

A New Water-soluble Singlet Oxygen Probe

Michel Giraud,^a Alain Valla,^a Marc Bazin,^b René Santus,^b and André Momzikoff^c

^a *Laboratoire de Chimie Appliquée aux Corps Organisés, Muséum National d'Histoire Naturelle, 63 rue de Buffon, 75005-Paris, France*

^b *Laboratoire de Physicochimie de l'Adaptation Biologique, E.R.A. 951 du CNRS, 43 rue Cuvier, 75231-Paris Cedex 05, France*

^c *Laboratoire de Physiologie des Etres Marins, Institut Océanographique, 195 rue Saint-Jacques, 75005-Paris, France*

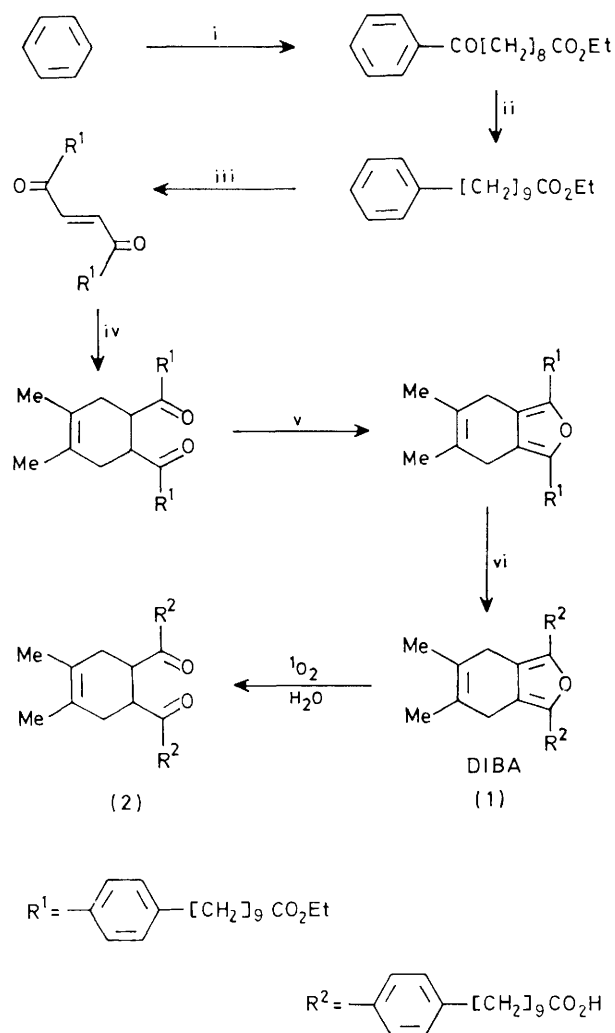
The synthesis of the dihydroisobenzofuran derivative (4,7-dihydro-5,6-dimethylisobenzofuran-1,3-diyl)bis(benzene-*p*-decanoic acid) is described; in buffered aqueous solution this compound readily reacts with singlet oxygen with rate constant $k = 7.1 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ as shown by the rapid bleaching of its 331 nm absorption band.

There has been considerable interest in the formation and biological implications of singlet oxygen ($^1\text{O}_2$) in aqueous media.¹ Consequently, efforts have been made to synthesize water-soluble $^1\text{O}_2$ traps²⁻⁵ whose bleaching can be followed by spectrophotometric detection. However, these water-soluble derivatives have a major flaw: their strong absorption is in the visible region ($\lambda \geq 400 \text{ nm}$). Thus, kinetic studies of photodynamic reactions induced by photodynamic sensitizers encountered in biological or natural environments and absorbing maximally in the 380–460 nm range (flavins,⁶ pterins,⁷ and porphyrins⁸) are complicated by direct photo-oxidation.⁹ Therefore we suggest use of the novel water-soluble dihydroisobenzofuran derivative; the potassium salt of (4,7-dihydro-5,6-dimethylisobenzofuran-1,3-diyl)bis(benzene-*p*-decanoic acid) (**1**) (DIBA). The long chains may be expected to favour ground state interactions with molecules, macromolecules, or

micelles bearing opposite charges. The DIBA concentration can be spectrophotometrically monitored by following the decay of its strong absorption band which peaks at 331 nm ($\epsilon = 1.6 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). DIBA was synthesised as shown in Scheme 1.[†]

