but less efficiently than the enzymic isomerization of **4** to **5**.

It is important to note that the enzymic nature of the epimerization of the 4β-methyl ketone **4** is evident from the results of incubations of **4** with a boiled (inactive) enzyme preparation. These control incubations were extracted without saponification, and the extracts were treated with an ethereal solution of lithium aluminum hydride. The reduction products, upon analysis by glpc,[10] were found to contain almost exclusively the 4β-methyl sterol **6**; less than 1% of the 4α-methyl sterol **7** was detected.

Taken together with our previous results,[1] the above findings suggest a pathway of demethylation of 4,4-dimethyl sterols (route "a") that entails the stepwise oxidation of the 4α-methyl group and its removal, presumably by decarboxylation of the 3-keto-4α-carboxylic acid.[11] The product of such a decarboxylation might be the 4β-methyl ketone **4** which on the basis of the present results would be expected to be isomerized to the 4α-methyl ketone **5** and reduced to the 4α-methyl sterol **7**. A second demethylation sequence could then convert **7**, *via* 4α-hydroxymethylcholestanol (**8**),[9] to **9**. The reported identification of a 4β-methyl sterol in skin[12] and our failure to detect any enzymic conversion of 4α- to 4β-methyl compounds in any of our experiments are consistent with a similar route of metabolism in the conversion of lanosterol to cholesterol. An alternative, however, would be route "b," involving essentially concomitant decarboxylation and epimerization to afford directly a 4α-methyl sterol ready for oxidative attack. The putative enol intermediate in the decarboxylation of a 3-keto-4α-carboxylic acid could afford either **4** or **5**.

We have synthesized[13] the 4β-methyl-4α-carboxylic acid **3** and found that it is metabolized to an approximately equimolar mixture of the 4α-methyl sterol **7** and cholestanol (**9**) with about the same efficiency as the corresponding 4β-methyl-4α-hydroxymethyl sterol **2**, which yields the same products in similar proportions.[1] However, our present data do not allow us to distinguish between the metabolism of either of these compounds *via* a unitary decarboxylation–epimerization mechanism ("b") or *via* stepwise decarboxylation and epimerization ("a") by separate enzymes. Further experiments will be required to elucidate these mechanistic details, but it is worth noting that an element of biological economy is suggested by the present results in that it is possible that each of the 4-methyl substituents is removed by the same enzyme system which is highly stereospecific for attack on a 4α-methyl group.

**Acknowledgments.** Financial support was provided by U. S. Public Health Service Grant AM-12855 (to T. A. S.) and the American Heart Association (to R. B. C.).

(10) Sterols 6 and 7 were easily separated (retention time of 6 = 1.27 × retention time of 7) on a 1% XE-60 column at 200°. This procedure for analysis of the 4-methyl-3-ketones was adopted in order to avoid epimerization which occurs when glpc of the methyl ketones is attempted.

(11) J. A. Olson, M. G. Lindberg, and K. Bloch, *J. Biol. Chem.*, 226, 94 (1957), first proposed the involvement of a 3-keto-4-carboxylic acid in the demethylation of lanosterol.

(12) A. Sanghvi, D. Balasubramanian, and A. Moscowitz, *Biochemistry*, 6, 869 (1967).

(13) Compound 3, mp 270° dec, was prepared from 4β-methyl-4α-carbomethoxycholestanone,[1] which had been labeled with tritium in the usual manner,[4] by successive treatment with sodium borohydride, dihydropyran containing hydrogen chloride, potassium hydroxide in refluxing aqueous methanol for 36 hr, and aqueous acid.

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*Received February 28, 1969*

## The Mechanism of the Addition of $^1\Delta_g$ Excited Oxygen to Olefins. Evidence for a 1,2-Dioxetane Intermediate[1]

*Sir:*

The dye-sensitized photooxidation of monoolefins and noncisoid polyolefins is a well-known process which usually leads *via* a stereospecific pathway to the formation of rearranged allylic hydroperoxides.[2] The formation of these products has been described as proceeding *via* a concerted "ene"-type mechanism.[3] A conspicuous number of examples exist, however, in which reaction with singlet oxygen leads to carbonyl fragments.[2a] To account for these observations, it has been suggested that the carbonyl fragments arise from secondary reactions of initially formed, unstable allylic hydroperoxides.[2a] In the work described here, we show conclusively that allylic hydroperoxides are not responsible for carbonyl fragment formation in the reactions of singlet oxygen with indene derivatives. Furthermore, based on the chemical evidence presented below, we propose that 1,2-dioxetanes are important intermediates in reactions of singlet oxygen with olefins.[4]

