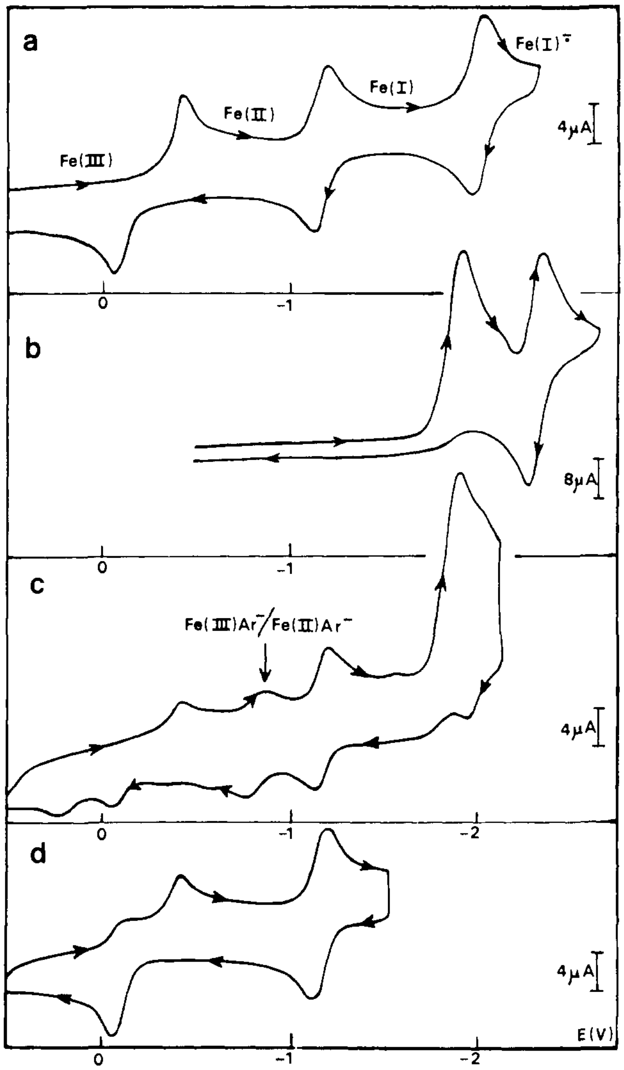

Figure 1. Cyclic voltammetry of $FeC_{12}(TPP)$ in the presence of 4-bromobenzonitrile in DMF and 0.1 M NBu_4BF_4 (working electrode, glassy carbon disc; reference electrode, aqueous SCE; sweep rate, 200 mV s^{-1}): (a) $FeC_{12}(TPP)$ (5×10^{-4} M) alone; (b) 4-bromobenzonitrile (7×10^{-4} M) alone; (c) $FeC_{12}(TPP)$ (5×10^{-4} M) and 4-bromobenzonitrile (7×10^{-4} M) starting the potential scan at -2.1 V and scanning first anodically then cathodically; (d) $FeC_{12}(TPP)$ (5×10^{-4} M) and 4-bromobenzonitrile (7×10^{-4} M) starting the potential scan at -1.5 V and scanning first anodically then cathodically.

radicals for the arylation of the iron porphyrin to occur. Arylation is, however, observed at this potential under spectroelectrochemical conditions that correspond to more efficient electrolysis than the cyclic voltammetric conditions. Aryl radicals can indeed be slowly generated at the Fe(II)/Fe(I) wave through redox catalysis of the reduction of 4-bromobenzonitrile.[9]

