Pergamon

Tetrahedron, Vol. 52, No. 6, pp. 2031-2046, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)01004-1

Synthesis and Photooxidation of Sodium 1,3-Cyclohexadiene-1,4-Diethanoate: a New Colorless and Water-Soluble Trap of Singlet Oxygen.

Véronique Nardello; Nathalie Azaroual; Isabelle Cervoise; Gaston Vermeersch; Jean-Marie Aubry*

Equipe de Recherches "Radicaux Libres et Oxygène Singulet", URA CNRS 351, Faculté de Pharmacie de Lille, BP 83, F-59006 Lille Cedex, France.

Abstract: The sodium 1,3-cyclohexadiene-1,4-diethanoate has been designed as a new colorless trap for the measurement of photogenerated singlet oxygen in aqueous solution. Kinetic studies carried out by flash and steady-state photolysis have shown that this compound interacts with singlet oxygen *via* a pure chemical quenching with a rate constant k_r equal to $(2.6 \times 10^7 \pm 0.3)$ $M^{-1}.s^{-1}$. The two products obtained by photooxidation have been identified. The major one (88 %) is a stable endoperoxide whereas the minor one (12 %) is an hydroperoxide which decomposes into a phenolic derivative by prolongated warming at 37 °C.

Introduction

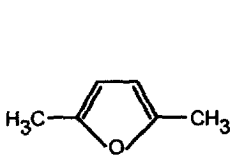
Since many years, singlet oxygen ($^1O_2$, $^1\Delta_g$), the lowest excited form of oxygen, has widely been used in organic synthesis as a powerful and selective oxidant.[1] More recently, a considerable interest has focused on the involvement of this species in many processes occuring in aqueous solutions: hydrogen peroxide disproportionation induced by metallic oxides,[2-6] degradation of natural organic compounds under sunlight,[7,8] generation of singlet oxygen by enzymes *in vivo*.[9-12] But above all, $^1O_2$ is a major intermediate in the photodynamic effect, namely the combined action of light, dye and oxygen on a biological substrate.[13-20] This effect has received several applications such as the phototherapic treatment of cancer[21,22] and the photoinactivation of virus in blood products.[23] For all these applications, it is necessary to resort to a method allowing the measurement of the amount of this transient species under given experimental conditions.

One of the most widely used and most sensitive methods consists in the chemical trapping of singlet oxygen with molecules which lead to a primary specific oxidation product. An ideal trap for aqueous media must have a number of properties: solubility in water, high reactivity toward singlet oxygen and low reactivity toward the other oxygen derivatives such as ground-state oxygen $^3O_2$, superoxide anion $O_2^{\cdot-}$ or hydrogen peroxide H_2O_2. However, it is difficult to avoid completely the oxidation of organic compounds by more powerful oxidants such as ozone O_3, hydroxyl radical $OH^{\cdot}$, peracids RCOOOH, peroxy $ROO^{\cdot}$ or alkoxy $RO^{\cdot}$ radicals. Therefore, a trap which would disappear only by reaction with singlet oxygen cannot exist. On the other hand, some molecules are able to give by reaction with singlet oxygen a primary oxidation product, stable and extremely specific of this species. Thus, Smith *et al.*[24-26] have used cholesterol which leads to a single product (the 5α-hydroperoxide) on reaction with singlet oxygen. The formation of two other products (7α and

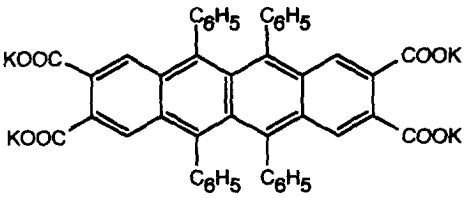
7β hydroperoxides) are indications of free radical processes.[27] Unfortunately, this natural compound is nearly completely insoluble in aqueous systems and exhibits a low reactivity toward singlet oxygen. Therefore, Foote[28,29] proposed to use a radiolabeled cholesterol bound to dispersible polymer beadlets which overcomes the solubility and sensitivity limitations. Another trap, largely used for the measurement of singlet oxygen in natural waters, is the 2,5-dimethylfuran (DIMF) **1**.[7,30-32] But despite its water-solubility and its high reactivity toward $^1O_2$, this compound and all the other furan derivatives, such as furfuryl alcohol[33-35] or 4,7-dihydro-5,6-dimethylisobenzofuran-1,3-diyl *bis*-(benzene-*p*-decanoic acid) (DIBA),[36] are poorly specific traps since they are also oxidized to diketones by many other strong oxidants.[32]

In fact, the traps meeting all the preceeding requirements belong to the series of polycyclic aromatic compounds. The water-soluble tetrapotassium rubrene-2,3,8,9-tetracarboxylate (RTC) **2**[37] effectively reacts with singlet oxygen by giving a specific endoperoxide but on account of its visible absorption until 620 nm, it may act itself as a photosensitizer and thus, cannot be used for the measurement of photogenerated singlet oxygen. On the other hand, the accessible wavelength area can slightly be increased by using anthracenic derivatives[38,39] such as the potassium or sodium salts of 3,3'-(anthracene-9,10-diyl) ethanesulfonic acid (AES) **3**[40,41], 3,3'-(anthracene-9,10-diyl) dipropionic acid (ADP) **4**,[42,43] and 9,10-diphenylanthracene-2,3,6,7-tetracarboxylic acid (DPATC) **5**.[44-46] Unfortunately, these compounds have still an absorption in the visible spectrum and as for RTC, singlet oxygen may be generated *via* the photosensitization of the trap. Such molecules can only be used with colored photosensitizers absorbing above 400 nm, e.g. rose bengal and porphyrin derivatives. But many photosensitizers, such as acridine, absorb in the near UV range. The naphthalene derivatives, e.g. 3,3'-(4-methyl-1,3-naphthyl) dipropionic acid (MNDP) **6**,[47] sodium 3-(4-methyl-1-naphthyl)-propionate (MNP) **7**[48,49] or disodium 3,3'-(naphthalene-1,4-diyl)-dipropionate (NDP) **8**,[50-52] do not absorb in the visible range but they are not ideally suited for the trapping of singlet oxygen too. Actually, they react slowly with singlet oxygen and the produced endoperoxides are unstable at room temperature and give back the initial naphthalene derivative and oxygen, a part of which being in the singlet state. They are used as "secondary" chemical sources of $^1O_2$ for biological systems rather than as traps.[53]

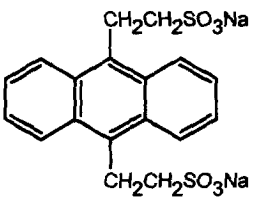
Therefore, we undertook the synthesis of a new water-soluble trap showing, besides the usual properties, a transparency in the visible and the near UV part of the spectra. This compound, designed on the basis of a cyclohexadienic core, is the sodium-1,3-cyclohexadiene-1,4-diethanoate (CHDDE) **13**. In this paper, we report its synthesis, a kinetic study of its reaction with singlet oxygen and the structures and the stabilities of the oxidation products.