The reported photooxidation of indene[2a] (I), leading to homophthaldehyde (IV), is a good example of exclusive carbonyl fragmentation. In our hands, methylene blue sensitized photooxidation of indene[9] in methylene chloride resulted only in the production of homophthaldehyde (IV). Similarly, when indene was treated with singlet oxygen generated by microwave discharge[10] in the vapor phase, only IV was obtained. In accordance with earlier views, the only possible allylic hydroperoxide, II, was postulated as the active

(1) This work was supported by grants from the American Cancer Society, California Division (to D. R. K.), and the Petroleum Research Fund (to P. R.), administered by the American Chemical Society.

(2) (a) K. Gollnick, *Advan. Photochem.*, 6, 1 (1968); (b) "Oxidation of Organic Compounds," Vol. III, Advances in Chemistry Series, No. 77, American Chemical Society, Washington, D. C., 1968.

(3) A. Nickon and J. F. Bagli, *J. Am. Chem. Soc.*, 83, 1498 (1961).

(4) 1,2-Dioxetanes have been considered as possible intermediates in the photooxidation of enamines[5,6] and in many reactions which exhibit chemiluminescence.[7,8]

(5) C. S. Foote and J. W.-P. Lin, *Tetrahedron Letters*, 3267 (1968).

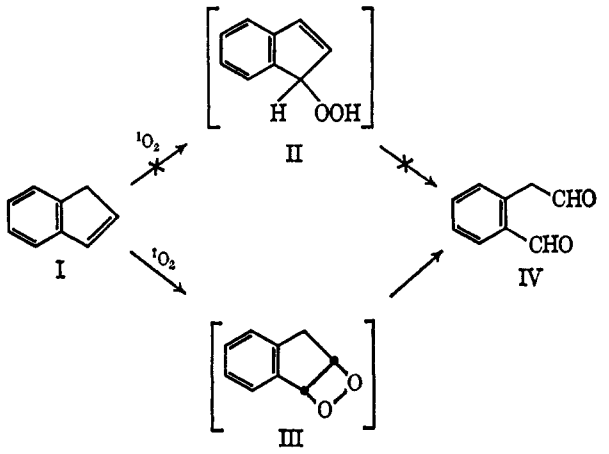
(6) J. E. Huber, *ibid.*, 3271 (1968).

(7) F. McCapra, *Quart. Rev.* (London), 20, 485 (1966).

(8) T. Goto and Y. Kishi, *Angew. Chem. Intern. Ed. Engl.*, 7, 407 (1968).

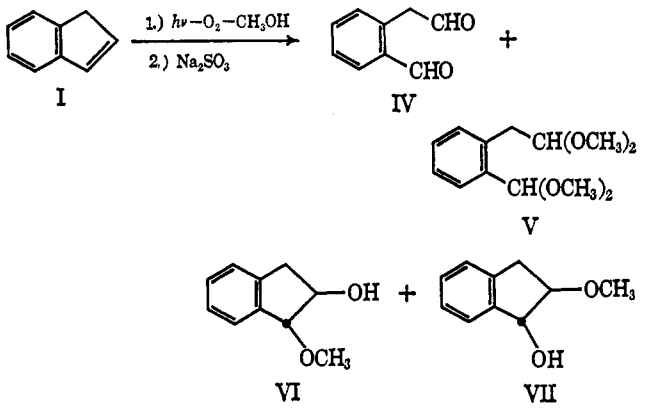
(9) All photooxidations were performed at 0°, using a 200-ml Pyrex immersion well apparatus fitted with an oxygen bubbler and a Sylvania DWY projection bulb. Methylene blue or rose bengal were used as dye sensitizers, and reagent grade methylene chloride or anhydrous methanol as solvents. The above-described reactions did not occur when oxygen, dye, or irradiation was omitted. Irradiation times varied from 50 min to 48 hr depending upon reactivities.