Similar behaviors were found with iodobenzene and 1-bromonaphthalene by using the same porphyrin. The spectra of the $Fe^{III}Ph^{-}$ and $Fe^{II}Ph^{-}$ complexes were found to be very similar to those of the 4-cyanophenyl and the alkyl complexes.[4] The spectrum found for $Fe^{III}Ph^{-}$ was practically the same as that previously described for $(TPP)Fe^{III}Ph^{-}$.[1c] Vinylation appears to occur in the same way. 1-Bromo-2,2-bis(4-chlorophenyl)ethylene gives rise to an irreversible wave located just behind the TPP $Fe(I)/Fe(I)^{-}\cdot$ wave which then becomes irreversible, indicating that redox catalysis of the reduction of the vinylic chloride by the $Fe(I)/Fe(I)^{-}\cdot$ couple is taking place. Starting the potential scan at the level of this wave or on the vinyl halide wave results in the formation of the σ-vinyliron porphyrin, which gives rise to a $(TPP)Fe^{III}Vi^{-}/Fe^{II}Vi^{-}$ reversible wave at $E° = -0.63$ V and an irreversible oxidation wave at $E_p = +0.6$ V ($v = 0.2$ V s^{-1}). The spectra of the $Fe^{III}Vi^{-}$ and $Fe^{II}Vi^{-}$ complexes were recorded under the same spectroelectrochemical conditions as above. The spectrum of the $(TPP)Fe^{II}Vi^{-}$ complex was found to be exactly the same as for the same compound (λ_{max} = 355 nm (ε 2.66 10^4 m^{-1} cm^{-1}), 428 (8.08×10^4), 510 (1.38×10^4), 800 (0.46×10^4)) obtained from electrochemical hydrogenation of the corresponding carbene complex: $Fe(II) \leftarrow C{=}C(p\text{-}ClC_6H_4)_2$. The generation of $Fe^{II}Vi^{-}$ at the Fe(II)/Fe(I) wave can also be observed as in the case of 4-bromobenzonitrile.

All these observations are compatible with a reaction mechanism involving the direct or indirect electrochemical generation of aryl (or vinyl) radical from the aryl (or vinyl) halide and its reaction with the electrochemically generated Fe(I) porphyrin yielding the Fe(II) σ-aryl (or -vinyl) porphyrins, which can then be reoxidized electrochemically into the Fe(III) σ-aryl (or -vinyl) complex. The possible interference of the Fe(II) and $Fe(I)^{-}\cdot$ complexes in the reaction should, however, be more carefully investigated. This study is underway in an effort to assess more soundly the reaction mechanism through the study of the competition between the reaction of the aryl (or vinyl) radicals with the iron porphyrins and of side reactions such as reduction at the electrode or in the solution and H atom abstraction from the solvent.[5] The ESR and NMR characteristics of these complexes are currently under investigation.

Acknowledgment. This work was supported in part by the CNRS (Equipe de Recherche Associée N°309 "Electrochimie Moléculaire"). Dr. M. Momenteau (Institut Curie, Orsay) is thanked for the gift of $C_{12}(TPP)$ samples and Dr. J. P. Battioni (Laboratoire de Chimie de l'Ecole Normale Supérieure-Paris) for the gift of a 1-bromo-2,2-bis(4-chlorophenyl)ethylene sample. We also thank Mrs. Jomain for her technical assistance in the spectroelectrochemical determinations.

Registry No. $FeC_{12}(TPP)$, 70196-65-5; 4-bromobenzonitrile, 623-00-7; iodobenzene, 591-50-4; 1-bromonaphthalene, 90-11-9; 1-bromo-2,2-bis-(4-chlorophenyl)ethylene, 23349-12-4.

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[9] Andrieux, C. P.; Blocman, C.; Dumas-Bouchiat, J. M.; M'Halla, F.; Savéant, J. M. *J. Am. Chem. Soc.* **1980**, *102*, 3806 and references cited therein.

Chemiluminescence from a Phenoxide-Substituted 1,2-Dioxetane: A Model for Firefly Bioluminescence

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The chemiluminescent decomposition of 1,2-dioxetanes has been an active area of investigation.[1] Simple, isolable dioxetanes such as tetramethyl-1,2-dioxetane are relatively stable and afford predominantly triplet excited products upon thermolysis. These properties are, however, in sharp contrast to those of the key intermediate **2a**[2] in firefly bioluminescence.[3] The bioexcitation efficiency for the formation of singlet excited **3a** from **2a** is at

