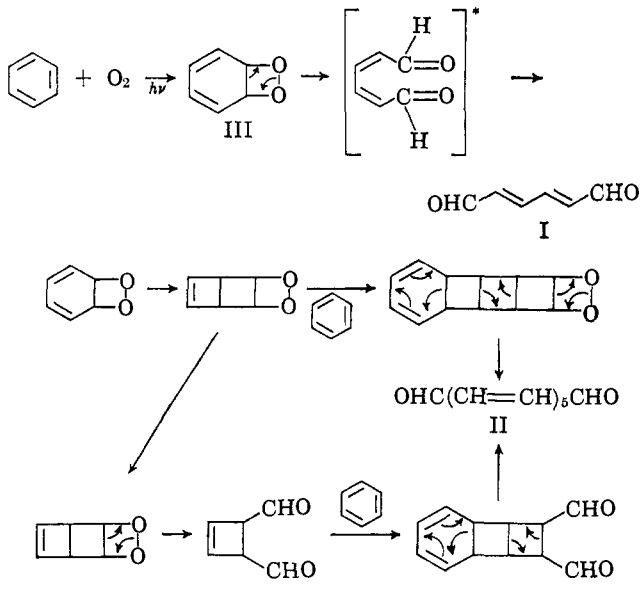
It is premature to speculate on the detailed mechanism of the reaction or the excited states of the species involved (including possibly a benzene isomer or excimer[8] and/or singlet oxygen). However, the reaction sequences given in Chart I[9] have an analogy[10] in known benzene photochemistry.[11] This photoinitiated

Chart I



attack of oxygen on benzene leading to ring opening and the formation of strongly absorbing, long-chain, conjugated dialdehydes has interesting implications in molecular biology. Thus, for example, enzymatic ring opening of pyrocatechol by oxygen gives *cis,cis*-muconic acid[12] while in another enzymatic oxygen fixation process with tryptophan, the oxygen opens the pyrrole ring to form N-formylkynurenine.[13] It is interesting to note that such attacks by "enzymatically activated oxygen"[13a] lead to ring opening and products similar to those we have observed in a photooxidation process.

Finally, the "yellowing" of polystyrene has long been a subject of investigation and speculation.[14] Mechanisms proposed to date concern reactions only of the skeletal chain. Our results with liquid benzene photooxidation suggest that ring opening by oxygen also may be involved.

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(8) Suggested by A. Chandross, Bell Telephone Laboratories, Inc.

(9) The valence isomerization of III should provide ample energy to lead to the all-*trans* form I.

(10) We are grateful to referees I and II for this approach.

(11) R. Srinivasan and K. A. Hill, *J. Am. Chem. Soc.*, **87**, 4653 (1965).

(12) O. Hayaishi, M. Katagiri, and S. Rothberg, *ibid.*, 77, 5450 (1955). We are investigating the photooxidation of pyrocatechol and preliminary results suggest that muconic acid is formed.

(13) (a) O. Hayaishi, "Oxygen in the Animal Organism," The Macmillan Co., New York, N. Y., 1964, p 151; (b) P. Feigelson, Y. Ishimura, and O. Hayaishi, *Biochem. Biophys. Acta*, **96**, 283 (1965); (c) B. Pullman and A. Pullman, "Quantum Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1963, p 327.

(14) N. Grassie and N. A. Weir, *J. Appl. Polymer Sci.*, **9**, 963, 975, 987, 999 (1965).

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Enzymatic Stereospecificity in the Hydration of Epoxy Fatty Acids. Stereospecific Incorporation of the Oxygen of Water[1]

Sir:

We have found that a soluble (100,000*g*) extract, prepared from a pseudomonad[2] (NRRL-2944), catalyzes the stereospecific hydration of the Δ^9-olefinic bond of oleic acid, yielding 10-D-hydroxystearic acid (or 10-*R*).[3-6] We now wish to report that the same enzyme preparation catalyzes the hydration of *cis*- and *trans*-9,10-epoxystearic acids, yielding *threo*- and *erythro*-9,10-dihydroxystearic acids,[7] respectively. These reactions are characterized by both substrate and product stereospecificity. The enzymatic hydration of the racemic epoxides proceeds only to the extent of ~50% utilization of the added substrates, yielding optically active dihydroxystearic acids.[8] In the case of the *trans*-epoxystearate the recovered, unreactive substrate after prolonged and repeated incubation with the enzyme preparation was also optically active.[9] This finding constitutes a clear example of the use of an enzyme to effect the resolution of a racemic epoxide, a result which would be difficult, if not impossible, to obtain by other methods.