Its reactivity towards $^1\text{O}_2$ was checked using $0.5 \times 10^{-5} \text{ M}$ hematoporphyrin (HP), a well known $^1\text{O}_2$ producer.⁸ Solutions of HP in carbonate buffer containing $1 \times 10^{-4} \text{ M}$ DIBA were irradiated at $\lambda > 398 \text{ nm}$ which does not excite DIBA but excites HP in its strongly absorbing Soret band. A fast and

[†] Spectroscopic data for DIBA: ^1H n.m.r. (CDCl_3) δ 1.30 (10 CH_2 , m), 1.62 (4 CH_3 , m), 1.82 (2 CH_3 , br.s), 2.35 (2 CH_2 , m), 2.62 (2 CH_2 , m), 3.35 (2 CH_2 , br.s), and 7.07 and 7.60 (8 H, AA'XX' system). U.v. λ_{max} (K_2CO_3 , 0.1 M) 364, 344, 331, and 239 nm (ϵ 9 200, 15 200, 16 000, and 10 800).



Scheme 1. Reagents: i, $\text{EtO}_2\text{C}[\text{CH}_2]_8\text{COCl-AlCl}_3$; ii, H_2 , Pd-C (5%) $\text{MeCO}_2\text{H-HCl}$ (2% v/v); iii, fumaroyl chloride- AlCl_3 -methylene dichloride; iv, 2,3-dimethylbutadiene, anhydrous benzene, heat; v, anhydrous benzene-toluene-*p*-sulphonic acid, heat; vi, KOH-EtOH (0.5 M), heat.

complete bleaching of DIBA occurred. The presence of isosbestic points at 297 and 246 nm suggests specific photosensitized oxidation leading to a single product whose absorption spectrum is in agreement with that of the acid (2) prepared independently by a chemical method. On the other hand, the direct photoexcitation of DIBA leads to numerous products.

The second-order rate constant ($k = 7.1 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) was determined using a comparative method in which the amino-acid, histidine, a recognized $^1\text{O}_2$ probe which reacts with $^1\text{O}_2$ at a fairly high rate ($k = 5 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) at neutral pH,¹⁰ replaced DIBA as the $^1\text{O}_2$ trap. The photosensitized degradation of histidine in pH 7 phosphate buffer and 0.1 M potassium carbonate was followed by h.p.l.c.

The rate of bleaching of DIBA was inhibited in solutions saturated with argon and dramatically increased ($k = 3 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) in 95% deuteriated water. These results point to the involvement of $^1\text{O}_2$ in the bleaching of DIBA.

We thank Dr. Morlière for the h.p.l.c. determination of the histidine concentration. This work has been supported by a grant from the C.N.R.S.

Received, 14th June 1982; Com. 675

References

- H. H. Wasserman and R. W. Murray, 'Singlet Oxygen,' Academic Press, New York, 1979.
- M. Botsivali and D. F. Evans, *J. Chem. Soc., Chem. Commun.*, 1979, 1114.
- B. A. Linding, M. A. J. Rodgers, and P. A. Schaap, *J. Am. Chem. Soc.*, 1980, **102**, 5580.
- P. A. Schaap, A. L. Thayer, G. R. Falor, K. Goda, and T. Kimura, *J. Am. Chem. Soc.*, 1974, **96**, 4025.
- J. M. Aubry, J. Rigaudy, and N. K. Cuong, *Photochem. Photobiol.*, 1981, **33**, 149.
- J. D. Spikes and R. Livingstone, *Adv. Radiat. Res.*, 1969, **3**, 29.
- C. Chahidi, M. Aubailly, A. Momzikoff, M. Bazin, and R. Santus, *Photochem. Photobiol.*, 1981, **33**, 641.
- J. D. Spikes, *Ann. N.Y. Acad. Sci.*, 1975, **244**, 496.
- G. Rio and M. J. Scholl, *J. Chem. Soc., Chem. Commun.*, 1975, 474; J. A. Howard and G. D. Mendenhall, *Can. J. Chem.*, 1975, **53**, 2199.
- K. Gollnick, 'Radiation Research: Biochemical, Chemical and Physical Perspectives,' Academic Press, New York, 1975, p. 590.