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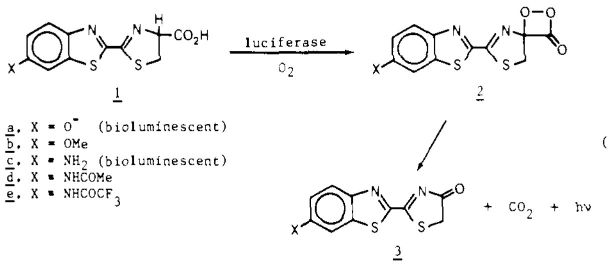
(1) For reviews of the chemiluminescent decomposition of 1,2-dioxetanes, see: (a) Wilson, T. *Int. Rev. Sci.: Phys. Chem. Ser. 2.* **1976**, *9*, 265-322. (b) Horn, K. A.; Koo, J.-K.; Schmidt, S. P.; Schuster, G. B. *Mol. Photochem.* **1978**, *9*, 1-23. (c) Bartlett, P. D.; Landis, M. E. In "Singlet Oxygen"; Wasserman, H. H., Murray, R. W. Eds.; Academic Press: New York, 1979; Chapter 7. (d) Turro, N. J.; Lechtken, P.; Shore, N. E.; Schuster, G. B.; Steinmetzer, H.-C.; Yekta, A. *Acc. Chem. Res.* **1974**, *7*, 97-105. (e) Adam, W. *Pure Appl. Chem.* **1980**, *52*, 2591-2608.

(2) (a) Shimomura, O.; Goto, T.; Johnson, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2799-2802. (b) Shimomura, O.; Johnson, F. H. *Photochem. Photobiol.* **1979**, *30*, 89-91.

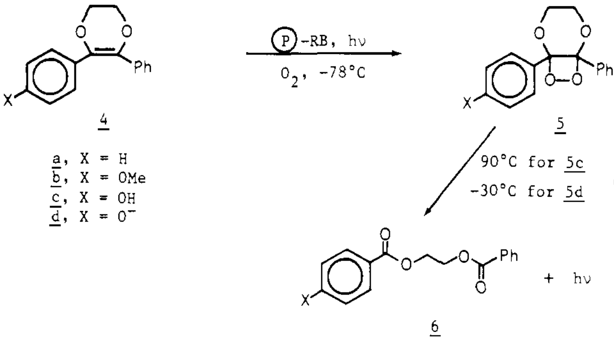
(3) For a review of the chemistry of firefly luminescence, see: McElroy, W. D.; DeLuca, M. In "Bioluminescence in Action"; Herring, P. J., Ed.; Academic Press: New York, 1978; Chapter 4.



(1)

least 88%.[4] Further, **2a** must be quite unstable as evidenced by the fact that the duration of the flash of the firefly can be as short as 40 ms.[5] In 1966 White described the effect of various substituents on in vitro firefly bioluminescence.[6] Although luciferins **1a–e** all oxidized to give the corresponding products **3a-e**, only in the case of the natural luciferin **1a** and the amino-substituted luciferin **1c** was the reaction attended by observable luminescence. We now present results of a study of similarly substituted 1,2-dioxetanes that provide additional insight into mechanisms of chemi- and bioluminescence. In particular, we have found that deprotonation of a phenolic substituent converts a stable, inefficiently luminescent dioxetane into one that exhibits properties more characteristic of the bioluminescent intermediate **2a**.

The phenol-substituted dioxetane **5c** was prepared by low-temperature photooxygenation of 2-phenyl-3-(4′-hydroxyphenyl)-1,4-dioxene (**4c**)[7] in acetone using polymer-bound Rose Bengal[8] (SENSITOX I), a 400-W high-pressure sodium lamp, and methods previously described.[9] After 75 min of irradiation, the solution was filtered and the solvent removed under vacuum at 0 °C to yield **5c** as an oil. Dioxetane **5c** was identified by ^{13}C NMR spectroscopy in $CDCl_3$ (dioxetane ring carbons at 109.8 and 110.0 ppm) and by its quantitative cleavage to diester **6c**, which was fully characterized.