These observations, indicative of notable stereospecificity with respect to substrate and product, raise an additional question: is the oxygen of water incorporated specifically at one carbon atom during the course of the enzymatic hydration of the epoxide? Accordingly, we have incubated the sodium salts of *cis*- and *trans*-9,10-epoxystearic acids with the enzyme preparation in water enriched with respect to ^{18}O. Mass spectrometry provides a powerful tool for the quantitation and localization of the isotope in the product. Methyl 9,10-dihydroxystearate (I), upon electron impact, does not give a significant molecular ion suitable for determination of the isotopic composition but it does give a significant peak at *m/e* 187 (III) corresponding to the fragment containing carbon atoms

(1) Supported by a grant (HE 09501) from the National Heart Institute, U. S. Public Health Service.

(2) L. L. Wallen, R. G. Benedict, and R. W. Jackson, *Arch. Biochem. Biophys.*, **99**, 249 (1962).

(3) G. J. Schroepfer, Jr., and K. Bloch, *J. Biol. Chem.*, **240**, 54 (1965).

(4) G. J. Schroepfer, Jr., *J. Am. Chem. Soc.*, **87**, 1411 (1965).

(5) W. G. Niehaus, Jr., and G. J. Schroepfer, Jr., *Biochem. Biophys. Res. Commun.*, **21**, 271 (1965).

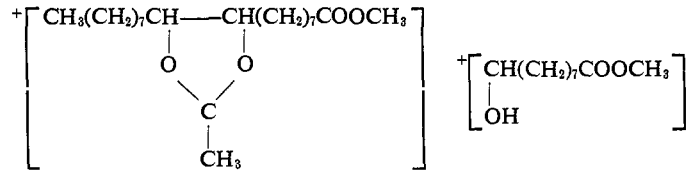
(6) G. J. Schroepfer, Jr., *J. Biol. Chem.*, **241**, 5441 (1966).

(7) Characterized as the methyl esters by mass spectrometric, infrared, and gas–liquid partition chromatographic (3.8% SE-30 on Diatoport S) analyses. The *threo* and *erythro* configurations of the respective diols were established by thin layer chromatographic analyses on silica gel G plates impregnated with boric acid (5%) using as solvent pentane–ether–acetic acid, 50:50:1. This system, a minor modification of that described by L. J. Morris (*Chem. Ind.* (London), 1238 (1962)), allows complete separation of the *threo* and *erythro* isomers.

(8) Methyl *threo*-9,10-dihydroxyoctadecanoate: $[\alpha]_{5890}$ + 27.0 ± 3.8° (*c* 0.74, methanol); methyl *erythro*-9,10-dihydroxyoctadecanoate: $[\alpha]_{2500}$ +1.38 ± 0.11° (*c* 1.71, methanol)

(9) *trans*-9,10-Epoxyoctadecanoic acid: $[\alpha]_{5890}$ −14.6 ± 0.4° (*c* 1.57, methanol).

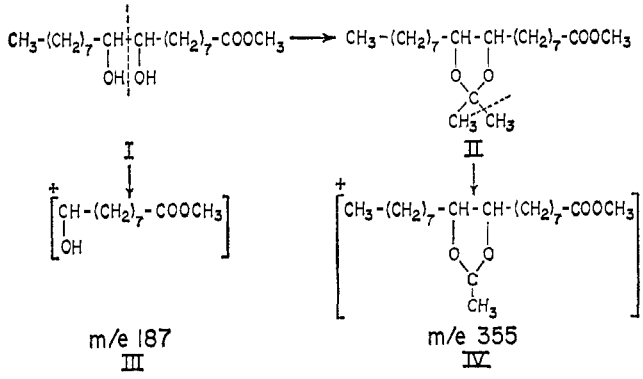
Table I. Mass Spectral Analysis of Isotopic Composition of ^{18}O-Labeled *threo*- and *erythro*-Dihydroxystearic Acids



No. of atoms of ^{18}O per molecule	% of molecules	% of molecules
threo-9,10-Dihydroxystearate[a]		
0	18.8	98.5
1	80.5	0.9
2	0.8	<0.3
3	0.0	<0.3
erythro-9,10-Dihydroxystearate[b]		
0	19.2	97.5
1	79.6	1.1
2	0.2	0.8
3	0.4	0.6

[a] Obtained from incubation of *dl-cis*-9,10-epoxystearate with enzyme in the presence of $H_2{}^{18}O$ (83.3% ^{18}O). [b] Obtained from incubation of *dl-trans*-9,10-epoxystearate with enzyme in the presence of $H_2{}^{18}O$ (85.0% ^{18}O).