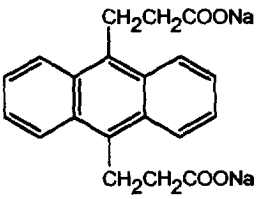
1 , DIMF



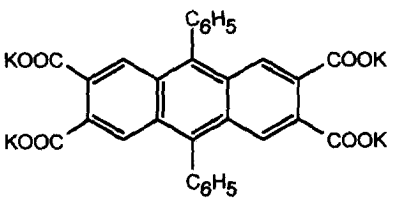
2 , RTC



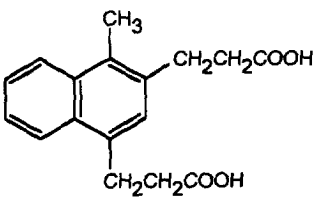
3 , AES



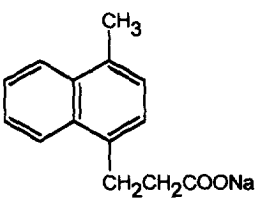
4 , ADP



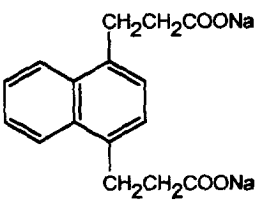
5 , DPATC



6 , MNDP



7, MNP



8 , NDP

Fig. 1. Structure of the main water-soluble chemical traps used for the detection of singlet oxygen in aqueous systems.

Results and Discussion

The most characteristic reaction of singlet oxygen ($^1O_2$, $^1\Delta_g$) is the [4+2] cycloaddition on 1,3-dienes giving a specific endoperoxide. When the diene is cyclic, the endoperoxide is quite stable and can be easily detected by NMR or HPLC. In order to minimize the absorption of UV/visible light by the trap, it is better to

resort to a cyclohexadienic moiety, the absorption maximum of which lies around 270 nm whereas the naphthalenes, the anthracenes and the tetracenes absorb at higher wavelengths (respectively 330 nm, 420 nm and 620 nm) (figure 2).

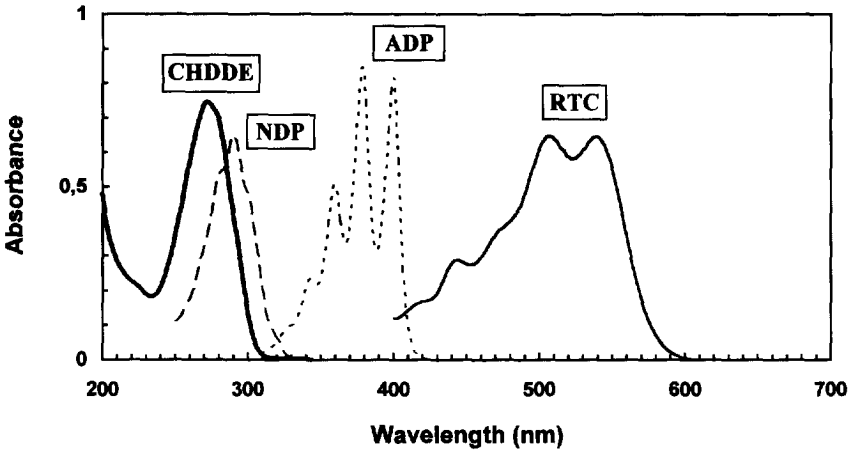


Fig. 2. UV/visible absorption spectra of RTC **2**, ADP **4**, NDP **8** and CHDDE **13** (10^{-4} M) in aqueous solution.

Moreover, the cyclohexadienic core must be substituted in positions 1 and 4 in order to stabilize the endoperoxide and to enhance reactivity toward singlet oxygen. A high water-solubility can be obtained by grafting two sodium carboxylate fonctions which do not interfere with singlet oxygen and which are resistant to usual oxidants. Finally, we preferred to keep the plane of symmetry of the cyclohexadiene core in order to facilitate the structural determination of the products and to avoid a surfactant behavior of the trap in aqueous solution. This led us to select sodium 1,3-cyclohexadiene-1,4-diethanoate (CHDDE, **13**) as an appropriate candidate (figure 3).

