

Cacalol and cacalol acetate as photoproducers of singlet oxygen and as free radical scavengers, evaluated by EPR spectroscopy and TBARS†

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Photodynamic therapy (PDT) is an emerging cancer treatment based on the production of singlet oxygen ($^1\text{O}_2$) upon illumination of a photosensitizer in the presence of oxygen. Antioxidants are primarily reducing agents prone to scavenge reactive species in one way or another. Cacalol (C) and cacalol acetate (CA) were examined and compared regarding to their capacity to produce singlet oxygen and as scavengers of free radicals. Their role as singlet oxygen photoproducers under UV-vis light irradiation was examined by electron paramagnetic resonance (EPR) using 2,2,6,6-tetramethyl-piperidine (TEMP) as spin-trapping material. The quantum yield to produce $^1\text{O}_2$ was found to be 0.4 ± 0.05 for CA and 0.13 ± 0.05 for C. Their properties as scavengers of hydroxyl (OH), nitrogen-centered (2,2-diphenyl-1-picrylhydrazyl radical, DPPH) and organic radicals (R and ROO) were evaluated using EPR and the thiobarbituric reactive substances (TBARS) method. C and CA differed in their abilities to trap DPPH. By contrast, both compounds showed similar activity to trap OH, R and ROO. A relationship between the redox potentials of the compounds and their activity as scavengers of DPPH was observed. The producing/inhibiting properties showed by C and CA make them interesting options for new therapeutic applications to treat tumors and other diseases.

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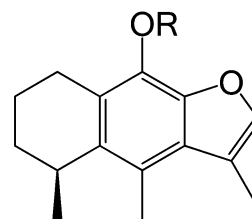
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Introduction

Photodynamic therapy (PDT) is a medical treatment that employs a combination of light and a photosensitizing agent to produce a cytotoxic or modifying effect on cancerous or other unwanted tissues. The initial photochemical processes that lead to cell death may follow two main pathways: upon light irradiation, the molecule (or photosensitizer) transfers the energy to O_2 , yielding $^1\text{O}_2$ (type II mechanism), or, alternatively, it engages in charge-transfer reactions with biomolecules (type I mechanism).^{1,2} Most photosensitizers for PDT are efficient producers of singlet oxygen in simple chemical systems, and it is assumed that, in most circumstances, Type II photochemistry is the dominant mechanism of PDT in cells and tissues.³ Accurately knowing the quantum yields of the production of singlet oxygen by sensitizers in different media is thus essential, not only to quantify the direct attack on biological substrates by them, but also because their products undergo free radical reactions, causing biological damage to other targets.

Cacalol (C) is the main constituent of *Psacalium decompositum* (A. Gray) H. Rob Brettell (Asteraceae), locally called “matarique” roots,⁴ and has attracted considerable attention because of its anti-microbial,⁵ hypoglycemic and anti-inflammatory⁶ activities; also there are reports about its role as electron transport inhibitor at the oxygen evolution level.⁷ The antioxidant properties of this natural compound have been studied on lipid peroxidation⁸ and through the use of several chemical reactions.⁹ Recently, cacalol showed a strong anti-proliferation effect against breast cancer cells, inducing apoptosis by activating a pro-apoptotic pathway.¹⁰ Its derivative compound, cacalol acetate (CA) (see Fig. 1) has shown



R=H Cacalol
R=COCH₃ Cacalol Acetate

Fig. 1 Structure of cacalol (C) and cacalol acetate (CA).

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antifungal⁴ and anti-inflammatory properties;^{6,11} however, its antioxidant activity is not well known yet.

There is a considerable emphasis in the need to find new photosensitizers for PDT. The lack of toxicity and the mutagenic properties of cacalol and its derivatives, make them attractive options for PDT. In addition, several research groups have reported that PDT enhances the activity of certain antioxidant molecules in the presence of different dyes, different tumor models and under visible light irradiation.^{12–14}

On the other hand, when plants are illuminated, their thylakoid membranes or sub-thylakoid fragments induce a complex set of stress reactions due to the generation of reactive oxygen species (ROS) such as singlet oxygen ($^1\text{O}_2$) and superoxide radical anions (O_2^-).¹⁵ These species attack many biological molecules with several consequences, including irreversible cellular damage and cell death. For example, singlet oxygen can oxidize lipids, amino acids and nucleic acids.¹⁶ Superoxide radical anions are relatively unreactive but can alter the low-density lipoprotein (LDL)¹⁷ and, through secondary pathways, produce hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\text{OH}\cdot$), which are more reactive species, better able to initiate the lipid peroxidation chain reaction.¹⁸ With this stimulating background and as part of our research program on the use of cacalol and its derivatives as medicinal compounds, we investigated their abilities to photo-produce singlet oxygen and to scavenge free radicals.

