Table II. Isomerization of 6 and 7

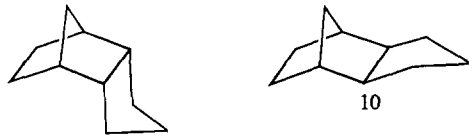
Temp, °C	6, %	7, %	8, %	Other products, %
222	47	53		
274	34	41	11	14
306	20	22	17	41

Table III. Isomerization of 8

Temp, °C	6, %	7, %	8, %	Other products, %
278	1	2	97	
306	2	4	96	
324	5	8	71	16

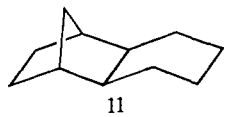
reversible. Again fragmentation predominates at higher temperatures.

Isomerization of di-*endo*-trimethylenenorbornane (**9**) into the di-*exo* isomer **10** was detected at 100° and was entirely selective at high conversions (≥90%) between 200 and 350°.[7] Isomerization of di-*exo*-tetramethylenenorbornane (**11**) to its *endo* isomer occurred at lower temperatures, but at 250° dehydrogenation became significant, and virtually complete conversion into benznorbornene (**12**) was recorded at 360°. Catalysts which had been deliberately contaminated with carbonaceous residues largely retained their activity for the dehydrogenation of **11** but showed greatly reduced isomerization activity, *e.g.*, of **9**. Normal isomerization activity was restored by heating the poisoned catalyst in oxygen at 430°, followed by hydrogen at 300°. Dehydrogenation of **11** must involve alkene- and alkenyl-type intermediates so the clear dichotomy of activity described above indicates that such species cannot account for the isomerization reactions.

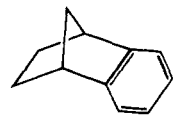


9

10



11



12

A further example of a reaction which can readily be explained by a cyclopropane intermediate, but not by any of the existing mechanisms, is the interconversion of bicyclo[3.2.2]nonane (**13**) and bicyclo[3.3.1]nonane (**14**) which we find to occur over palladium at 200° or above.

In summary, some of the above rearrangements can be explained by a combination of the previously proposed mechanisms A and B. However, all the rearrangements can be explained in terms of cyclopropyl intermediates analogous to **4**. Previous mechanisms cannot explain the interconversions of **6**, **7**, and **8**; of **13** and **14**; or the simultaneous formation of **2** and **3** from **1** in equal amounts. Our proposed mechanism for this latter result parallels the formation of equal amounts of *cis*- and *trans*-9-methyldecalins when tricyclo[4.4.1.0]undecane is hydrogenolyzed over a platinum catalyst.[8]

(7) This isomerization constitutes a very convenient method for the preparation of 10. We have also found that **10** when treated with aluminum chloride gives adamantane in yields superior to those obtained from the *endo* isomer **9**: H. Hamill, unpublished observations; *cf.* R. C. Fort, Jr., and P. v. R. Schleyer, *Chem. Rev.*, **64**, 277 (1964).

Acknowledgments. H. A. Q. thanks the Northern Ireland Ministry of Education for a Research Studentship. We thank Dr. W. Parker, The New University of Ulster, Northern Ireland, for gifts of **13** and **14**.

(8) Z. Majerski and P. v. R. Schleyer, *Tetrahedron Lett.*, 6195 (1968).

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Kinetic Analysis of the Action of Pancreatic Lipase on Lipid Monolayers[1]

Sir:

Pancreatic lipase catalyzes the hydrolysis of fatty acid esters of glycerol and of other simple alcohols.[2] While the enzyme acts readily on emulsions of lipids (the state of dispersion of lipase substrates under physiological conditions), it is by no means clear whether phase heterogeneity is a necessary requirement for enzymatic catalysis.[3] Even if the action of the enzyme were limited to interphase layers, the use of emulsions for mechanistic studies would present several theoretical and experimental difficulties: emulsions contain extraneous material of often ill-defined composition (*e.g.*, gum arabic), and cannot be prepared in reproducible fashion with homogeneous particle size. Furthermore, different substrates yield emulsions of different particle size and composition and the presence of a large lipid phase renders the substrate concentration available to the enzyme at any given time difficult to evaluate. Finally, the possible mechanistic role of the emulsifier, its interaction with the enzyme and the substrate, and its possible effect on transport across the phase boundaries are rather difficult to assess.