(2)

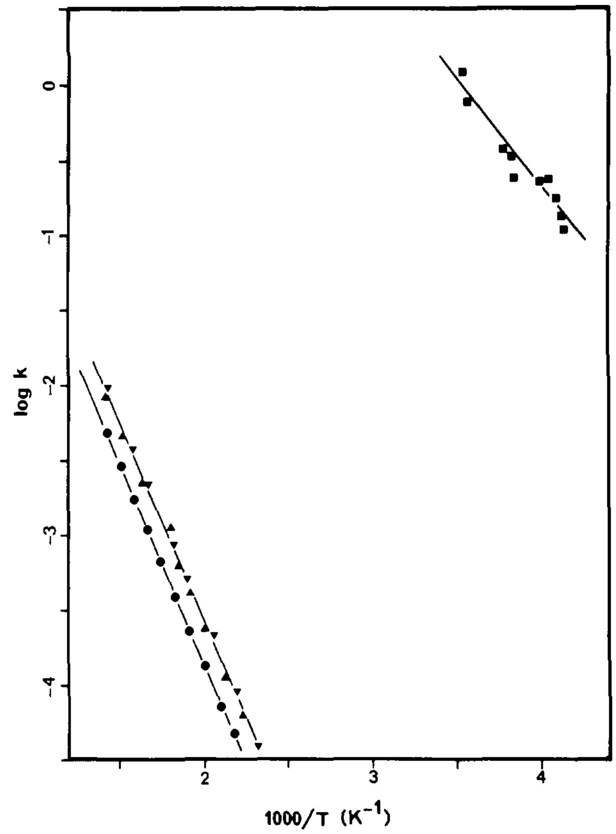


Figure 1. Arrhenius plot for the decomposition of dioxetanes **5a** (●), **5b** (▲), and **5c** (▼) in *o*-xylene from 45–90 °C and of dioxetane **5d** (■) in toluene from −35 to 2 °C.

Table I. Activation Parameters, Rates of Decomposition, and Chemiluminescence Efficiencies for 1,2-Dioxetanes 5a–d

dioxetane (X)	E_a, kcal/mol	log *A*	k_{rel} (25 °C)	$\tau_{1/2}$ (25 °C)	$^1\phi_{CE}$, %[b]	$^3\phi_{CE}$, %[c]
5a (H)	24.8	12.39	0.48	120 h	0.02	36
5b (OMe)	24.5	12.50	0.99	56 h		
5c (OH)	24.4	12.38	1.00[a]	57 h	0.006	1.5
5d (O⁻)	13.4	11.0	4.4×10^6	46 ms	1[d]	

[a] Corresponding to a rate constant in *o*-xylene at 25 °C of 3.40×10^{-6} s^{-1}. [b] Chemiluminescence efficiency for the formation of singlet excited **6** at 90 °C. [c] Chemiluminescence efficiency for the formation of triplet excited **6** at 90 °C with ϕ_F for DBA = 0.043 and ϕ_{TS} taken as 0.2.[10] Efficiencies are based on a calibration with the Hastings ^{14}C radioactive light standard.[12] Use of the luminol standard[13] would give efficiencies lower by a factor of approximately 2.[14] Errors in ϕ_{CE} are estimated to be ±20%. [d] At 3 °C in toluene with KO-*t*-Bu/18-crown-6 and purging with nitrogen.

Rate constants for the decomposition of dioxetane **5c** were obtained at 45–90 °C from measurements of the decay of chemiluminescence intensity of 10^{-4} *M* solutions in *o*-xylene. Rates showed variations of less than 3% and gave excellent Arrhenius plots (Figure 1 and Table I). As ester **6c** is very weakly fluorescent, singlet chemiexcitation yields ($^1\phi_{CE}$) from **5c** were determined from Stern–Volmer plots with 9,10-diphenylanthracene as an energy acceptor.[10] Triplet yields ($^3\phi_{CE}$) were obtained by energy transfer to 9,10-dibromoanthracene.[10] The results for dioxetane **5c** are compared in Table I to those for the unsubstituted dioxetane **5a**[11] and methoxy-substituted dioxetane **5b**. As anticipated, **5c** is quite stable and decomposes with a low singlet chemiexcitation efficiency that is typical of most simple dioxetanes

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(4) Seliger, H. H.; McElroy, W. D. *Arch. Biochem. Biophys.* **1960**, *88*, 136–141.