(10) E. J. Corey and W. C. Taylor, *J. Am. Chem. Soc.*, 86, 3881 (1964).



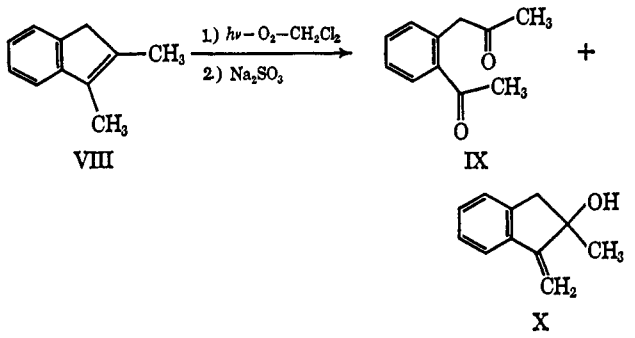

precursor to IV.[2a] To test this suggestion, the proposed intermediate was prepared by the method of Hock and Ernst[11] and found quite stable thermally. II distilled at 73–75° (0.3 mm) and showed little tendency toward decomposition at 125°. Furthermore, when II was subjected to the identical conditions required for the photooxidation of indene it was slowly converted to 1-indanone. No trace of IV was detected in the reaction mixture. Consequently, II can be ruled out as the precursor to IV, and therefore the significance of "ene"-type reactions of singlet oxygen with olefins to produce carbonyl products must be reconsidered.

When the photooxidation of indene was conducted in methanol solution, IV (6%), V (74%), VI (16%), and VII (4%) were obtained in high yield after sulfite




reduction of the hydroperoxide mixture.[12] The bisacetal V was shown to be rapidly formed from IV under the conditions of the photooxidation. The formation of significant amounts of both VI and VII cannot be accounted for by previously described mechanisms. The formation of a dioxetane intermediate (III) and its subsequent reaction with solvent readily account for these products.

When 2,3-dimethylindene[13] (VIII) was photooxidized in methylene chloride and the hydroperoxide mixture reduced with sodium sulfite, the carbonyl fragment IX[14] (30%) and the allylic alcohol X[15] (65%) were formed in 95% yield. This provides the first example in which significant quantities of both the expected allylic hydroperoxide and a carbonyl cleavage product have been reported. When VIII was photooxidized in




methanol solution, IX and X were obtained, but in addition a mixture of methanol-incorporated hydroperoxides was also obtained, with structures analogous to VI and VII.

For comparison, 2-methyl- and 3-methylindene were also prepared and photooxidized under identical conditions.[16] In methylene chloride, each hydrocarbon led to two products after reduction: the allylic methylene alcohol analogous to X and the carbonyl cleavage product analogous to IX. Again, when the photooxidations were conducted in methanol solution products resulting from solvent addition were obtained in significant amounts (*ca.* 25%). The possibility that radical side reactions were responsible for carbonyl products was discounted since identical products and product ratios were obtained when VIII was photooxidized in methylene chloride, 0.1 *M* in 2,6-di-*t*-butylphenol.[17]

In view of these results we propose that $^1\Delta_g$ oxygen reacts with olefins to form dioxetane[18] intermediates which cleave to the observed carbonyl products. From our studies and those of Kopecky[20] we further believe that the mechanism of allylic hydroperoxide formation is one or both of the possibilities: (1) stereospecific intramolecular hydrogen abstraction from an intermediate dioxetane, (2) the commonly accepted "ene" mechanism. Presently there are no data which conclusively differentiate between these two possible mechanisms.

(11) H. Hock and F. Ernst, *Chem. Ber.*, **92**, 2723 (1959).

(12) The structures of VI and VII were confirmed by comparison of their respective infrared and nmr spectra with those obtained from known samples prepared by a procedure described by W. Treibs and W. Schroth, *Ann.*, **639**, 204 (1960).

(13) 2,3-Dimethylindene was prepared by the procedure of F. Plenat and G. Bergson, *Arkiv Kemi*, **25**, 109 (1965).

(14) The structure of IX was confirmed by its nmr spectrum which consisted of a multiplet at $\tau$ 2.85 (4 H) and singlets at 6.12 (2 H), 7.60 (3 H), and 7.88 (3 H).