In contrast to emulsions, insoluble surface monolayers of substrate molecules would provide a system more amenable to quantitative experimentation. Stable homogeneous monolayers can be prepared with exceedingly small amounts of substrate esters, without the addition of any extraneous material. The surface concentration of the substrate and its change during the reaction can be evaluated accurately and with high sensitivity by monitoring changes in surface pressure. Finally, use of insoluble substrate monolayers automatically defines the site of the reaction at the interface. For nonenzymatic reactions at monolayers, detailed kinetic analyses have been carried out,[4] and semiquantitative studies indicate the feasibility of such an approach for phospholipase catalyzed reactions.[5] We

(1) This research was supported by grants from the U.S. Public Health Service, Medical Research Grants No. GM 13863 and GM 13885.

(2) P. Desnuelle, *Advan. Enzymol. Relat. Subj. Biochem.*, **23**, 129 (1961).

(3) (a) R. G. H. Morgan, J. Barrowman, H. Filipek-Wender, and B. Borgstrom, *Biochem. Biophys. Acta*, **167**, 355 (1968); (b) B. Entressangles and P. Desnuelle, *ibid.*, **159**, 285 (1968).

(4) G. L. Gaines, Jr., "Insoluble Monolayers at Liquid-Gas Interfaces," Interscience Publishers, New York, N. Y., 1966.

(5) (a) D. O. Shah and J. H. Schulman, *J. Colloid Interface Sci.*, **25**, 107 (1967); (b) R. H. Quarles and R. M. C. Dawson, *Biochem. J.*, **113**, 697 (1969); (c) G. Colacicco and M. M. Rapport, *J. Lipid Res.*, **7**, 258 (1966); R. M. C. Dawson, *Methods Enzymol.*, **14**, 633 (1969).

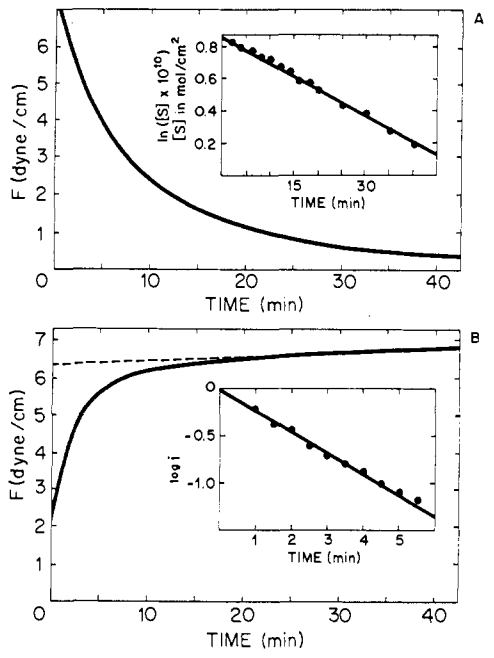
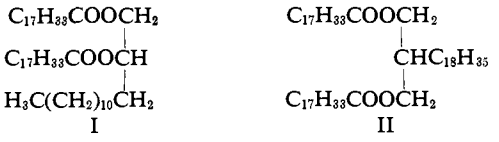


Figure 1. Time course of pancreatic lipase reaction on surface monolayers; solid curves, recorder tracings: (A) 1,2-dioctanoin, [S] = 2.4×10^{-10} mol/cm², [E] = 5.8×10^{-2} units/ml, 0.1 *M* phosphate buffer, pH 7.6; (B) compound II, [S] = 1.3×10^{-10} mol/cm², [E] = 6.0×10^{-2} unit/ml, 0.1 *M* phosphate buffer, pH 8.0.

have therefore undertaken the determination of the kinetics of the reaction of pancreatic lipase on substrate monolayers.