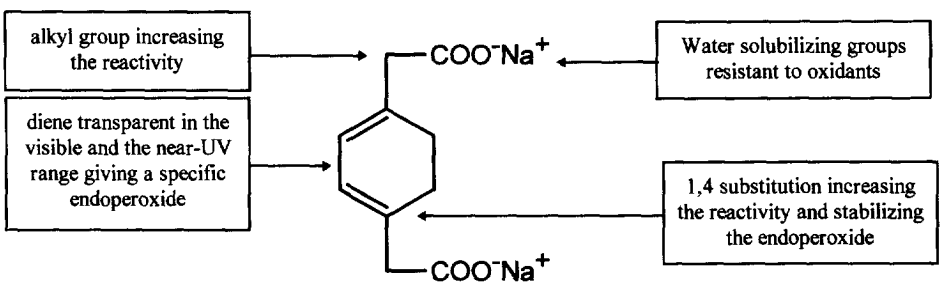
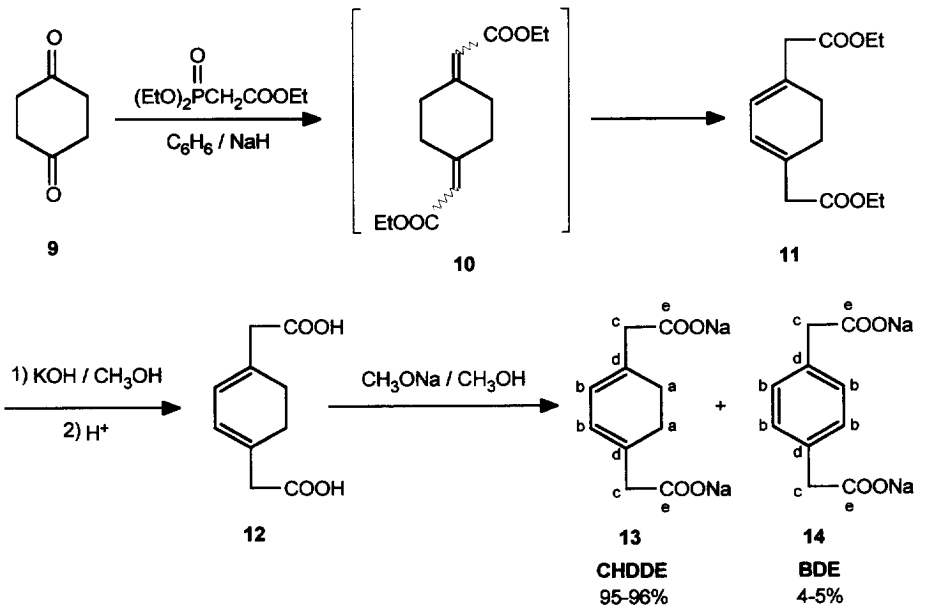


Fig. 3. Main features of sodium 1,3-cyclohexadiene-1,4-diethanoate (CHDDE) **13**.

1. Synthesis

1,3-Cyclohexadiene-1,4-diethylethanoate **11** was prepared following the method of Engel *et al.*[54] by a double Wittig reaction of triethyl phosphonoacetate on 1,4-cyclohexanedione **9**. Under the experimental conditions, the expected diadduct **10** was isomerized into **11**. Saponification and addition of an excess of acid yielded the diacid **12** which was dried and neutralized with two equivalents of sodium methoxide leading to anhydrous sodium 1,3-cyclohexadiene-1,4-diethanoate (CHDDE) **13** (scheme 1).



scheme 1

During this preparation, a small amount (4-5 %) of sodium 1,4-benzenediethanoate (BDE) **14** was formed. Since this compound does not react with $^1O_2$, we did not try to remove it because it might be used as an internal standard for the trapping of singlet oxygen by CHDDE. This compound is highly soluble in basic and neutral (pH ≥ 6) aqueous medium and in methanol (> 1 M) but insoluble in apolar organic solvents. On the other hand, its solubility is low in acidic water (2.10^{-2} M at pH 2) because of the predominance of the free acid form **12**. CHDDE does not absorb above 310 nm but shows a well-defined absorption maximum at 270 nm (ε = 7340 M^{-1}. cm^{-1}) characteristic of its cyclohexadienic structure (figure 2). The ^1H and ^{13}C NMR spectra of this compound are very simple according to the high symmetry of this molecule.

2. Kinetics of the interaction between CHDDE and singlet oxygen

When singlet oxygen is generated in an aqueous solution of CHDDE, its disappearance may occur through three main pathways (eq. 1 to 3).[56] The first one is the deactivation by the molecules of solvent, the electronic excitation energy of $^1O_2$ being transferred to the vibrational-rotational levels of water (eq. 1). The corresponding rate constant k_d was accurately determined by flash photolysis and was found to be 22.7×10^4 s^{-1} in H_2O (τ_Δ = 4.4 μs) and 1.5×10^4 s^{-1} in D_2O (τ_Δ = 67 μs).[57] The two other reactions are the physical and chemical quenchings of singlet oxygen by CHDDE. In the first case, CHDDE is left unchanged but $^1O_2$ is deactivated into $^3O_2$ (eq. 2) whereas in the second case, an irreversible oxidation of CHDDE occurs (eq. 3).

quenching by solvent	$^1O_2 + \text{solvent} \xrightarrow{k_d} {}^3O_2$	(1)
physical quenching by CHDDE	$^1O_2 + \text{CHDDE} \xrightarrow{k_q} {}^3O_2 + \text{CHDDE}$	(2)
chemical reaction of CHDDE	$^1O_2 + \text{CHDDE} \xrightarrow{k_r} \text{CHDDEO}_2$	(3)
chemical reaction of RTC	$^1O_2 + \text{RTC} \xrightarrow{k_r'} \text{RTCO}_2$	(4)

The overall rate constant $k_r + k_q$ had previously been measured by flash photolysis by monitoring the time-resolved decay of singlet oxygen luminescence at 1270 nm in aqueous solutions (D_2O) containing increasing concentrations of CHDDE. This method led to a value of 2.6×10^7 $M^{-1}.s^{-1}$.[58] The measurement of k_r was more difficult since one needed to know the concentration of the oxidation product. We determined this rate constant by comparison with another trap of known reactivity, the tetrapotassium rubrene-2,3,8,9-tetracarboxylate (RTC). This red compound is highly reactive toward singlet oxygen and can act itself as a photosensitizer.[59] Air saturated aqueous (D_2O) solutions of RTC, alone or with some CHDDE, were irradiated in a sealed cell, filled to the top, until no more evolution of the concentration of RTC could be detected from its absorption at 520 nm. Under these conditions, the singlet oxygen formed chemically reacted either with CHDDE (eq. 3) or with RTC (eq. 4). The rates of disappearance of these two traps depend on their relative reactivities and on their concentrations. They can be expressed by the relations (5) and (6):[56]

$$\frac{d[\text{CHDDE}]}{dt} = - k_r [\text{CHDDE}] [^1O_2] \quad (5)$$

$$\frac{d[\text{RTC}]}{dt} = - k_r' [\text{RTC}] [^1O_2] \quad (6)$$

By elimination of the concentration of singlet oxygen between the two equations, we obtain the differential equation (7) which, after integration, leads to the relation (8):

$$k_r' \times \frac{d[CHDDE]}{[CHDDE]} = k_r \times \frac{d[RTC]}{[RTC]} \quad (7)$$

$$k_r' \times Ln\frac{[CHDDE]_o}{[CHDDE]_\infty} = k_r \times Ln\frac{[RTC]_o}{[RTC]_\infty} \quad (8)$$

where the subscripts $_o$ and $_\infty$ refer respectively to the initial and to the final concentrations of the traps CHDDE and RTC when all dissolved oxygen is consumed. This technique avoids measuring the remaining concentration of CHDDE since it may be calculated from the relation (9) which indicates that all oxygen, initially present, has been trapped either by RTC or by CHDDE.

