Scheme II



[†] University of Maryland.
[‡] University of California.
[§] American Foundation for Pharmaceutical Education H.A.B. Dunning Memorial Fellow.
[⊥] American Cancer Society Faculty Research Awardee (1983–1988).

(1) Benzoylformate decarboxylase was isolated according to: Hegeman, G. D. *Methods Enzymol.* **1970**, *17A*, 674. The enzyme had a final specific activity of 34 units/mg and is stored in the presence of 1 mg/mL bovine serum albumin.[2] A coupled assay[2] with equine liver alcohol dehydrogenase was used. Pyruvate is neither a substrate nor an inhibitor of BFD.[2]

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(4) (*p*-Methylbenzoyl)formic acid (Barnish, I. T.; Cross, P. E.; Danilewicz, J. C.; Dickinson, R. P.; Stopher, D. A. *J. Med. Chem.* **1981**, *24*, 399) was treated with 1.1 equiv of *N*-bromosuccinimide in refluxing CCl_4 for 90 min in the presence of a trace of benzoyl peroxide and Pyrex-filtered UV light (275 W). The organic layer was washed with H_2O, driev over anhydrous Na_2SO_4, and concentrated in vacuo. Recrystallization from hot toluene/hexane afforded pale yellow crystals (mp 68–70 °C; yield 54%). UV (H_2O λ_{max} 265 nm; ϵ 22 500 M^{-1} cm^{-1}. 1H NMR (acetone-d_6) δ 7.5 (2 H, d), 7.15 (2 H, d), 4.2 (2 H, s). Elemental analysis for $C_9H_7O_3Br \cdot H_2O$. Theoretical: C, 41.41; H, 3.47. Found: C, 41.14; H, 3.20.

(5) Bromide ion release was measured on an Orion Model 811 pH meter equipped with an Orion Model 94-35 bromide electrode and a Model 90-01 single-junction reference electrode. Experiments were performed on 2 mL of assay solution (see Figure 1) containing 0.1 M potassium phosphate buffer (pH 7.0) with 0.1 M sodium nitrate to stabilize electrode performance. The enzyme was dialyzed against 0.05 M potassium phosphate (pH 6.0) to remove excess thiamine pyrophosphate and chloride, the latter of which affects the electrode. The electrode was not disturbed during the course of each experiment.

(6) The rate of consumption of **1** is ~1% that of benzoylformate (turnover number ~70 s^{-1})[2] under the same reaction conditions.

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