Selective Inhibition of Prostaglandin Synthetase by a Bicyclo[2.2.1]heptene Derivative

Sir:

The enzymatic conversion of 8,11,14-eicosatrienoic acid (**1**) into prostaglandin E_1 (PGE_1) (**4**) and $PGF_{1\alpha}$ (**5**) by the prostaglandin synthetase complex has been demonstrated to involve initial elimination of the pro-*S* hydrogen at C-13 and oxygenation at C-11 to give the peroxy acid **2**, which is converted into endoperoxide **3**. Removal of a hydrogen at C-9 in **3** with opening of the peroxide gives PGE_1 (**4**), whereas reduction of the endoperoxide affords $PGF_{1\alpha}$ (**5**); **3** can also be transformed into the C_{17}-hydroxy acid **6** by elimination of malonaldehyde. It has also been shown that **2** can give rise to hydroxyeicosatrienoic acid **7** by reduction of the peroxy group.[1,2] Reports on the inhibition of the overall conversion by various unsaturated fatty acids have appeared.[3,4]

The present report describes experiments on the effect of racemic diastereomers in the bicyclo[2.2.1]-heptene series of structure **8**[5] on the transformation of eicosatrienoic acid by the enzymatic system from the vesicular gland of sheep. The selection of **8** for these studies was based on the clear structural relationship to the endoperoxide **3**. The enzyme preparation and the analysis of the product have been described in detail previously.[6]

The effect of adding increasing amounts of inhibitor **8** on the conversion of **1** into PGE_1, $PGF_{1\alpha}$, and the monohydroxy acids **6** and **7** is shown in Table I. The

Table I. Effect of Inhibitor **8** on the Transformation of 8,11,14-Eicosatrienoic Acid[a]

Inhibitor concn, m*M*	Products formed, nmol		
	PGE_1	$PGF_{1\alpha}$	Monohydroxy acids **6** and **7**
0	75	14	42
0.3	60	18	60
0.9	50	24	68
1.7	24	21	57

[a] The incubation mixture consisted of 0.05 *M* phosphate buffer at pH 7.8, reduced glutathione, and hydroquinone (5×10^{-4} *M* each), 0.32 m*M* substrate (ammonium salt of [2-^{14}C]8,11,14-eicosatrienoic acid containing about 20,000 cpm), microsomes corresponding to 0.5 g of tissue and varying concentrations of inhibitor **8**, in a total volume of 1 ml. Incubations were carried out for 30 min at 37° and terminated with 7 ml of methanol–chloroform 1:1. Lipids were extracted and analyzed as described previously.[6]

two diastereomeric racemates **8** were found to afford the same results within the precision of the experimental data. The bicyclic acid **8** specifically inhibited the formation of PGE_1, whereas the conversion to $PGF_{1\alpha}$ and the monohydroxy acids **6** and **7** was slightly increased. The inhibition of PGE_1 formation at different substrate concentrations is given in Table II.

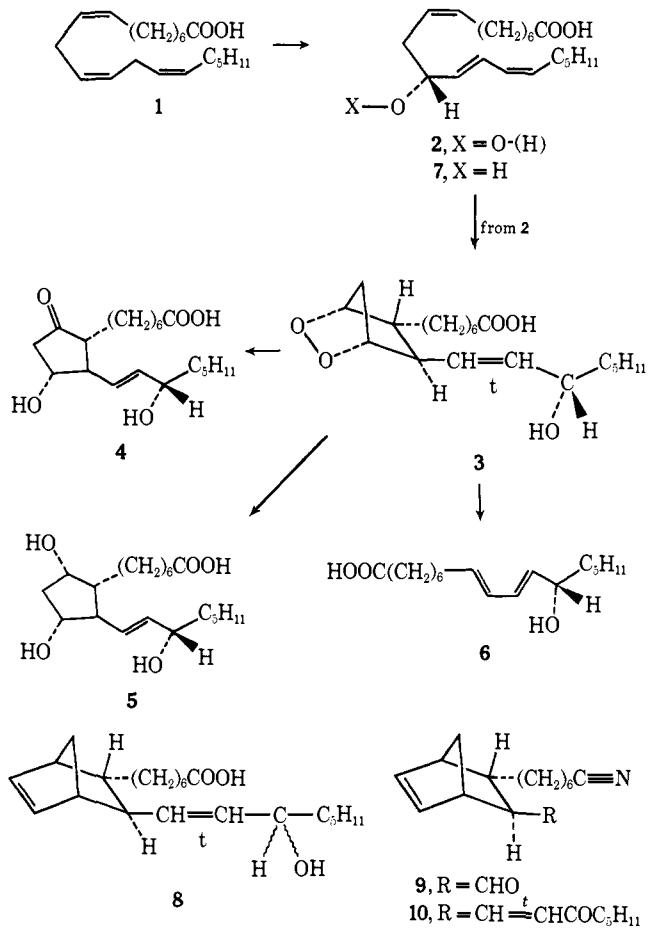
Table II. Effect of Inhibitor **8** on PGE_1 Formation at Various Substrate Concentrations[a]