(15) The structure of the allylic alcohol X was determined by its nmr spectrum which consisted of a multiplet at $\tau$ 2.80 (4 H), a singlet at 4.61 (1 H), a singlet at 4.81 (1 H), a multiplet at 6.48 (1 H), a multiplet at 7.07 (2 H), and a sharp singlet at 8.67 (3 H), and by its ultraviolet absorption which was similar to that of styrene with maxima (m$\mu$ ($\epsilon$)) at 300 (1160), 288 (1360), 258 (sh, 3990), and 250 (5400).

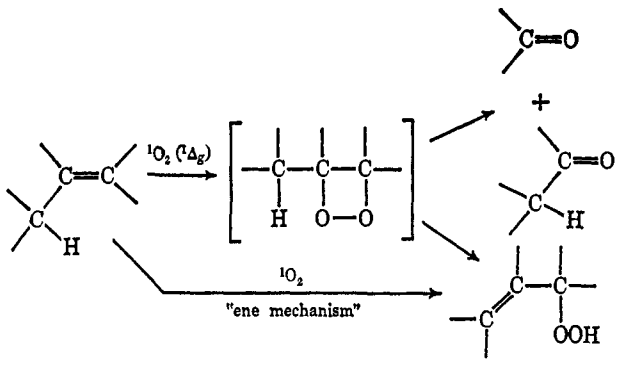
(16) 3-Methylindene was prepared by base-catalyzed isomerization of 1-methylindene prepared by the method of A. Weidler and G. Bergson, *Acta Chem. Scand.*, **18**, 1483 (1964). 2-Methylindene was prepared *via* addition of methylmagnesium iodide to 2-indanone followed by acid-catalyzed dehydration.

(17) The efficiency of various substituted phenols as alkoxy radical inhibitors is discussed in detail by C. Walling, "Free Radicals in Solution," John Wiley & Sons, Inc., New York, N. Y., 1957, p 430.

(18) Since $^1O_2$ contributes a pair of electrons in $\pi$-antibonding orbitals, the addition of oxygen to an olefin is analogous to an allowed photochemical $2\pi + 2\pi$ addition reaction.[19]

(19) D. R. Kearns, presented at the 5th International Congress on Photobiology, Hanover, N. H., Aug 1968.

(20) K. R. Kopecky and J. H. Van De Sande, *Can. J. Chem.*, **46**, 25 (1968), and personal communication.




We are continuing our investigations on these reactions in the hope that we may be able to resolve this problem.

(21) Alfred P. Sloan Fellow.

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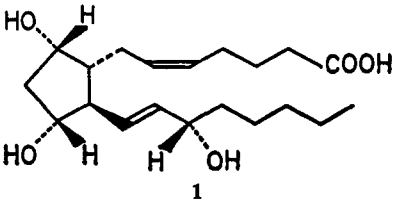
## The Structure of a Urinary Metabolite of Prostaglandin $F_{2\alpha}$ in Man

*Sir:*

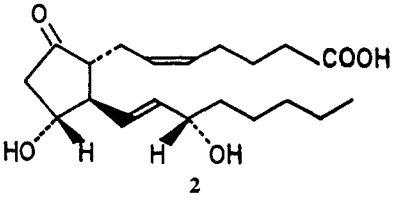
The main urinary metabolite of prostaglandin $F_{2\alpha}$[1,2] (**1**) in the guinea pig was recently identified as 5α,7α-dihydroxy-11-ketotetranorprostanoic acid (**12**). Studies on the metabolism of prostaglandin $E_2$[3] (**2**) in man led to the identification of a dicarboxylic acid as the major urinary metabolite[4] (**3**). We now wish to report the structure of a urinary metabolite (**4**) of prostaglandin $F_{2\alpha}$ (**1**) in man. [9β-$^3$H]Prostaglandin $F_{2\alpha}$[2] (35 μg, 200 μCi/μmole) was injected intravenously into female subjects. The urinary excretion of radioactive material was completed in about 5 hr; 85–95% of the administered radioactivity had then been excreted. To obtain larger amounts of the urinary metabolites, unlabeled prostaglandin $F_{2\alpha}$ was administered to female subjects by intravenous infusion at a rate of 4–12 μg/min for several hours.[5] Urine was collected from the beginning of the infusion to 5 hr after the administration of prostaglandin $F_{2\alpha}$ was completed. The urine thus obtained was added to the urine containing the tritium-labeled metabolites, and samples of this pool were processed as described below.