$$[^1O_2]_{aq} = ([RTC]_o - [RTC]_\infty) + ([CHDDE]_o - [CHDDE]_\infty) \quad (9)$$

Practically, we first performed an experiment using RTC alone in order to measure precisely the concentration of dissolved oxygen which was equal to the concentration of RTC having disappeared. Then, we did again the same experiment using variable concentrations of CHDDE in order to infer the ratio k_r' / k_r. The chemical rate constant of RTC , k_r', is equal to $(1.5 \pm 0.3) \times 10^8$ $M^{-1}. s^{-1}$. It is a reliable value since it has been determined by several different techniques.[6,59] From this data, the rate constant of the chemical quenching by CHDDE, k_r, was estimated to $(2.6 \pm 0.3) \times 10^7$ $M^{-1}. s^{-1}$. This value is equal to the overall rate constant $k_r + k_q$ determined by flash photolysis.[58] This result means that, at least in water, CHDDE interacts with singlet oxygen by a pure chemical process ($k_r >> k_q$). A similar conclusion had been drawn by Matusch and Schmidt[60,61] for another cyclohexadienic derivative, α-terpinene.

3.Preparative oxidation of CHDDE by singlet oxygen

CHDDE was oxidized by singlet oxygen photogenerated by irradiation (sodium lamp) of an aqueous (D_2O) solution containing rose bengal and maintained at 5 °C. The disappearance of the trap and the appearance of the oxidation products were followed by HPLC coupled to a UV-detector locked at 200 nm. Contrary to what was expected, two oxidation products ("per 1" and "per 2") were detected by HPLC (figure 4).

In order to ensure that singlet oxygen was really the species involved in the process, we carried out the photooxidation in deuterated water. Actually, on account of the exceptional deuterium effect on the lifetime of $^1O_2$ in water (67 μs in D_2O compared with 4.4 μs in H_2O), the rate of an oxidation proceeding *via* singlet oxygen should increase when D_2O is used instead of H_2O. We actually observed such a phenomenon since the photooxidation of CHDDE 10^{-4} M occured ten times faster in D_2O than in H_2O. In order to check that both oxidation products arose from a singlet oxygen reaction, CHDDE was chemically oxidized by the system H_2O_2 / MoO_4^{2-} which is known to be a very efficient chemical source of singlet oxygen.[2,6] In this case too, we

observed the formation of "per 1" and "per 2", in the same ratio that those obtained by photooxygenation. This result confirmed that both products were characteristic of the reaction of CHDDE with singlet oxygen. The structural identification of the oxidation products was realized by using ^{1}H NMR in one and two dimensions.

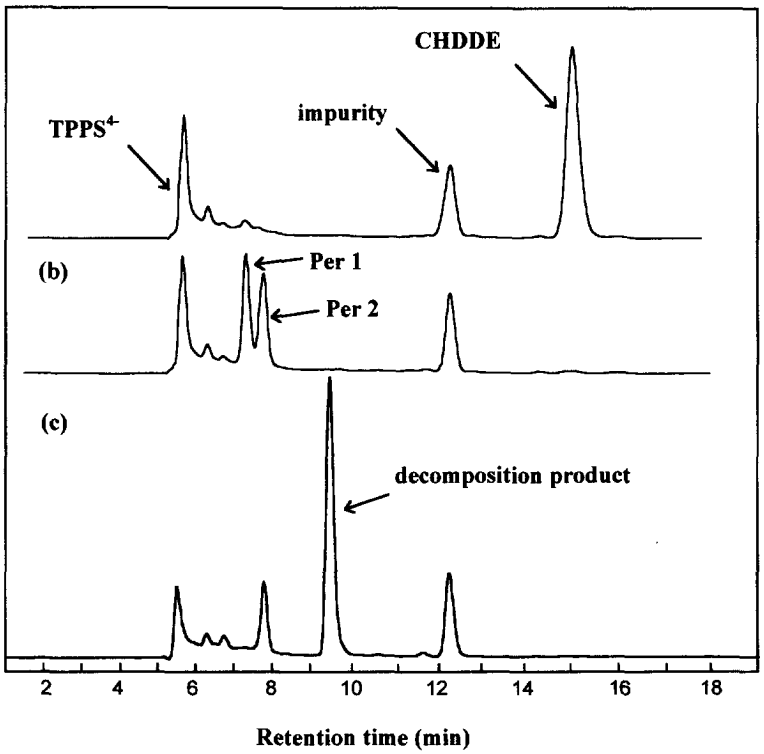
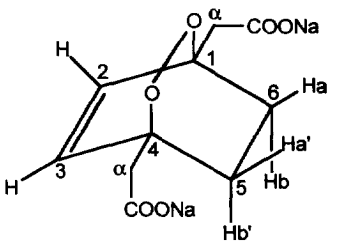


Fig. 4. HPLC chromatograms: (a) CHDDE (10^{-2} M) with its impurity (BDE), (b) products obtained by photooxidation, (c) photooxidized reaction medium after incubation (one night at 37 °C).

Integration values in the ^{1}H one dimensional NMR spectrum showed that one of the products was highly prevalent (88 %). It was identified as the expected endoperoxide $CHDDEO_2$ **15** (scheme 2).

