of β-keto esters, III[2] and IV.[2] Two-stage condensation ((i) 0.25 equiv of potassium *tert*-butoxide-*tert*-BuOH, room temperature, 48 hr; (ii) 1.8 equiv of TsOH–AcOH 90°, 3 hr) of III (purified by preparative glc) with I gave a mixture of trienedione V[2] (mp 94–95°; $\lambda_{\max}^{\mathrm{EtOH}}$ 307 mμ (ε 18,200)) and salicylate VI[2,3] (mp 134–135°) in 39 and 14% yields, respectively.

Model experiments[4] in the propionylacetate series pointed to the method for avoiding unwanted salicylates such as VI, which arise from tautomerization of a tricyclic dienone intermediate[5–8] in which the β-keto ester arrangement has survived. Condensation of ethyl propionylacetate with I under the aforementioned conditions gave a mixture of VII[7] and VIII[2,3] (mp 96–96.5°). Identical condensation with *tert*-butyl propionylacetate gave only VII in 73% yield. Clearly the greater lability of the carbo-*tert*-butoxy group to the acidic cyclization conditions is a useful property in avoiding salicylate formation.

Accordingly, we prepared compound XII[2] starting with the unsaturated acid IX. Treatment of the latter with thionyl chloride gave the labile acid chloride X which served to acylate the sodium salt of *tert*-butylacetoacetate. Crude XI so produced was subjected to the action of ammonia to give keto ester XII (10% from IX). The value of the *tert*-butyl ester grouping is seen in that condensation of XII with enetrione I gave V in 56% yield with no evidence for aromatic by-products. These successes pinpoint the need for efficiently generated partners for condensation with I.

This objective was successfully realized. Treatment of 3-oxoglutaric acid with isobutylene under the following conditions (10 g of freshly prepared diacid, 1 ml of concentrated H_2SO_4, 100 ml of isobutylene shaken in a bomb at room temperature for 18 hr) gave XIII[9] in 90% yield. Monoalkylation of XIII (sodium hydride–THF–*trans*-1,3-dichloro-2-butene) gave XIV[2] in 64% yield. Two-stage condensation of keto diester XIV with I under the same conditions used for the monoesters gave V in 64% yield.[10,11] The objective of the smooth assemblage of an intermediate containing the requisite functionality for the elaboration of a wide variety of steroids has thus been reached.

Hydrolysis (concentrated H_2SO_4–methylene chloride, 0°, 0.5 hr) of V gave trienedione XV[2] (mp 146–147°; $\lambda_{\max}^{\mathrm{EtOH}}$ 308 mμ (ε 17,200)) in 95%[12,13] yield. Base-catalyzed (3% KOH–dioxane–H_2O, 41–43°, 1 hr) cyclization of XV gave the tetracyclic XVI[14] in 68% yield. The latter has been converted[15] to 8^{14}-dehydroestrone.

The power of this new methodology is seen in the fact that the overall conversion (*i.e.*, trisannelation) of 2-methylcyclopentane-1,3-dione to XVI is executed in 34% yield. The uses of compounds V and XV in the preparation of other steroids will be reported shortly.

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(2) Combustion analysis within 0.3% of theory; infrared, nmr, and mass spectra are in full agreement with the proposed structure.

(3) Although its sharp melting point and nmr spectrum point to this being a homogeneous substance, the stereochemistry of the junction is unassigned.

(4) Conducted by B. H. Migdalof in these laboratories.

(5) While acid-catalyzed aromatization of dienones such as V is also precedented,[6] the rate of this process as distinct from the β-keto ester series is sufficiently slow as to allow for their survival under the acidic conditions of their formation.[1,7,8]

(6) C. Sannié and J. J. Panouse, *Bull. Soc. Chim. Fr.*, 1435 (1956).

(7) A. Frey, "Promotionsarbeit," E. T. H. Zurich, 1954.

(8) J. J. Panouse and C. Sannié, *Bull. Soc. Chim. Fr.*, 1429 (1956).