Substrate concn, m*M*	—PGE_1 production, nmol— No additions	+ inhibitor	% inhibition
0.16	50	24	52
0.32	96	49	49
0.64	142	83	42
1.00	165	96	41
1.62	148	90	35

[a] Conditions of incubation were as in Table I, except that the concentrations of substrate ([2-^{14}C]8,11,14-eicosatrienoate, 20,000 cpm) were varied as indicated. Inhibitor, when present, was 0.9 m*M*.

The bicyclo[2.2.1]heptenes of structure **8** *thus inhibit the component isomerase enzyme forming* PGE_1 *but not the reductase, which catalyzes the formation of* $PGF_{1\alpha}$. Further work is in progress to study the effect of those bicyclic acids on components of the multienzyme complex.

The synthesis of the diastereomeric racemates **8** was accomplished starting with the Diels–Alder reaction of cyclopentadiene and *trans*-9-cyano-2-nonenal[7] which gave two isomeric adducts (reaction conditions, diene



aldehyde ratio 3:1, refluxing toluene as solvent, 4 hr under argon in the presence of 2,6-di-*tert*-butyl-4-methylphenol) in 80% yield in a ratio of 1.5:1. The two adducts were separated by preparative thin-layer chromatography (tlc) (silica gel–benzene, multiple development).[8] The predominating adduct (higher R_f)

(1) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **242**, 5336 (1967).
(2) M. Hamberg and B. Samuelsson, *ibid.*, **242**, 5329 (1967).
(3) C. Pace-Asciak and L. S. Wolfe, *Biochim. Biophys. Acta*, **152**, 784 (1968).
(4) D. H. Nugteren, *ibid.*, **210**, 171 (1970).
(5) All synthetic derivatives described herein were racemic. The two synthetic racemates of **8** which were obtained differ with regard to the stereocenter corresponding to C-15 in prostaglandin numbering.
(6) E. Granström, W. E. M. Lands, and B. Samuelsson, *J. Biol. Chem.*, **243**, 4104 (1968).
(7) E. J. Corey, I. Vlattas, N. H. Andersen, and K. Harding, *J. Amer. Chem. Soc.*, **90**, 3247 (1968).

was shown to possess an exo formyl group, and the other adduct was found to possess an endo formyl function by nmr spectroscopy.[9] Especially revealing was the chemical shift of the proton α to the formyl group (assignment confirmed by irradiation of the formyl proton) which occurred (in $CDCl_3$, parts per million downfield from internal tetramethylsilane) at 1.66 and 2.36 for the adducts of higher and lower R_f, respectively. The nmr data and the known stereochemistry of the Diels–Alder reaction thus allowed the assignment of structure **9** to the predominating (higher R_f) adduct. Reaction of **9** with the sodio derivative of dimethyl 2-oxoheptylphosphonate[7] produced the cyanoenone **10**.[8] Reduction of **10** with sodium borohydride in methanol at 0° produced a 1:1 mixture of two racemates[8] differing with regard to the newly created stereocenter and separable by tlc (silica gel, 85:15 petroleum ether ether, multiple development). Hydrolysis of the isomeric, racemic carbinols using potassium hydroxide (10 equiv, 0.3 *M*) in 4:1 ethanol–water at reflux (argon atm) for 48 hr and isolation in the usual way afforded each of the oily isomeric acids **8**.[10]

(8) The infrared, nmr, and mass spectra were in accord with the structure assigned to this oily substance.

(9) See J. C. Davis, Jr., and T. V. Van Auken, *J. Amer. Chem. Soc.*, **87**, 3900 (1965).

(10) This work was assisted financially by a study award to S. M. A. from CNICT (Argentina), a grant to Harvard from the National Institutes of Health, and a grant from the Swedish Medical Research Council (13X-217).