Two types of substrates were chosen to explore the experimental technique: octanoyl glycerides, in which the fatty acid product is instantaneously soluble, and oleoyl glyceride analogs I and II.



Surface tension measurements were made with a du Noüy ring suspended from the beam of an automatic recording Cahn electrobalance, Model RG. Monolayers were spread from petroleum ether (bp 30–60°) solutions with a Gilmont microburet onto enzyme solutions contained in an all-Teflon trough (S = 124 cm²). All kinetic measurements were performed at constant area. Force–area measurements indicated that the monolayers were in the expanded phase throughout our working range (0.5–10 dyn/cm). Preliminary studies showed that the octanoyl di- and triglycerides as well as I, II, and oleic acid all formed insoluble monolayers, while octanoic acid and the monooctanoins were completely soluble. Insoluble reaction products were collected quantitatively for analysis by thin layer chromatography (tlc) after flooding the surface with 2,2,4-trimethylpentane, collecting the organic layer by aspiration, and removing the solvent *in vacuo*.

When 1,2-dioctanoin was spread onto an enzyme solution, the surface pressure showed a substantial time-dependent decrease (Figure 1A). Since both of the hydrolysis products are readily soluble, the surface concentration [S] of the unreacted diester can be derived by the use of standard force–area diagrams. The ln [S] *vs.* time plots gave excellent straight lines (see insert, Figure 1).

For the oleoyl glyceride analogs, where all enzymatic reaction products remained on the surface, surface pressure increased with time (Figure 1B). Assuming additivity of the partial surface pressures, the percentage of hydrolysis was calculated according to the equation $i = (1/F_t - 1/F_\infty)/(1/F_0 - 1/F_\infty)$, where F_t, F_0, and F_∞ are the surface pressures of the reaction mixture at times t, 0, and ∞, respectively, and i is the fraction of unreacted material at time t. In the case of hydrolysis of compound II, a correction was made for the slower hydrolysis of the monoester. Again, the log i *vs.* t plots yielded excellent straight lines. Similar techniques were used to analyze the results of trioctanoin hydrolysis, where a decrease in surface pressure resulted because of the solubility of the fatty acid product. A small correction was necessary for the much slower reaction of 1,2-dioctanoin. In all cases reaction products were verified by tlc.

Experimental rate constants for various substrates and different enzyme concentrations are shown in Table I.

Table I. Hydrolysis of Lipid Monolayers by Pancreatic Lipase[a]

Substrate	[Enzyme] × 10^2 units/ml	[Substrate] × 10^{10} mol/cm²	k_{expt} × 10^4 sec^{-1}	$k_{expt}/[E]_{corr}$[e] × 10^3 ml/unit sec
1,2-Dioctanoin[b]	1.9	2.0	0.49	2.6
	5.8	2.4	1.4	2.3
	9.7	2.4	2.3	2.4
Trioctanoin	0.95	1.6	6.9	38
	1.9	1.5	15	35
	3.8	1.5	28	37
Trioctanoin[c]	0.8	1.5	9.6	59
I[c,d]	2.0	1.4	17	96
	6.0	1.4	57	83
II[c,d]	2.0	1.3	30	77
	6.0	1.3	89	74
	6.0	1.4	87	73
	10.0	1.3	154	76

[a] Worthington Batch No. PLI 7GA, 78 Worthington units/mg, in 0.1 *M* phosphate, pH 7.6; 20° ± 0.5. [b] Prepared by partial enzymatic hydrolysis of trioctanoin in emulsion followed by preparative thin layer chromatography. Structure was verified by mass spectrometry. [c] At pH 8.0. [d] Compounds I and II were prepared by acylation of the corresponding diols with oleoyl chloride. The 1,2-diol was prepared by $LiAlH_4$ reduction of α-hydroxymyristic acid. The 1,3-diol was prepared by alkylation of diethyl malonate with oleyl bromide followed by $LiAlH_4$ reduction. The monoester corresponding to II was prepared by limited $LiAlH_4$ reduction of II. All structures were verified by elemental analysis and mass spectrometry. [e] These values were corrected by dividing the rate constants by the number of equivalent primary ester groups in the molecule.

