suggests that some process effecting a thermodynamic equilibration is operative.

Possible mechanisms for the photoisomerization which do not involve energy transfer include eq 1-5,[5]

Path A. Ion-Chain Process

$$MPL \xrightarrow{h\nu} MPL^* \longrightarrow MP^+ + L^- \quad (1)$$

$$L^- \rightleftharpoons L'^- \quad (2)$$

$$L'^- + L \longrightarrow L' + L^- \quad (3)$$

Path B. Photocatalysis

$$MPL \xrightarrow{h\nu} MPL^* \rightleftharpoons MPL'^* \quad (4)$$

$$MPL'^* + L \rightleftharpoons MPL^* + L' \quad (5)$$

where MP = metalloporphyrin, L = *cis* ligand, L′ = *trans* ligand. Path A is reasonable since photoexcited metalloporphyrins can serve as electron donors[7] and since radical ions of olefins such as stilbene undergo *cis–trans* isomerization and electron-transfer reactions.[8] Quinone could interrupt path A by abstracting electrons from MPL* or L⁻. However, ionic processes such as reaction 1 are unlikely in nonpolar solvents such as benzene, and we see no evidence for formation of radicals upon irradiation of benzene–zinc etioporphyrin I–NPE solutions or even benzene–zinc etioporphyrin I–quinone solutions in an esr cavity.[9] Solvent effects provide evidence against path A. The reaction is less than one-tenth as fast in polar solvents such as acetonitrile and ethanol which should facilitate ionic processes. Flash spectroscopic studies (Table II) reveal no transients other than the zinc porphyrin triplet.[11]

Table II. Flash Spectroscopic Study of Zinc Etioporphyrin I Complexes

Sample[a]	Quencher	Lifetime, μsec	k_q, l. mole^{-1} sec^{-1}
Zinc etio I		200	
Zinc etio I–10^{-4} *M* pyridine		475	
Zinc etio I–10^{-4} *M* NPE[b]		480	
Zinc etio I–10^{-2} *M* piperidine		420	
Zinc etio I–10^{-4} *M* NPE[b]	4.33×10^{-6} *M* azulene	70	3×10^9
Zinc etio I–10^{-4} *M* NPE[b]	3.74×10^{-6} *M* quinone	25	1×10^{10}

[a] All samples 10^{-6} *M* in porphyrin, degassed benzene solutions, temperature 25°. [b] Starting material *cis*-NPE; initial data agree with those obtained after several flashes.

Path B seems possible since excited states of zinc porphyrin undergo rapid ligand exchange.[3] Flash studies indicate that triplets of the zinc porphyrin are not quenched by NPE; in fact the porphyrin triplets have longer lifetimes in the presence of NPE and pyridine. Triplet–triplet spectra of the zinc porphyrin are shifted to longer wavelengths with pyridine or NPE, but the change is slight. The lifetimes listed in Table II are long enough so that many exchanges occur in excess of the number required by the quantum efficiency listed in Table I. Although quinone quenches the isomerization at concentrations where fluorescence quenching is unimportant,[3] it appears unlikely that quinone, triplet energy 53 kcal/mole,[12] is quenching the 40–42-kcal/mole porphyrin triplet *via* energy transfer. A reasonable possibility is that quinone quenches the porphyrin triplet *via* charge-transfer complex formation at close to the diffusion-controlled rate.[13] If path B is operative, compounds with low-lying triplet states such as azulene and naphthacene should quench the isomerization nearly as well as quinone. Quenching by quinone and azulene is detectable in flash experiments (Table II) and correlates with isomerization experiments. In typical experiments with 10^{-4} *M* quencher, the isomerization of 5×10^{-3} *M* *cis*-NPE is 97% quenched by azulene and 99% quenched by quinone.

These results suggest that path B provides the most likely mechanism for the photoisomerization. The results are remarkable for the following reasons: spectral evidence suggests little delocalization of the porphyrin excited states to the ligand, yet isomerization occurs; *cis–trans* isomerization and ligand exchange occur in the excited state without concurrent deactivation. The observed phenomena imply that the activation barrier for *cis–trans* isomerization is sharply reduced in the excited porphyrin–NPE complexes. Results of studies with other metal complexes and kinetic details of the photoisomerization will be developed in the full paper.[14]

(5) Interesting, but probably unrelated, redox and isomerization phenomena involving olefins and cobalt porphyrins have been reported.[6]

(6) M. Tsutsui, R. Velapoldi, K. Suzuki, and A. Ferrari, *J. Am. Chem. Soc.*, **90**, 2723 (1968).

(7) K. P. Quinlan, *J. Phys. Chem.*, **72**, 1797 (1968), and earlier references; G. R. Seely, *ibid.*, **69**, 2779 (1965); L. P. Vernon and E. R. Shaw, *Biochemistry*, **4**, 132 (1965); V. B. Estigneev, *ibid.*, **5**, 171 (1966).

(8) R. Chang and C. S. Johnson, Jr., *J. Chem. Phys.*, **46**, 2314 (1967); C. S. Johnson, Jr., and R. Chang, *ibid.*, **43**, 3183 (1965).