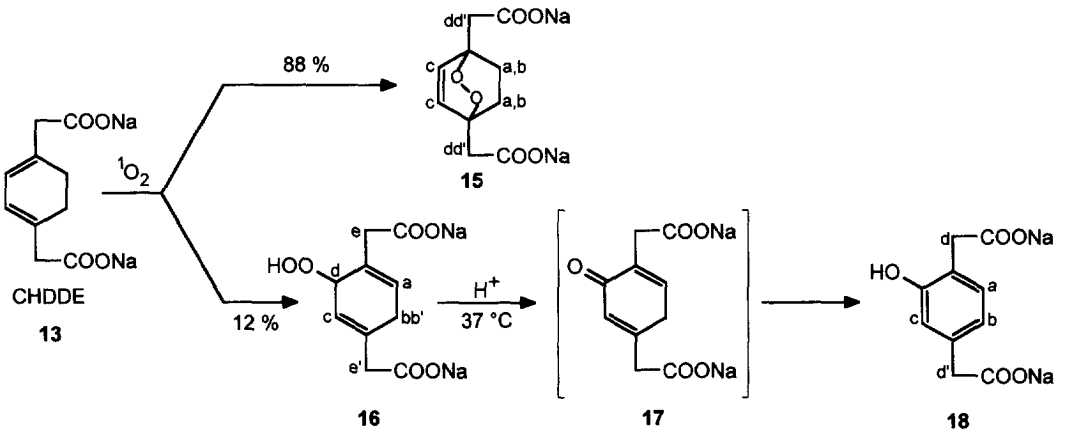


CHDDEO$_2$, 15

scheme 2

The identification of the other peroxide was less straightforward because this secondary product was present in a low ratio (12 %) and its separation was difficult owing to its thermal instability. Therefore, we determined its structure from the untreated reaction medium by resorting to the two dimensional ^{1}H-^{1}H correlation NMR spectrum. This compound was identified as an hydroperoxide (**16**).

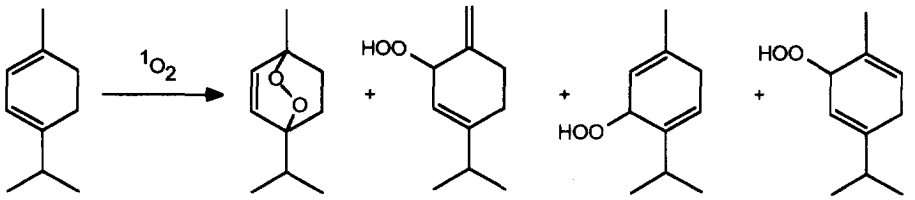
Therefore, the peroxidation of CHDDE by singlet oxygen occured *via* two competitive pathways. The main one was a [4+2] cycloaddition of $^1O_2$ on the diene moeity leading to the endoperoxide **15** whereas the secondary reaction was an ene-reaction (Schenck reaction) which consisted in an addition of singlet oxygen on a single double bond giving the hydroperoxide **16** with concomitant migration of the double bond (scheme 3).



scheme 3

The minor product **16**, present at 12 %, has no more the plane symmetry of the initial diene **13** whereas the endoperoxide **15** has retained it. The hydroperoxide **16** was easily detected by HPLC since it absorbs UV radiation at 200 nm much more efficiently than the endoperoxide. Finally, both oxidation products were

produced at the same time and exhibited a ratio characteristic of singlet oxygen. We could also notice that the initial impurity (sodium 1,4-benzenediethanoate **14**) was not altered by the photooxidation and might be used as an internal standard. Actually, a few years ago, Matusch and Schmidt[60,61] had shown that both [4+2] cycloaddition and ene-reaction could compete during the photoperoxidation of other cyclic 1,3-dienes such as α-terpinene **19** (scheme 4).



19 **20** 90 % **21** 0.5 % **22** 2.2 % **23** 2.2 %

scheme 4.

They had observed the formation of four different products (one endoperoxide and three hydroperoxides). This result is qualitatively in agreement with those obtained with CHDDE. In our case, the isomers **22** and **23** reduced to only one hydroperoxide **16** on account of the symmetry of our molecule. Moreover, as for α-terpinene, the endoperoxide was the main oxidation product (88 % of **15** compared with 90 % of ascaridol **20**). However, the lack of sensitivity of our analytical techniques (NMR and HPLC) did not allow us to identify the analogous of the minor hydroperoxide **21** (0.5 %). Finally, the hydroperoxides **22** and **23** were reported to decompose thermically by giving the 1-methyl-4-isopropyl benzene. In our case, the hydroperoxide **16** should have given compound **14**. This was not the case since **14** was already present as impurity before photooxidation and thermolysis of **16** clearly showed the appearance of a distinct peak on the chromatogram.

4. Stability of the oxidation products

One of our aims was to establish whether the oxidation products of CHDDE were sufficiently stable under biological conditions (pH= 7, 37 °C, water) to be detected using HPLC. When an aqueous solution of the oxidized products was incubated at 37 °C, the NMR spectrum showed the disappearance of the hydroperoxide **16** whereas the endoperoxide **15** was kept unchanged. In the same manner, after one night of heating, we could see by HPLC that the peak having the shortest retention time had disappeared and another peak having a much higher absorption at 200 nm had appeared, whereas the second oxidation peak was left unchanged (figure 4). This transformation occured according to a first order kinetic with a half time equal to

5 h. The structure of the decomposition product was established by NMR analysis of the untreated photooxidized reaction medium heated overnight at 37 °C.

A rough analysis of the NMR spectrum showed that both the impurity **14** and the endoperoxide **15** had not been affected by the heating, they were still present in the same ratio. On the other hand, we no more observed the peak corresponding to the hydroperoxide **16** but a new compound had appeared. This was equally accounted by the HPLC chromatograms (figure 4) for which the peak appearing at t = 7.2 min was no more observed whereas a new peak at t = 9.5 min was clearly distinguished. This allowed us to assign the so-called "per 1" to the hydroperoxide **16** and "per 2" to the endoperoxide **15**.

Finally, the ^1H one dimensional NMR spectrum led us to assign to the decomposition product the structure of sodium 2-hydroxy-1,4-benzenediethanoate **18**. In addition, the high UV absorption at 200 nm was in agreement with the phenolic structure of the product. Actually, an acid catalyzed dehydration of the allylic hydroperoxide **16** might proceed on thermolysis and lead to the cyclohexadienone **17**, which would rearrange into its more stable phenolic mesomer **18** (scheme 3).