(9) For a recent but less convenient preparation of XIII see H. Paul and J. Polyczynski, *J. Prakt. Chim.*, **312**, 240 (1970).

(10) Also isolated from this reaction was a 10% yield of II which undoubtedly arises from acidic degradation of unreacted XIII. We were unable to detect any of the isomer of V containing the side chain on the α′ side of the dienone. This is another illustration[11] of the tendency of the monoalkylated derivative of acetonedicarboxylates to undergo base-induced alkylation at the methylene carbon.

(11) *Cf.* G. Schroeter, *Chem. Ber.*, **49**, 2711 (1916).

(12) The use of methylene chloride as diluent is responsible for this unusually high yield for an enol chloride → ketone transformation. The yield under the standard conditions[13] is 61% yield. We thank Dr. U. Eder of Schering AG (Berlin) for bringing this method to our attention.

(13) J. A. Marshall and D. J. Schaeffer, *J. Org. Chem.*, **30**, 3642 (1965).

(14) T. B. Windholz, J. H. Fried, H. Schwam, and A. A. Patchett, *J. Amer. Chem. Soc.*, **85**, 1707 (1963).

(15) D. B. R. Johnson, D. Taub, and T. B. Windholz, *Steroids*, **8**, 365 (1966).

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Singlet Oxygen Analogs in Biological Systems. Coupled Oxygenation of 1,3-Dienes by Soybean Lipoxidase

Sir:

The formation of allylic hydroperoxides from alkenes with allylic hydrogens and the addition of oxygen to 1,3-dienoid systems are two well-established reactions of singlet molecular oxygen.[1] Similar processes of oxygenation have been postulated in enzyme-mediated reactions although direct evidence is still lacking. Soybean lipoxidase catalyzes the conversion of *cis,cis*-1,4-pentadienes to the hydroperoxides of *cis,trans*-1,3-dienes by molecular oxygen[2]—a process directly analogous to the action of singlet oxygen on alkenes. In the presence of its substrate (linoleic acid or its methyl or ethyl ester), this enzyme also catalyzes the coupled oxidation of carotene.[3] The possibility that the mode of catalysis by lipoxidase mimics the reactions of singlet oxygen is being investigated and results on the coupled oxygenation of 1,3-dienoid systems are reported here.

In general, 0.125 mmol of the dienoid compound[4] and 0.5 mmol of ethyl linoleate[5] are suspended in 1.5 l. of 0.05 *M* pH 9 Tris buffer. The incubation, which is carried out at 30° and in the dark, is initiated by the addition of the enzyme (240,000 units[6] or sufficient so that the reaction is complete in 1 hr). Thus, tetraphenylcyclopentadienone yielded its endoperoxide (I) (20%; uv and ir spectra identical with those of an

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(1) C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968), and references cited therein.

(2) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **242**, 5329 (1967).

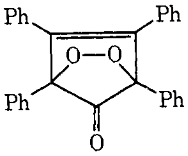
(3) R. T. Holman, *Arch. Biochem.*, **10**, 519 (1946).

(4) The choice of dienoid compounds is limited by the inability of most of such compounds to form a stable fine suspension in aqueous media.

(5) No reaction was observed in the absence of ethyl linoleate.

(6) As defined by the supplier (Sigma Co.).

authentic specimen) which has been isolated recently and characterized.[7] The reaction using 1,3-diphenylisobenzofuran as the diene had a rate 30 times greater than that of tetraphenylcyclopentadienone and gave a 30% yield of 1,2-dibenzoylbenzene (mp 146–147° (lit. 148°) undepressed in admixture with an authentic specimen obtained by photosensitized oxygenation). A control incubation performed under identical conditions but with boiled enzyme gave, in each case, no detectable reaction.