(5) Lloyd, J. E. In "Bioluminescence in Action"; Herring, P. J., Ed.; Academic Press: New York, 1978; Chapter 8.

(6) (a) White, E. H.; Worther, H.; Seliger, H. H.; McElroy, W. D. *J. Am. Chem. Soc.* **1966**, *88*, 2015–2019. (b) White, E. H.; Worther, H. *J. Org. Chem.* **1966**, *31*, 1484–1488.

(7) Vigorous stirring of a mixture of 4-methoxybenzoin, ethylene glycol ditosylate, and tetrabutylammonium bromide in refluxing benzene and aqueous KOH gave 2-phenyl-3-(4′-methoxyphenyl)-1,4-dioxene (mp 55–56 °C) in 65% yield. Demethylation of this olefin with EtSNa in DMF at 150 °C gave dioxene **4c** (mp 110.5–111.5 °C) in 83% yield.

(8) (a) Schaap, A. P.; Thayer, A. L.; Blossey, E. C.; Neckers, D. C. *J. Am. Chem. Soc.* **1975**, *97*, 3741–3745. (b) Schaap, A. P.; Thayer, A. L.; Zaklika, K. A.; Valenti, P. C. *Ibid.* **1979**, *101*, 4016–4017. (c) This sensitizer is available under the tradename SENSITOX I from Hydron Laboratories, Inc., Chemical Sales Dept., New Brunswick, NJ 08902.

(9) (a) Schaap, A. P.; Burns, P. A.; Zaklika, K. A. *J. Am. Chem. Soc.* **1977**, *99*, 1270–1272. (b) Zaklika, K. A.; Burns, P. A.; Schaap, A. P. *Ibid.* **1978**, *100*, 318–320.

(10) (a) Wilson, T.; Schaap, A. P. *J. Am. Chem. Soc.* **1971**, *93*, 4126–4136. (b) Wilson, T. *Photochem. Photobiol.* **1979**, *30*, 177–183. (c) Wilson, T.; Halpern, A. M. *J. Am. Chem. Soc.* **1980**, *102*, 7272–7279. (d) Wilson, T.; Halpern, A. M. *Ibid.* **1980**, *102*, 7279–7283.

(11) The value[18a] originally published for $^3\phi_{CE}$ of dioxetane **5a** was low as a result of reabsorption of chemiluminescence with concentrated DBA solutions. Use of an appropriate cutoff filter has minimized this problem; see ref 10b.

(12) Hastings, J. W.; Weber, G. *J. Opt. Soc. Am.* **1963**, *53*, 1410–1415.

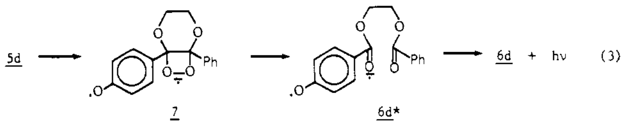
(13) Lee, J.; Seliger, H. H. *Photochem. Photobiol.* **1965**, *4*, 1015–1048.

(14) Michael, P. R.; Faulkner, L. R. *Anal. Chem.* **1976**, *48*, 1188–1192.

and dioxetanones.[1] The modest rate enhancements provided by the hydroxy and methoxy substituents are consistent with a biradical mechanism for the decomposition of these dioxetanes involving rate-limiting O–O bond homolysis.[15]

More significantly, we find that deprotonation of **5c** to give the phenoxide-substitued dioxetane **5d** dramatically changes the properties of **5** to those more typical of the biological intermediate **2a**. While **5c** has a half-life of 17 years at −30 °C, treatment of a solution of **5c** in toluene at −30 °C with the hindered base $(Me_3Si)_2MeCONa$ results in a flash of brilliant bluish luminescence. Rate constants for the decomposition of **5d** were measured by injection of 1 equiv of base in toluene into a prethermostated solution of **5c** (10^{-4} M in toluene) at temperatures from −35 to 2 °C. Control experiments have shown that the rates are unaffected by an excess of base but are reduced significantly with less than 1 equiv. Within experimental error, the following bases give rise to identical rates of decomposition for **5d**: $CsHCO_3$ with 24-crown-8, KOH or KO-*t*-Bu with 18-crown-6, and $(Me_3Si)_2MeCONa$ with 15-crown-5. An Arrhenius plot (Figure 1) gave an activation energy of 13.4 kcal/mol, with a calculated half-life at 25 °C of only 46 ms. *The relative rate of decomposition of* **5d** *vs.* **5c** ($k_{O^-}/_{OH}$) *at 25 °C is 4.4 × 10^6.*