Conclusion

The synthesis of sodium 1,3-cyclohexadiene-1,4-diethanoate (CHDDE) **13** was simple and allowed a straightforward access to a water-soluble cyclohexadienic trap of $^1O_2$ accompanied by minute amount of sodium 1,4-benzenediethanoate (BDE) which was not altered by the photooxidation and which might be used as an internal standard in the HPLC analysis. The photooxidation led to two well-defined products, highly specific of singlet oxygen: a major one (88 %), the endoperoxide $CHDDEO_2$ **15** and a minor one (12 %), the hydroperoxide **16** which decomposed into sodium 2-hydroxy-1,4-benzenediethanoate **18** on thermolysis at 37 °C. In any case, the secondary product did not prevent the use of CHDDE as a specific trap of singlet oxygen, usable for the measurement of this species in aqueous systems. Moreover, CHDDE is highly water-soluble in neutral and basic medium, it rapidly reacts with $^1O_2$ ($k_r = 2.6 \times 10^7\ M^{-1}.\ s^{-1}$) and is particularly well-adapted for the measurement of singlet oxygen photogenerated from water-soluble sensitizers because of its transparency in the visible and near UV range of the spectrum.

Experimental Section

Reagents:

1,4-cyclohexanedione (98 %), triethyl phosphonoacetate (99 %), 1,4-phenylenediethanoic acid (97 %), benzene (99 %), sodium hydride (60 %), sodium methoxide (97.5 %) and sodium molybdate (VI) dihydrate ($Na_2MoO_4.2H_2O$) were purchased from Aldrich-Chemie. Stabilizer-free hydrogen peroxide (30 %, Merck,

Perhydrol Suprapur), potassium hydroxide (Rectapur) and methanol (R.P. Normapur) were from Prolabo. The dye sensitizer, rose Bengal (93 %), was purchased from Janssen. The photooxidations were carried out into deuteriated solvents: D_2O (99.8 %) and CD_3OD (99.8 %) respectively from Aldrich-Chemie and CEA. The aqueous solutions were buffered at pH 7 with phosphate buffer (Titrisol) from Merck.

Synthesis:

Tetrapotassium rubrene-2,3,8,9-tetracarboxylate (RTC),[37] disodium 3,3'-(anthracene-9,10-diyl)-dipropionate (ADP)[42] and disodium 3,3'-(naphthalene-1,4-diyl)-dipropionate (NDP)[51] were prepared according to known procedures.

Sodium 1,3-cyclohexadiene-1,4-diethanoate **13** (CHDDE) was prepared from 1,4-cyclohexanedione **9** (scheme 1). The first step, consisting in a Wittig reaction followed by an isomerization, led to the 1,3-cyclohexadiene-1,4-diethylethanoate **11** according to the procedure given by Engel *et al.*.[54] Then, 1 g (4 mmol) of this diester was treated for one hour with a solution of potassium hydroxide (50 mmol) in methanol (50 ml). Removal of the solvent provided a white solid which was redissolved into H_2O (100 ml) and acidified with H_3PO_4 (pH ≈ 2). After three extractions with ether, drying on $MgSO_4$ and evaporation of the solvent, the 1,3-cyclohexadiene-1,4-diethanoic acid **12** was quantitatively obtained. Then, 3.77 g (20 mmol) of **12** was dissolved into THF and cooled with an ice-bath. 10 ml of a solution of CH_3ONa (40 mmol) in methanol was added drop by drop. After evaporation *in vacuo* of the solvents, the residue was triturated three times with ether yielding quantitatively the sodium 1,3-cyclohexadiene-1,4-diethanoate **13** (CHDDE) as a white powder.

CHDDE **13**: ^1H NMR (D_2O, 300 MHz) 2.15 (4H, s, a), 2.95 (4H, s, c), 5.71 (2H, s, b); ^{13}C NMR (D_2O, 75 MHz) 29.40 (a), 48.92 (c), 124.01 (b), 136 (d).

BDE **14**: ^1H NMR (D_2O, 300 MHz) 3.49 (4H, s, c), 7.20 (4H, s, b); ^{13}C NMR (D_2O, 75 MHz) 47.02 (c), 132.19 (b), 138.1 (d), 183.89 (e).

Instrumentation:

High Performance Liquid Chromatography (HPLC) analysis were carried out with a reversed-phase column (Spherisorb RP 18-5ODS) using a Gilson model 303 pump, a mixture of CH_3OH 40/ H_2O 60/ H_3PO_4 0.2 as eluent and a UV detection at 200 nm with an Holochrom H/MD Gilson detector.

NMR spectra were recorded in D_2O on a Bruker AC 300 FT-spectrometer (Laboratoire d'Applications RMN-Université de Lille II) (300 MHz for ^1H, 75.46 MHz for ^{13}C) at 278 K. All chemical shifts were referenced with respect to $TSPd_4$ signal at $\delta = 0$ ppm. One dimensional spectra were obtained by operating in the pulse Fourier-transform mode with quadripole detection. In ^1H NMR spectra, the water signal was suppressed by applying a gate secondary irradiation field at the water resonance frequency during the relaxation delay. ^{13}C spectra were recorded with complete decoupling of protons. Two dimensional ^1H-^1H correlation spectra (COSY 45) were obtained at 300 MHz with the following pulse sequence (D-90°-t_1-45°-acquisition). Solvent saturation was achieved using irradiation at the water resonance during the relaxation delay D = 1 s. The period t_1 was incremented to allow exchange of magnetization of spin-spin coupled protons. Data were collected into 2048 data points in F_2 using 128 scans per increment with a spectral width of 2941 Hz and 256

increments of F_1. The FID was weighted using a sinebell function in t_1 and t_2 prior to Fourier transformation and magnetude spectrum calculation.

UV/visible spectroscopic analysis were performed with a Milton Roy Spectronic 3000 spectrophotometer equipped with a diode array photodetector.

Continuous irradiations were performed at 5 °C under oxygen bubbling using either a 150 W high pressure sodium lamp (Philipps Son T) for preparative photooxidation or the 365 nm line of a 150 W high pressure Hg-Xe lamp (ORIEL) selected with two band pass filters (CORNING 7.51 and SCHOTT SFK 19) for kinetic measurements.