The urine was acidified to pH 3 and extracted four times with ethyl acetate and subsequently with butanol. About 50% of the urinary radioactivity was extracted with ethyl acetate and 45% with butanol. The ethyl acetate extract was purified by reversed-phase partition chromatography.[6] Two peaks of radioactivity (I, 110–180 ml of effluent, and II, 180–240 ml of effluent) appeared. The material in peaks I and II was esterified with diazomethane and purified by reversed-phase partition chromatography. Chromatography of the esterified material in peak I gave two peaks of radioactivity, *viz.* Ia (30–40 ml of effluent) and Ib (42–58 ml of effluent). The methyl ester of metabolite II was eluted with 40–53 ml of effluent.

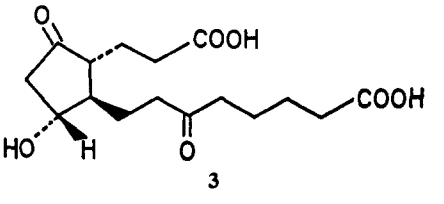
Metabolite Ib (**5**) was further purified by silicic acid chromatography (eluted with ethyl acetate–benzene 60:40) and subsequently converted into four derivatives for glpc and mass spectrometry: acetate **6**, trimethylsilyl ether **7**, O-methyloxime (methoxime) acetate **8**, and methoxime trimethylsilyl ether **9**.[7] Deuterated trimethylsilyl ether derivatives were also prepared using trimethylchlorosilane-$d_9$ (**10**, **11**).[8] The derivatives **13**, **14**, **15**, and **16** were prepared[2] for use as references in the analysis by glpc–mass spectrometry.




**1**




**2**




**3**

The retention times of the four derivatives of metabolite Ib (**6**, **7**, **8**, and **9**) found on glpc analysis were converted into *C* values[7] and are listed in Table I. The difference between the retention times of the acetates and the trimethylsilyl ethers (between **6** and **7**, $C = 1.0$; between **8** and **9**, $C = 1.1$) indicated the presence of two hydroxyl groups in the metabolite.[4]

**Table I.** *C* Values Found on Gas–Liquid Chromatography (1% SE-30)

| Derivative | *C* value | Derivative | *C* value |
|---|---|---|---|
| **6** | 24.9 | **13** | 21.9 |
| **7** | 23.9 | **14** | 20.9 |
| **8** | 24.9 | **15** | 21.9 |
| **9** | 23.8 | **16** | 20.9 |

In the mass spectrum of **8**, the ion with the highest *m/e* value was found at *m/e* 471. This corresponds to the molecular ion of a C-16 dicarboxylic acid containing two acetoxy groups and one methoxime group.

(1) Prostaglandin $F_{2\alpha}$ is the trivial name for 9α,11α,15-trihydroxyprosta-5(*cis*),13(*trans*)-dienoic acid.
(2) E. Granström and B. Samuelsson, submitted for publication.
(3) Prostaglandin $E_2$ is the trivial name for 11α,15-dihydroxy-9-ketoprosta-5(*cis*),13(*trans*)-dienoic acid.
(4) M. Hamberg and B. Samuelsson, *J. Am. Chem. Soc.*, **91**, 2177 (1969).
(5) The infusion of prostaglandin $F_{2\alpha}$ to healthy female subjects (20–30 years) was carried out by Dr. M. Bygdeman.
(6) Ethyl acetate extracts of urine were purified by reversed phase partition chromatography using solvent system D containing acetic acid[7] and columns of 27 g of hydrophobic Hyflo Super-Cel. Reversed-phase partition chromatography of the methyl esters of the metabolites was performed with columns of 4.5 g of hydrophobic Hyflo Super-Cel and solvent system F-50 (moving phase: methanol–water (150:150, v/v); stationary phase: chloroform–heptane (45:5, v/v)).
(7) M. Hamberg, *European J. Biochem.*, 6, 135 (1968).
(8) K. Gréen, submitted for publication.