These observations demonstrate for the first time that pancreatic lipase acts upon insoluble monolayers of lipid substrates, even in the absence of the usual auxiliary factors such as calcium ion or emulsifiers. The reaction is first order with respect to enzyme and substrate and independent of the compression in the surface pressure range investigated. Thus, the rate is proportional not to the "concentration of interface,"[6] but to the total number of substrate molecules at the in-

(6) G. Benzonana and P. Desnuelle, *Biochem. Biophys. Acta*, **105**, 121 (1965).

terface. Since the rate constant is independent of the compression and rather insensitive to the nature of the acyl group, the enzymatic reaction most likely occurs at the interface without penetration of the enzyme.

Orientation of diglycerides dominated by the polar hydroxyl group should make the primary ester group more inaccessible at the interface than in the case of triglycerides, and this is reflected by a large rate difference observed between dioctanoin and trioctanoin.

We believe that this simple experimental technique, which is sensitive, convenient, reproducible, and amenable to quantitative analysis, opens the way to rigorous quantitative studies on the behavior and mechanism of pancreatic lipase. The method can undoubtedly be adapted to the study of the action of enzymes at interfaces, such as biological membranes.

(7) Predoctoral trainee of the National Aeronautics and Space Administration.

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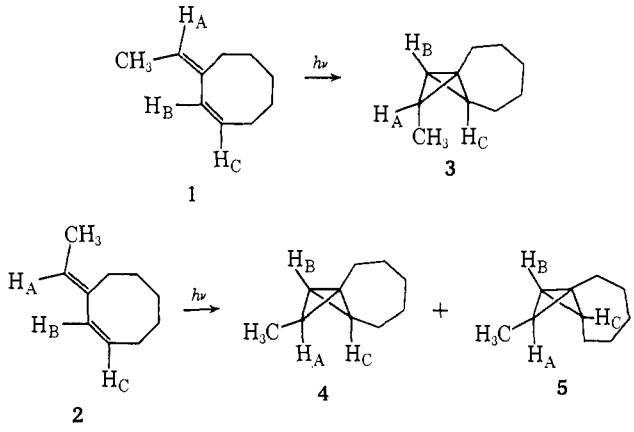
Photochemistry of Ethylidenecyclooctenes. Mechanism of Bicyclobutane Formation.[1]

Sir:

We wish to report the stereochemistry of bicyclobutanes formed from *cis*- and *trans*-3-ethylidenecyclooctene (**1** and **2**) and the mechanistic implications to the photocyclization of butadienes to bicyclobutanes.