Flash photolysis experiments were conducted in air saturated D_2O using sodium tetraphenylporphinesulfonate ($TPPS^{4-}$) as photosensitizer. A short (6 ns) flash of light at 532 nm was emitted by a Nd-Yag laser. Infra-red phosphorescence of singlet oxygen at 1270 nm was detected perpendicularly with a Ge photodiode (JUDSON J16) and the signal was recorded with an oscilloscope (TEKTRONIX 556) and analyzed according to a first order decay. The apparatus was described in detail before.[55]

Preparative photooxidation of CHDDE:

A solution of CHDDE **13** (12 mg) in water or in methanol (5 ml) containing rose bengal (2.10^{-5} M) was irradiated at 5 °C with a sodium lamp (515 nm < λ < 650 nm), maintaining a continuous bubbling of oxygen. In H_2O, the pH was maintained at 7.0 by using directly 5 ml of phosphate buffer (Titrisol) as solvent whereas in D_2O, the same pD was obtained by addition of solid $NaH_2PO_4.H_2O$ (0.05 M) and NaOH (0.03 M) to D_2O. The evolution of the reaction medium was followed by HPLC at 200 nm without dilution. The photooxidation was completed within 40 min in H_2O, 15 min in D_2O, 45 min in CH_3OH and 30 min in CD_3OD.

Sodium 2,2'-(1,4-epidioxy-2-cyclohexen-1,4-yl) diethanoate **15**: ^1H NMR (D_2O, 300 MHz) 1.72 (2H, dd, J = 16 and 6 Hz, b), 2.06 (2H, dd, J = 16 and 6 Hz, a), 2.50 (2H, d, J = 14 Hz, d), 2.57 (2H, d, J = 14 Hz, d'), 6.68 (2H, s, c).

Sodium 2,2'-(hydroperoxide-2,5-cyclohexadien-3,6-yl) diethanoate **16**: ^1H NMR (D_2O, 300 MHz) 2.72 (2H, m, e'), 2.97 (1H, m, b), 3.02 (2H, m, e), 3.16 (1H, m, b'), 4.89 (1H, m, d), 5.72 (1H, d, J = 3.5 Hz, c), 5.99 (1H, t, J = 3.3 Hz, a).

Chemical oxidation of CHDDE by MoO_4^{2-} / H_2O_2:

20 µl of hydrogen peroxide 30 % (2.10^{-4} mole) was added to an aqueous solution (10 ml) of CHDDE (24 mg, 10^{-4} mole) containing sodium molybdate (12 mg, 5.10^{-5} mole) and carbonate buffer (10^{-3} mole). The reaction, followed by HPLC, was completed within 15 min.

Measurement of the rate constant k_r of CHDDE:

An aqueous solution (D_2O) of RTC (6×10^{-4} M), saturated with air, is introduced into a sealed cell, filled to the top. The solution is irradiated at 5 °C with a sodium lamp until no more disappearance of RTC is observed by spectrophotometry at 520 nm. This experiment is then carried out again by adding various concentrations of CHDDE and the disappearance of RTC is measured.

Thermolysis of the oxidation products of CHDDE:

The photooxidized reaction medium was heated at 37 °C during several hours. The reaction was followed by HPLC under the usual conditions.

Sodium 2-hydroxy-1,4-benzenediethanoate **18**: ^{1}H NMR (D_2O, 300 MHz) 3.45 (2H, s, d'), 3.49 (2H, s, d), 6.78 (1H, d, J = 1.9 Hz, c), 6.79 (1H, dd, J = 8.1 and 1.9 Hz, b), 7.09 (1H, d, J = 8.1 Hz, a).

References

1. Matsumoto, M. *Singlet Oxygen*, Frimer, A. A. Ed. CRC Press, Boca Raton, Fl. **1985,** *2*, 205.
2. Aubry J. M. *J. Am. Chem. Soc.* **1985**, *107*, 5844-5849.
3. Murray, R. W. *Singlet Oxygen*, Wasserman, H. H.; Murray, R. W. Eds., Academic Press: New York, 1979, pp 59-114.
4. Barton, D. H. R.; Magnus P. D.; Quinney J. C.; *J. Chem. Soc. Perkin Trans.* **1975**, *1*, 1610-1614.
5. Sugimoto, H.; Sawyer, D. T. *J. Am. Chem. Soc.* **1984**, *106*, 4283-4285.
6. Aubry J. M.; Cazin B. *Inorg. Chem.* **1988**, *27*, 2013.
7. Zepp, R. G.; Wolfe, N. L.; Baughman, G. L.; Hollis, R. C. *Nature*, **1977**, *267*, 421-423.
8. Garcia, N. A. *J. Photochem. Photobiol. B: Biol.* **1994**, *22*, 185-196.
9. Khan, A. U. *J. Am. Chem. Soc.* **1983**, *105*, 7195.
10. Kanofsky, J. R. *J. Biol. Chem.* **1983**, *258*, 5991.
11. Kanofsky, J. R. *J. Photochem.* **1984**, *25*, 105.
12. Kanofsky, J. R.; Wright, J.; Miles-Richardson, G. E.; Tauber, A. I. *J. Clin. Invest.* **1984**, *74*, 1489.
13. Gallo, U.; Santamaria, L. *'Research Progress in Organic Biological and Medicinal Chemistry'*, Am. Elsevier, New York, 1972, *3*.
14. Spikes, J. D.; Livingston, R. *Adv. Radiat. Biol.* **1969**, *3*, 29.
15. Spikes, J.; Macknight, M. L. *Ann. N. Y. Acad. Sci.* **1970**, *171*, 149.
16. Foote, C. S. *Free Radicals in Biology*, Pryor, W. A. Ed. Academic Press, New York, 1979, *1*.
17. Gross Weiner, L. I. *Curr. Top. Radiat. Res. Q.* **1976**, *11*, 141.
18. Gorman, A. A.; Rodgers, M. A. J. *J. Photochem. Photobiol. B: Biol.* **1992**, *14*, 159-176.
19. Foote, C. S. *Free Radicals in Biology*, Pryor, W. A. Ed. Academic Press, New York, 1976, *2*, 85.
20. Straight, R. C.; Spikes, J. D. *Singlet Oxygen*, Frimer, A. A. Ed. CRC Press, Boca Raton, Fl, 1985, *4*, pp 91-143.
21. Weishaupf, K. R.; Gomer, C. J.; Dougherty, T. J. *Cancer Res.* **1976**, *36*, 2326-2329.
22. Photodynamic Therapy of Neoplastic Disease, Kessel, D. Ed., CRC Press, Boston, 1990.
23. North, J.; Neyndorff, H.; Levy, J. G. J. *Photochem. Photobiol. B.* **1993**, *17*, 99-108.
24. Smith, L.L.; Kulig, M. J. *J. Am. Chem. Soc.* **1976**, *98*, 1027.
25. Smith, L. L.; Shoud, J. P. *Photochem. Photobiol.* **1978**, *28*, 479-485.