There is also a significant increase in the singlet chemiexcitation efficiency upon deprotonation (Table I). Cleavage product **6d** is sufficiently fluorescent ($\phi_F = 0.002$)[16] so that $^1\phi_{CE}$ could be determined directly. As the phenoxide ion **6d** is chemically unstable in the presence of oxygen, both the fluorescence quantum yield of **6d** and the chemiluminescence of **5d** were measured in N_2-bubbled solutions.[17]



Intramolecular electron-transfer mechanisms have been proposed for the efficient chemiluminescence from dioxetanes bearing easily oxidized substituents.[18] Chemiluminescence has also been observed by Schuster[19] and Adam[20] from intermolecular electron-transfer reactions between peroxides and fluorescent hydrocarbons with low oxidation potentials. On the basis of these results, similar mechanisms have been suggested for the bioexcitation process in the firefly system.[18a,21] For the present case, we suggest that cleavage of dioxetane **5d** is initiated by the transfer of an electron from the phenoxide substituent to the peroxide σ^* orbital. Subsequent decomposition of intermediate **7** can yield directly a charge-transfer excited state of **6d**. The contrast between dioxetanes **5d** and **5a–c** together with the results of an earlier study of a related amino-substituted dioxetane[18a] now provides an explanation for the observations of White of the substituted luciferins **1a–e**. Further, a comparison of the efficiencies and stabilities of **5c** and **5d** prompts us to speculate about a possible control mechanism for the rapid flashing of the firefly involving initiation of luminescence by deprotonation of **2**.

Acknowledgment. Support from the U.S. Army Research Office is gratefully acknowledged.

Registry No. 4c, 81616-86-6; **5a**, 67592-95-4; **5b**, 81616-87-7; **5c**, 81616-88-8; **5d**, 81616-89-9; **6c**, 81616-90-2; **6d**, 81616-91-3; 2-phenyl-3-(4′-methoxyphenyl)-1,4-dioxene, 73260-63-6; 4-methoxybenzoin, 1889-84-5; ethylene glycol ditosylate, 6315-52-2.

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[15] Richardson, W. H.; Montgomery, G. C.; Yelvington, M. B.; O'Neal, H. E. *J. Am. Chem. Soc.* **1974**, *96*, 7525–7532.

[16] Determined by adding 1 equiv of KO-*t*-Bu/18-crown-6 to a nitrogen-purged toluene solution of **6c** at 3 °C. ϕ_F for methyl *p*-hydroxybenzoate under the same conditions is 0.038.

[17] A referee suggested that singlet emission could result from triplet-triplet annihilation. However, the chemiluminescence efficiencies and rates are identical within experimental error in aerated solution, in the presence of 0.2 M 2,3-dimethyl-1,3-butadiene or under N_2 and Ar.

[18] (a) Zaklika, K. A.; Thayer, A. L.; Schaap, A. P. *J. Am. Chem. Soc.* **1978**, *100*, 4916–4918. (b) McCapra, F.; Beheshti, I.; Burford, A.; Hann, R. A.; Zaklika, K. A. *J. Chem. Soc., Chem. Commun.* **1977**, 944–946. (c) Nakamura, H.; Goto, T. *Photochem. Photobiol.* **1979**, *30*, 27–30. (d) Lee, C.; Singer, L. A. *J. Am. Chem. Soc.* **1980**, *102*, 3823–3829.

[19] Schuster, G. B. *Acc. Chem. Res.* **1979**, *12*, 366–373.

[20] Adam, W.; Cueto, O. *J. Am. Chem. Soc.* **1979**, *101*, 6511–6515.