(9) We have detected radicals from zinc etioporphyrin I–quinone–ethanol solutions upon irradiation in agreement with Tollin,[10] but we obtain only very weak signals from ethanol solutions of zinc etioporphyrin I–NPE.

(10) G. Tollin, K. K. Chatterjee, and G. Green, *Photochem. Photobiol.*, **4**, 593 (1965); G. Tollin and G. Green, *Biochim. Biophys. Acta.*, **60**, 524 (1962).

(11) A mercury–xenon flash having a fall time of *ca.* 10 μsec was used with Corning filters to activate the porphyrin visible bands. A tungsten steady-state lamp was used to monitor transients. Degassed solutions were irradiated in cylindrical cells with absorbance adjusted to low values (*ca.* 0.1) to ensure uniform production of transients.

(12) M. G. Jayswal and R. S. Singh, *Spectrochim. Acta*, **21**, 1597 (1965).

(13) H. Beens and A. Weller in "Molecular Luminescence," E. C. Lim, Ed., W. A. Benjamin, New York, N. Y., 1969, p 203.

(14) Support of this work by the National Institutes of Health (Grant No. GM 15,238-01,2) and the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

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David G. Whitten, Peter D. Wildes, Irene G. Lopp[15]

Department of Chemistry, University of North Carolina Chapel Hill, North Carolina 27514

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Biological Demethylation of 4,4-Dimethyl Sterols. Evidence for Enzymic Epimerization of the 4β-Methyl Group Prior to Its Oxidative Removal

Sir:

In a previous report we have shown that in the oxidative demethylation of 4,4-dimethylcholestanol (**1**) to cholestanol (**9**) by enzymes of rat liver, the 4α-hydroxymethyl-4β-methyl sterol **2**, but not its 4β-hydroxymethyl isomer, behaved as an intermediate, and the conclusion was drawn that the 4α-methyl group of **1** was the first to be attacked.[1] It was noted that Gaylor and Delwiche had arrived at the opposite conclusion in

(1) K. B. Sharpless, T. E. Snyder, T. A. Spencer, K. K. Maheshwari, G. Guhn, and R. B. Clayton, *J. Amer. Chem. Soc.*, **90**, 6874 (1968).

a study of the sequence of removal of the 4α- and 4β-methyl groups of lanosterol in the course of its metabolism to cholesterol.[2]

In support of our earlier findings we now demonstrate that rat liver contains an enzyme system that epimerizes the 4β-methyl group of 4β-methylcholestanone (**4**) and that the 4α-methyl sterol **7** is demethylated much more efficiently than the 4β-methyl sterol **6**. On the basis of these and other results discussed herein, we propose that, following the initial loss of the 4α-methyl group of a 4,4-dimethyl sterol, the oxidative removal of the remaining methyl substitutent occurs only after its inversion into the 4α configuration.

The compounds used in the present study were prepared from 4-methyl-Δ^4-cholesten-3-one[3] (**10**) which had been labeled at C_2 and C_6 by exposure to acidic ^3H_2O in tetrahydrofuran.[4] Hydrogenation of **10** afforded 4β-methylcholestanone (**4**)[5] (specific activity 7.0×10^4 dpm of $^3H/\mu g$), and hydride reduction of **4** gave 4β-methylcholestanol (**6**)[6] (specific activity 6.5×10^4 dpm of $^3H/\mu g$). The 4α-methyl ketone **5** was obtained (with specific activity 4.5×10^4 dpm of $^3H/\mu g$) by epimerization of **4**.[7] Hydride reduction of the resulting **5**[7] afforded **7** with the same specific activity.

The substrates indicated in Table I were incubated aerobically in duplicate with aliquots of the same rat liver homogenate under experimental conditions similar to those used for our earlier studies, and, unless otherwise stated, the products were also isolated and analyzed as previously described.[1] The results are shown in Table I.