26. Smith, L. L.; Teng, J. I.; Kulig, M. J.; Hill, F. L. *J. Org. Chem.* **1973**, *38*, 1763.
27. Korytowski, W; Bachowski, G. J.; Girotti, A. W. Photochem. Photobiol. **1992**, *1*, 1-8.
28. Foote, C. S.; Shook, F. C.; Abakerli, R. B. *Methods in enzymology*, **1984**, *105*, 36-47.
29. Foote, C. S.; Shook, F. C.; Abakerli, R. B. *J. Am. Chem. Soc.*, **1980**, *102*, 2503.
30. Held, A. M.; Halko, D. J; Hurst, J. K. *J. Am. Chem. Soc.* **1978**, *100*, 5732.
31. Usui, Y.; Kamogawa, K. *Photochem. Photobiol.* **1974**, *19*, 245-247.
32. Foote, C. S. *Biochemical and Clinical Aspects of Oxygen*, Caughey, W. S. Ed. Academic Press, New York, 1979, pp 603-632.
33. Graf, G. A.; Braun, A. M.; Faure, J. *Chimia*, **1980**, *34*, 234-236.
34. Maurette, M. T.; Oliveros, E.; Infelta, P. P.; Ramsteiner, K.; Braun, A. M. *Helv. Chim. Acta*, **1983**, *66*, 722-723.
35. Murasecco, P.; Oliveros, E.; Braun, A. M.; Monnier, P. *Photochemistry and Photobiophysics*, **1985**, *9*, 193-201.
36. Giraud, M.; Valla, A.; Bazin, M.; Santus, R.; Momzikoff, A. *J. Chem. Soc. Chem. Comm.* **1982**, 1147-1148.
37. Aubry, J. M.; Rigaudy, J.; Cuong, N. K. *Photochem. Photobiol.* **1981**, *33*, 149-153.
38. Schaap, A. P.; Thayer, A. L.; Faler, G. R.; Goda, K.; Kimura, T. *J. Am. Chem. Soc.* **1974**, *96*, 4025.
39. Aubry, J. M.; Rigaudy, J.; Ferradini, G.; Pucheault, J. *J. Am. Chem. Soc.* **1971**, *103*, 4965.
40. Botsivali, M.; Evans, D. F. *J. Chem. Soc. Chem. Comm.* **1979**, 1114-1116.
41. Evans, D. F.; Upton, M. W. *J. Chem. Soc. Dalton-Trans.* **1985**, 2525.
42. Linding, B. A.; Rodgers, M. A. J.; Schaap, A. P. *J. Am. Chem. Soc.* **1980**, *102*, 5590-5593.
43. Schaap, A. P.; Thayer, A. L.; Zaklika, K. A.; Valenti, P. C. *Ibid.* **1979**, *101*, 4016.
44. Rigaudy, J.; Guillaume, J.; Cuong, N. K. *C.R. Acad. Sci. Paris*, **1964**, *259*, 4729.
45. Rigaudy, J.; Ricard, M. *Tetrahedron*, **1968**, *24*, 3241.
46. Schmitz, C.; Aubry, J. M.; Rigaudy, J. *Tetrahedron*, **1982**, *38*, 1425-1430.
47. Müller, K.; Ziereis, K. *Arch. Pharm.* **1993**, *326*, 369-371.
48. Saito, I.; Matsuura, I.; Inoue, K. *J. Am. Chem. Soc.* **1981**, *103*, 188.
49. Saito, I.; Matsuura, I.; Inoue, K. *J. Am. Chem. Soc.* **1983**, *105*, 3200.
50. Nieuwint, A. W. M.; Aubry, J. M.; Arwert, F.; Kortbeek, U.; Herzberg, S.; Joenje, H. *Free Radical Res. Commun.* **1985**, *1*, 1.
51. Aubry, J. M.; Cazin, B.; Duprat, F. *J. Org. Chem.* **1989**, *54*, 726.
52. Marcel, C. S.; Wilson, B. D. *J. Org. Chem.* **1958**, *23*, 1483-1488.
53. Aubry, J. M. New Chemical Sources of Singlet Oxygen. *Menbrane Lipid Oxidation*, Vol. II, Vigo-Pelfrey, Ed. CRC Press, Boca Raton, 1991, pp 65-102.
54. Engel, P. S.; Allgren, R. L.; Chae, W-K.; Leckonby R. A.; Marron, N. A. *J. Org. Chem.* **1979**, *44*, 4233.

55. Vever-Bizet, C.; Dellinger, M.; Brault, D.; Rougee, M.; Bensasson, R. V. *Photochem. Photobiol.* **1989**, *50*, 321.

56. Wilkinson, F.; Brummer, J. G. *J. Phys. Chem. Ref. Data*, **1981**, *10*, 809-999.

57. Gormann, A. A.; Rodgers, M. A. J. *Singlet Oxygen in Handbook of Organic Photochemistry, Vol II*; Scaiano, J. C.,Ed. CRC Press, Boca Raton, 1989, pp 229-247.

58. Aubry, J. M.; Mandard-Cazin, B.; Rougee, M.; Bensasson, R. *J. Am. Chem. Soc.* **1995**, *117*, 9159-9164.

59. Aubry, J. M.; Rigaudy, J.; Cuong, N. K. *Photochem. Photobiol.* **1981**, *33*, 155-158.

60. Matusch, R.; Schmidt, G. *Angew. Chem. Int. Ed. Eng.* **1988**, *27*, 717-718.

61. Clennan, E. L. *Tetrahedron*, **1991**, *47*, 1343-1382.

(*Received in Belgium* 11 *July* 1995; *accepted* 11 *November* 1995)