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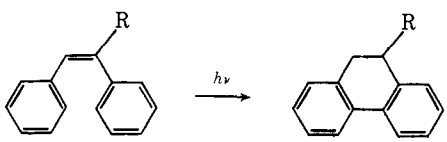
Received March 13, 1971

Photoisomerization of Certain Stilbenes to 9,10-Dihydrophenanthrenes

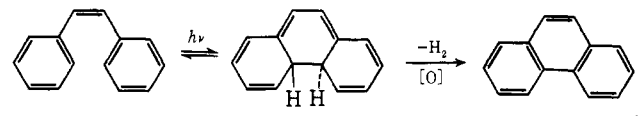
Sir:

Several years ago Sargent and Timmons reported[1] that the irradiation in the absence of oxidizing agents of certain stilbenes with one or more electron-withdrawing substituents on the central double bond, I,



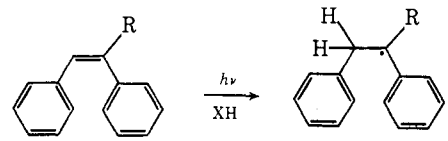
Ia, R = $COOCH_3$ IIa, R = $COOCH_3$ (1)

led to the formation of the isomeric 9,10-dihydrophenanthrenes (II). In view of the known, well-documented oxidative photocyclization of stilbene to phenanthrene (reaction 2)[2-4] which proceeds through the initial isomerization of the stilbene to the dihydrophenanthrene III it was suggested[1a] that in the case of I, the initial photoproduct was also a derivative of III which under oxidizing conditions gave the corresponding phenanthrene but otherwise rearranged to the 9,10-dihydrophenanthrene (II). However, in a more recent review, Blackburn and Timmons[5] have commented that the "mechanistic implications of this reaction (reaction 1) are not clear."



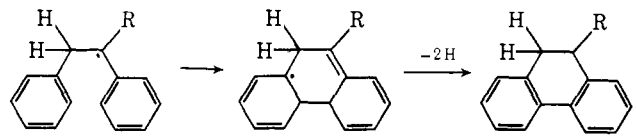
III (2)

We suggest that reaction 1 is most probably a free-radical process consisting of several steps which may be initiated by the abstraction of a hydrogen atom from a donor molecule (XH) by the stilbene I in an electronically excited state (eq 3). The donor molecule



IV (3)

can be the solvent and it is noteworthy that this reaction has been observed[1] usually in good hydrogen donating solvents such as chloroform, methanol, and ethanol. The radical IV can cyclize to V which will



IV V VI (4)

tend to lose 2 H atoms in order to aromatize again.[6] Hence, it can also serve as a hydrogen donor to another stilbene molecule. The process will end with the abstraction of a hydrogen atom by the radical VI to give the observed product.

We have carried out some tests of this mechanism with methyl α-phenylcinnamate (Ia) which is photoisomerized in methanol (in a nitrogen atmosphere) at 313.0 nm to methyl 9,10-dihydrophenanthrene-9-carboxylate (IIa)[7] in 72% yield, as well as with 1,2-diphenylfumaronitrile which was investigated by Sargent and Timmons.[1]

The first test was to conduct the irradiation in a fully deuterated solvent to determine the extent to which the 9,10 positions in the product were labeled.

A solution of Ia (4.2×10^{-2} *M*) in CD_3OD was irradiated to about 70% conversion. The product IIa that was formed was separated by vapor phase chromatography and characterized by its spectra. The mass spectrum showed a parent peak at *m/e* 240 which corresponded to the presence of two deuterium atoms in the molecule. The nmr spectrum (τ 2.40 (2 H),

(1) (a) M. V. Sargent and C. J. Timmons, *J. Amer. Chem. Soc.*, **85**, 2186 (1963); (b) M. V. Sargent and C. J. Timmons, *J. Chem. Soc.*, 5544 (1964).

(2) F. B. Mallory, C. S. Wood, and J. T. Gordon, *J. Amer. Chem. Soc.*, **86**, 3094 (1964), and earlier references therein.

(3) W. M. Moore, D. D. Morgan, and F. R. Stermitz, *ibid.*, **85**, 828 (1963).

(4) K. A. Muszkat and E. Fisher, *J. Chem. Soc. B*, 662 (1967), and earlier references therein.

(5) E. V. Blackburn and C. J. Timmons, *Quart. Rev., Chem. Soc.*, **23**, 482 (1969).

(6) Molecular elimination of hydrogen is not considered because gas evolution was not observed during photolysis.

(7) Identified as the acid,[8] mp 123.5–125.5°; satisfactory elemental analysis was obtained for the ester.

(8) Literature value 123–124°: H. De Konig, K. Wiedhaup, U. K. Pandit, and H. O. Huisman, *Recl. Trav. Chim. Pays-Bas*, **83**, 364 (1964).