I

The enzyme-catalyzed formation of the endoperoxide I from tetraphenylcyclopentadienone finds direct analogy in the addition of singlet oxygen to 1,3-dienoid systems. In this case, the mild conditions of the enzymic reaction led to the isolation of the endoperoxide which undergoes facile reactions under more drastic conditions.[7] The conversion of 1,3-diphenylisobenzofuran to 1,2-dibenzoylbenzene, here catalyzed by lipoxidase, is a known reaction of singlet oxygen[8] involving its addition to the 1,3-dienoid system present in the furan. That the oxygenation of the dienes is not due to the product(s) of lipoxidation of ethyl linoleate is shown by incubation of ethyl linoleate, under the same conditions, prior to the addition of the diene; this led to at least a tenfold decrease in the rate of oxygenation of the dienes. Kinetic studies further indicated that the disappearance of the diene had a maximum initial rate that occurs concurrently with the maximum rate of the oxidation of ethyl linoleate. The results reported here are therefore consistent with a reaction sequence in which the dienes trap, in a fast reaction, an intermediate formed during the oxidation of ethyl linoleate.

The observation that two modes of oxygenation analogous to the reactions of singlet oxygen are catalyzed by soybean lipoxidase raises the interesting question whether such excited molecular species, either free or bound to a group on the enzyme, are involved in the catalysis of this enzyme. As oxygen is usually the only high-energy reactant present in reactions catalyzed by lipoxidase, the generation of singlet oxygen would involve raising one molecule of oxygen to this excited state by the free energy released upon the reduction of another.[9] Previous studies[10] have given no indication that more than 1 mol of oxygen is consumed per mole of linoleate oxidized. They have, on the other hand, shown the absence of prosthetic groups and heavy metal ions on the enzyme[11] and have pointed to the participation of free radicals which are not involved in chain reactions.[12] Apart from these investigations, little is known about the way oxygen is involved in reactions catalyzed by soybean lipoxidase. The results discussed here indicate that an intermediate is generated during the lipoxidase-catalyzed oxidation of ethyl linoleate that is a close analog of singlet oxygen. Thus, it is reasonable to assume the existence of enzymes that catalyze the addition of the elements of molecular oxygen to 1,3-dienoid systems in specific substrates in the biosynthesis of natural products having structures that suggest this mode of derivation. Further studies on the relationship between the reactions of singlet oxygen and enzymic oxygenations including the possible presence of singlet molecular oxygen in biological systems is in progress.

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(7) H. W.-S. Chan. *Chem. Commun.*, 1550 (1970).

(8) C. Dufraisse and S. Ecary, *C. R. Acad. Sci.*, **223**, 735 (1946).

(9) This only applies to free molecular singlet oxygen. There can, of course, be oxygen bound to the enzyme that is "singlet-like" without having an excess of 22 kcal of electronic energy. This would fall into the category of oxygen activation that is often invoked in the discussion of reactions catalyzed by dioxygenases and may be involved in the present case.

(10) (a) R. T. Holman, *Arch. Biochem.*, **15**, 403 (1947); (b) H. L. Tookey, R. G. Wilson, R. L. Lohmar, and H. J. Dutton, *J. Biol. Chem.*, **230**, 65 (1958).

(11) A. L. Tappel, *Enzymes*, **8**, 275 (1963).

(12) A. L. Tappel, P. D. Boyer, and W. O. Lundberg, *J. Biol. Chem.*, **199**, 267 (1952).

*Book Reviews**

Physical Organic Chemistry. Second Edition. By LOUIS P. HAMMETT, Columbia University. McGraw-Hill Book Co., New York, N. Y. 1970. 420 pp. $13.95.

Second editions usually closely resemble first editions, but this is not the case with the book under review. Instead of being satisfied with assessing progress in the great contributions of the first edition (sigma-rho theory, H_0 acidity function, *i*-factors), Hammett has chosen a more ambitious goal. Briefly, it is to examine the consequences of thermodynamics and transition-state theory on reaction rates.

This second edition is unusual in another way. It is published thirty years after the first.

It will be convenient to review Chapters 11 and 12 first, since they stand somewhat apart from the rest of the book. Chapter 11 is entitled "Quantitative Relationships Involving Structure and Reactivity." This is Hammett's sigma-rho theory, and it must give the author tremendous paternal pride to see how well it has

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* Unsigned book reviews are by the Book Review Editor.