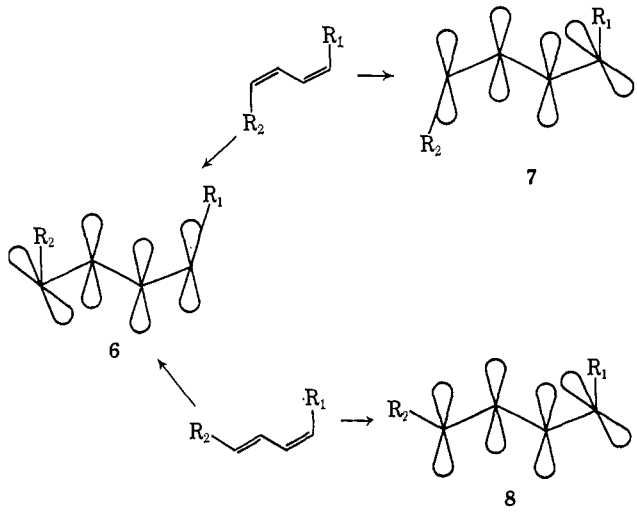


Ethylidenetriphenylphosphorane upon reaction with 2-cycloocten-1-one afforded a mixture of two isomeric dienes (**1**:**2**, 40:60), readily separable by vpc:[2] nmr (δ, CCl_4) **1**, 6.27 (H_B, d, J = 12 Hz), 5.49 (H_C, d of t, J = 12, 8 Hz), 5.20 (H_A, q, J = 7 Hz); **2**, 6.03 (H_B, d, J = 12 Hz), 5.42 (H_A, q, J = 7 Hz), 5.24 (H_C, d of t, J = 12, 8 Hz). Isomer **2** was assigned the *trans* configuration since the H_A of **2** is at lower field due to the greater deshielding by the endocyclic double bond and the H_B and H_C of **1** are at lower field due to deshielding by the methyl group.[3]

(1) This work was supported in part by PHS Grant No. AM 00709, National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.
(2) 5% Carbowax 20M, 5% KOH on Chromosorb G.
(3) C. G. Cárdenas, *Tetrahedron Lett.*, 4013 (1969).

Typical of *s-trans* dienes,[4a,b] irradiation[5] of **1** and **2** gave bicyclobutanes **3**–**5**. Concentration studies showed that **3** was derived from **1**, and **4** and **5** (3:1) from **2**: nmr (δ, CCl_4) **3**, 1.10 (H_C, br d, J = 6 Hz), 0.77 (3 H, d, J = 6 Hz); **4**, 0.93 (5 H, m); **5**, 0.92 (4 H, m). In **3**, an *endo*-methyl group is indicated by its chemical shift (δ 0.77), and its almost symmetrical doublet pattern requires H_A to be at much lower field ($\Delta\nu \gg J$)[6] and thus *exo*. The presence of the high-field broad doublet indicates that H_C has the *endo* configuration.[7] Hence, **3** is *endo*-9-methyl-*cis*-tricyclo[6.1.0.0^{1,7}]nonane. In **4** and **5**, the multiplet patterns agree well with the calculated[6] AB_3 spectra for $\Delta\nu/J$ = 1. The presence of 5-H above δ 1.1 permits assignment of **4** as the *exo*-9-methyl-*cis* isomer and the 4-H above δ 1.1 in **5** confirms the *exo*-9-methyl-*trans* structure.

The orbital symmetry concept of the cyclization proceeding in a concerted manner beginning with two planar double bonds and following *cis–cis* or *trans–trans* (2 + 2) cycloaddition[8] requires any pair of bicyclobutanes produced from a *single* diene such as **1** or **2** to be epimeric at *two* centers. This prediction is incompatible with our results which show that a single diene, **2**, yields two bicyclobutanes epimeric at only *one* center. This result is, however, easily accommodated by considering the cyclization to be initiated from the vibrationally relaxed (nonplanar or orthogonal) first excited singlet state.[9] If two epimeric dienes are excited to their first excited electronic level, vibrational relaxation can lead to identical (**6**) or to epimeric species (**7** and **8**).



In the present study, it has been found that isomerization about the endocyclic double bond in ethylidenecyclooctenes occurs faster by a factor of 3–5 than exocyclic bond isomerization though, indeed, the product of the dominant path is highly strained. Hence, vi-

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(6) K. B. Wiberg and B. J. Nist, "The Interpretation of NMR Spectra," W. A. Benjamin, Inc., New York, N. Y., 1962.
(7) (a) K. B. Wiberg, G. M. Lampman, R. P. Ciula, D. S. Connor, P. Schertler, and J. Lavanish, *Tetrahedron*, **21**, 2749 (1965); (b) K. B. Wiberg, *Advan. Alicyclic Chem.*, **2**, 185 (1968).
(8) R. B. Woodward and R. Hoffmann, *Angew. Chem. Int. Ed. Engl.*, **8**, 781 (1969).
(9) (a) L. Burnelle, *J. Chem. Phys.*, **43**, S29 (1965); (b) R. Hoffmann, *Tetrahedron*, **22**, 521 (1966).