1 through 9.[10] This peak can therefore be used to quantitate the labeled oxygen on carbon atom 9. The isopropylidene derivative II, while it does not yield a significant molecular ion upon electron impact, does yield a fragment (*m/e* 355; IV)[11] containing the oxygen at both carbon atoms 9 and 10.



The sodium salt of *dl-cis*-9,10-epoxystearic acid-1-^{14}C (20 mg; 9×10^3 cpm/mg) in water (1 ml) was added to the enzyme solution (50 mg of protein; 2 ml; 0.02 *M* Tris chloride buffer, pH 7.8; 0.002 *M* mercaptoethanol), frozen immediately, and subjected to lyophilization. The residue was incubated with $H_2{}^{18}O$ (2.0 ml, 83.3% ^{18}O)[12] for 4 hr at 30°. After recovery of the labeled water by lyophilization, the fatty acids were isolated and subjected to chromatography on an activated silicic acid column, yielding 10 mg of the epoxystearate and 4.4 mg of *threo*-9,10-dihydroxystearic acid. The methyl ester of the diol was prepared, dissolved in methanol (0.8 ml), and heated at 65° with 5 *N* HCl (0.2 ml) for 50 hr.[13] The reaction mixture was allowed to stand at room temperature for an additional 50 hr. The isolated diol methyl ester and the isopropylidene derivative (prepared according to McCloskey and McClelland[11] and purified by column chromatography) were analyzed by mass spectrometry (Table I). These results indicate that, in the enzymatic hydration of the *cis*-epoxystearate, the oxygen of water is specifically incorporated at carbon atom 10.

In a similar fashion the sodium salt of *dl-trans*-9,10-epoxystearic acid was incubated with the enzyme preparation in the presence of $H_2{}^{18}O$ (85% ^{18}O). After treatment of the methyl ester with methanolic HCl as outlined above, the methyl ester and the isopropylidene derivative were analyzed by mass spectrometry. The results (Table I) indicate that the oxygen of the water is specifically incorporated at carbon atom 10 in the case of the enzyme-catalyzed hydration of the *trans*-epoxide.

The fact that this enzyme preparation which catalyzes the hydration of oleic acid (with the incorporation of the oxygen of water at carbon atom 10) also catalyzes the hydration of the corresponding *cis*- and *trans*-epoxystearates (with the specific incorporation of the oxygen of water at carbon atom 10) raises the possibility that we are dealing with a single enzyme. Favoring this view are the results of preliminary studies which indicate marked and parallel thermolability of the two activities (hydration of the olefin and hydration of the epoxide). Further studies directed at this question and at the determination of the absolute configurations of the isolated epoxy- and dihydroxystearates are in progress.

(10) R. Ryhage and E. Stenhagen, *Arkiv Kemi*, 15, 545 (1960).

(11) J. A. McCloskey and M. J. McClelland, *J. Am. Chem. Soc.*, 87, 5090 (1965).

(12) Purchased from Yeda Research and Development Co., Ltd., Rehovoth, Israel.

(13) This treatment was necessary because of the presence of a significant amount of labeled oxygen in both oxygens of the carboxyl group. Analysis of the mass spectra of the isopropylidene derivative of the isolated diol indicated the following percentage composition (number of atoms of ^{18}O per molecule, % of molecules): 0, 7.0; 1, 39.4; 2, 37.2; 3, 16.5. Analysis of the methyl ester of the *threo*-diol indicated the following percentage isotopic composition in fragment III (number of atoms of ^{18}O per molecule, % of molecules): 0, 42.0; 1, 38.1; 2, 19.8; 3, 0.0. The relatively large extent of labeling of both oxygens at the carboxyl group under the relatively mild incubation conditions suggests the possibility that the exchange might well be catalyzed by the enzyme preparation. Further studies along this line are planned.

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