[21] (a) McCapra, F. *J. Chem. Soc., Chem. Commun.* **1977**, 946–948. (b) Koo, J.-Y.; Schmidt, S. P.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 30–33. (c) Zaklika, K. A.; Kissel, T.; Thayer, A. L.; Burns, P. A.; Schaap, A. P. *Photochem. Photobiol.* **1979**, *30*, 35–44.

Stereochemistry of Casbene Biosynthesis

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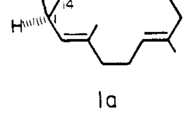
The isolation of casbene (**1a**) from a mixture of diterpene



1a

hydrocarbons produced by incubation of mevalonate (**2a**) or geranylgeranyl pyrophosphate (GGPP, **3a**) with an enzyme extract from castor bean (*Ricinus communis* L.) seedlings was reported by Robinson and West in 1970.[2] The structure proposed for casbene has been confirmed and its stereochemistry (1*R*, 14*S*) established by total synthesis from methyl (+)-*cis*-chrysanthemate.[3] Further studies by West and co-workers have shown that casbene is a phytoalexin for *R. communis*,[4] i.e., a fungal-elicited host metabolite that exhibits significant antifungal activity. In the context of the continuing interest on the mechanism and stereochemistry of the biosynthesis of cyclopropane-containing natural products,[5] we report experimental results that elucidate the stereochemistry of casbene biosynthesis.

Soluble enzyme extracts (S-150 fraction) were prepared from 2.5–3-day-old castor bean seedlings according to the procedures of Robinson and West.[2,6] In many germinations, the seedlings were inoculated with a spore suspension of *Rhizopus Stolonifer* 12 h before harvesting to enhance casbene synthetase activity.[4] Large-scale incubations of [2-^{14}C]mevalonic acid (**2b**) and ATP or of [1-$^3H_1$]- or [1-^{14}C]GGPP[7] with 300–700 mL of the S-150 enzyme preparations gave rise to the usual mixture of five diterpene hydrocarbons,[8] from which casbene was separated by column chromatography on silver nitrate impregated silica gel. The incorporation of radioactivity into casbene was typically 5–15% (200–800 μg) from mevalonate and 10–30% (100–300 μg)

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[1] 3M Co. Fellow, 1977–1978; Eastman Kodak Fellow, 1978–1979; Merck and Company Fellow, 1979–1980.

[2] (a) Robinson, D. R.; West, C. A. *Biochemistry* **1970**, *9*, 70–79. (b) Robinson, D. R.; West, C. A. *Ibid.* **1970**, *9*, 80–89. (c) Robinson, D. R. Ph.D. Thesis, University of California, Los Angeles, 1967.

[3] Crombie, L.; Kneen, G.; Pattenden, G.; Whybrow, D. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1711–1717.

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[5] (a) Presqualene pyrophosphate: Poulter, C. D.; Rilling, H. C. In "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley-Interscience: New York, 1981; Chapter 8. (b) Cycloartenol: Altman, L. J.; Han, C. Y.; Bertolino, A.; Handy, G.; Laungani, D.; Muller, W.; Schwartz, S.; Shanker, D.; deWolf, W. H.; Yang, F. *J. Am. Chem. Soc.* **1978**, *100*, 3235–3237. Blätter, W. A. Ph.D. Thesis, ETH, Zürich, 1978. (c) Δ^3-Carene: Akhila, A.; Banthorpe, D. V. *Phytochemistry* **1980**, *19*, 1691–1694. (d) Cyclopropane-containing fatty acids: Law, J. H. *Acc. Chem. Res.* **1971**, *4*, 199–203. Buist, P. H.; MacLean, D. B. *Can. J. Chem.* **1981**, *59*, 828–838; Obrecht, J. Ph.D. Thesis, ETH, Zürich, 1982.

[6] Drengler, K. A.; Coates, R. M. *J. Chem. Soc., Chem. Commun.* **1980**, 856–857.

[7] Typical conditions: 0.3 mM potassium mevalonate (6 μM GGPP), 3.1 mM ATP, 12 mM $MgCl_2$, 0.3 mM $MnCl_2$, 100 mM Tris-bicarbonate buffer, pH 7.3; 4 h at 30 °C.

[8] Trachylobane, kaurene, sandaracopimaradiene, beyerene, and casbene.