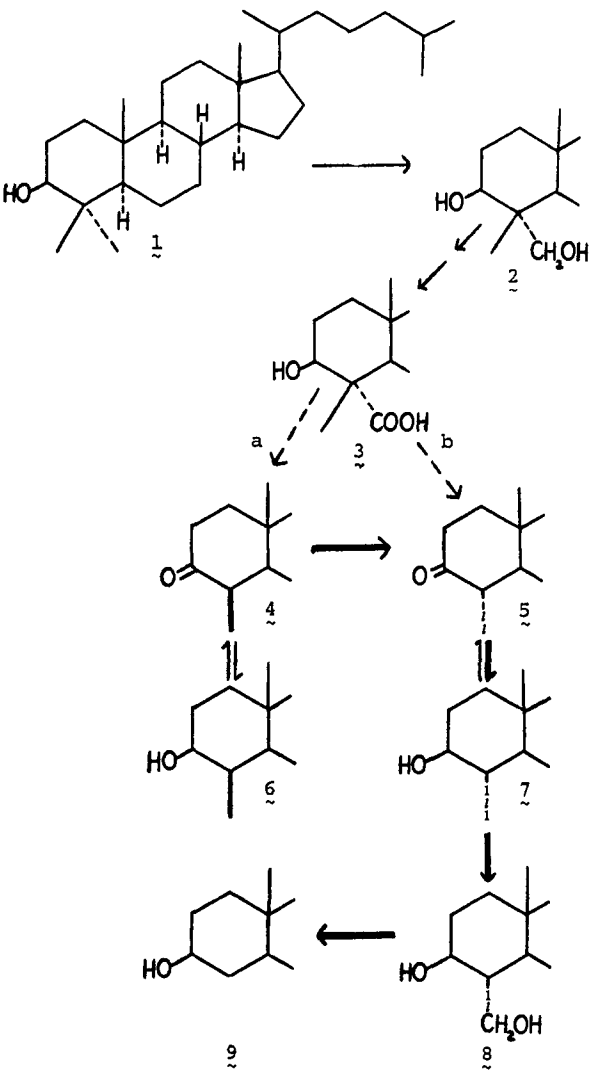
Table I

Substrate	Recovered substrates[a]	4-Methyl cholestanols 4α (7)	4β (6)	Cholestanol (9)
4,4-Dimethylcholestanol (**1**)				7.3
4α-Methylcholestanone (**5**)	8.0	64.1		27.9
4α-Methylcholestanol (**7**)	71.8			28.2
4β-Methylcholestanone (**4**)	24.3	43.2	18.4	14.1
4β-Methylcholestanol (**6**)	96.5			3.5

[a] For experimental conditions see ref 1. Values refer to per cent of total recovered labeled materials. (Total recoveries were 80–90% of incubated labeled substrate.)

The 4α-methyl ketone **5** and 4α-methyl sterol **7** were both converted in similar yield (*ca.* 28%) to cholestanol (**9**), and the major portion of **5** that was not demethylated (64.1%) was reduced to **7**. These results suggest that the rate of enzymic 3-keto → 3β-ol reduction exceeds the rate of demethylation and are consistent with the conclusion of Swindell and Gaylor that a 4α-methyl-3-ketone must be reduced to the corresponding 3β-ol prior to oxidative attack on the 4α-methyl group.[8]

By contrast, the 4β-methyl ketone **4** and the corresponding 4β-methyl sterol **6** were much less efficiently converted to cholestanol. The sterol **6** was converted to cholestanol with only half the efficiency of 4,4-dimethylcholestanol (**1**) (see Table I) and therefore is unlikely to be an intermediate in the demethylation of **1**. The 4β-methyl-3-ketone **4** yielded the 4α-methyl sterol **7** as the major product, together with lesser amounts of the 4β-methyl sterol **6** and cholestanol (**9**) (14%). In view of the extremely poor metabolism of **6** and the relatively efficient conversion of the 4α-methyl ketone **5** to the 4α-methyl sterol **7**, and of both **5** and **7** to **9**, the results obtained on metabolism of **4** imply its initial isomerization to the 4α-methyl ketone **5** as a major pathway (heavy arrows), followed by conversion of **5** to **7** and thence to **9**.[9] The observed formation of some 4β-methyl sterol **6** from the ketone **4** is best accounted for by assuming that under these experimental conditions a 3β-reductase is capable of reducing the 3-ketone in the 4β-methyl compound **4**,



(2) J. L. Gaylor and C. V. Delwiche, *Steroids*, **4**, 207 (1964).
(3) N. W. Atwater, *J. Amer. Chem. Soc.*, **82**, 2852 (1960).
(4) R. G. Nadeau and R. P. Hanzlik, *Methods Enzymol.*, **15**, in press.
(5) D. Rosenthal, A. O. Niedermeyer, and J. Fried, *J. Org. Chem.*, **30**, 510 (1965).
(6) S. Julia and J.-P. Lavaux, *Bull. Soc. Chim. Fr.*, 1223 (1963), report that reduction of **4** with lithium tri-*t*-butoxyaluminum hydride afforded material with mp 142–143° to which they assigned structure **6**, but which gave unsatisfactory elemental analyses. Repetition of their procedure in our laboratory yielded a substance, mp 160–162°, with properties, including elemental analysis, consistent with struture **6**.
(7) Y. Mazur and F. Sondheimer, *J. Amer. Chem. Soc.*, **80**, 5220 (1958).
(8) A. C. Swindell and J. L. Gaylor, *J. Biol. Chem.*, **243**, 5546 (1968).
(9) We have previously demonstrated the efficient conversion of 4α-hydroxymethylcholestanol (**8**), a presumed intermediate in this process, to **9**.[1]

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.

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(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.

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(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.

K. B. Sharpless, T. E. Snyder
Department of Chemistry, Stanford University
Stanford, California 94305

T. A. Spencer, K. K. Maheshwari, J. A. Nelson
Department of Chemistry, Dartmouth College
Hanover, New Hampshire 03755

R. B. Clayton
Department of Psychiatry, Stanford University School of Medicine
Stanford, California 94305

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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).

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(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.

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