Results and discussion

Photoproduction of $^1\text{O}_2$

In accordance to the photosensitization mechanism proposed by Wilkinson,¹⁹ the quantum yield of $^1\text{O}_2$ formation, $\phi(^1\text{O}_2)$, is defined as the number of molecules of $^1\text{O}_2$ generated for each photon absorbed by the photosensitizer. This measurement is an indication of the relative capability of drugs to generate singlet oxygen. $\phi(^1\text{O}_2)$ was determined from the generation rate of singlet oxygen, $R(^1\text{O}_2)$, and the flux of absorbed photons (I_a): $\phi(^1\text{O}_2) = R(^1\text{O}_2)/I_a$.¹² EPR spectroscopy, using TEMP as spin-trapping material, was employed for determining $R(^1\text{O}_2)$. TEMP reacts selectively with $^1\text{O}_2$, yielding the stable TEMPO adduct.²⁰ I_a was determined to be $1.5 \times 10^{-7} \text{ M s}^{-1}$ under irradiation with $\lambda > 300 \text{ nm}$ (see the ESI,† Section A1 for details on the determination of I_a).

A time-accumulating EPR spectrum was obtained upon irradiating solutions of TEMP containing cacalol and cacalol acetate in air-saturated ethanol. This spectrum is characteristic of TEMPO, consisting of three equally intense lines, where the hyperfine coupling constant (hfcc) a_N was 16.3 G (a_N is the nitrogen hyperfine coupling constant in gauss, G) and the gyromagnetic constant (g) was 2.006 (Fig. 2A).

When samples were degassed by bubbling N_2 for 10 min, the intensity of EPR signal significantly decreased (Fig. 2B), showing that oxygen is indispensable for generating the signal. No TEMPO signal appeared in control experiments: in the TEMP solution with cacalol systems but in dark conditions (Fig. 2C), in the irradiated TEMP solution without cacalol

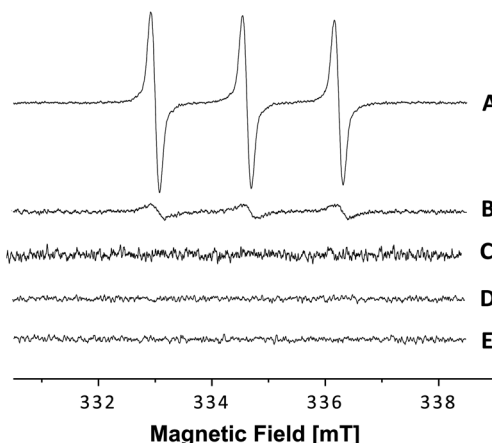


Fig. 2 EPR spectra at room temperature of TEMP from C and CA in ethanol solutions, under several experimental conditions: (A) UV-light in O_2 presence, (B) UV-light with N_2 . Control experiments: (C) TEMP solution with cacalol systems in dark conditions, (D) TEMP solution without C/CA with UV-light, (E) in the absence of TEMP but with C/CA and UV-light.

systems (Fig. 2D) or in the absence of TEMP but with C or CA under irradiation (Fig. 2E).

Fig. 3 shows TEMPO formed as a function of the illumination time for C and CA. During the measurement, the absorbance at $\lambda > 300 \text{ nm}$ of the cacalol samples was adjusted to be the same and concentrations of C and CA were in the range where the incident light is totally absorbed (fraction $I_a/I_0 = 1$). According to the results, $R(^1\text{O}_2)$ was found to be $6 \times 10^{-8} \text{ M s}^{-1}$ for CA and $2 \times 10^{-8} \text{ M s}^{-1}$ for C (steady state, zero-order kinetics conditions), while $\phi(^1\text{O}_2)$ was 0.4 ± 0.05 for CA and 0.13 ± 0.05 for C. Thus, the acetate derivative was approximately four times as fast in the production of $^1\text{O}_2$ species in ethanol solutions when compared to cacalol.

The excitation of C and CA can simultaneously generate O_2^- through an electron transfer reaction (Type I mechanism). To determine the ability of cacalol compounds to produce O_2^- ,

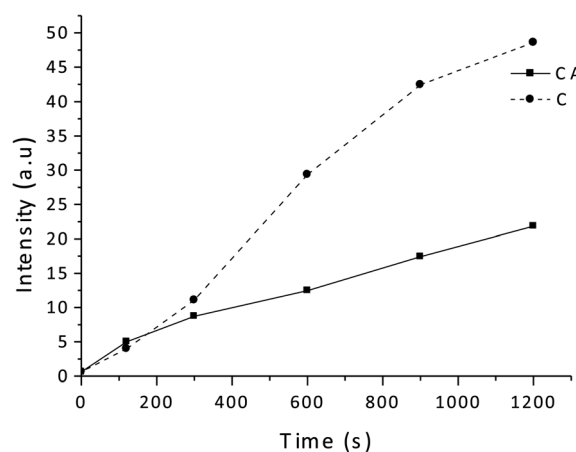


Fig. 3 Formation of TEMPO as a function of irradiation time by using C (1 mM) and CA (1.4 mM) in aerated ethanol solutions, with $\lambda > 300 \text{ nm}$ ($I_0 = 1.5 \times 10^{-7} \pm 0.03 \text{ M s}^{-1}$).

5,5 dimethyl-1-pyrroline-*N*-oxide (DMPO) was employed as spin-trap. The reaction of this spin-trap with $O_2^{\cdot -}$ produces a spin-trapped adduct with a characteristic EPR spectrum.²¹ Thus, when solutions of DMPO with **C** and **CA** in air-saturated DMSO were irradiated with $\lambda > 300$ nm (experimental conditions were similar to those employed in 1O_2 photoproduction), no DMPO- $O_2^{\cdot -}$ adduct was obtained (not shown) and the baseline was observed.

The results from those depicted in Fig. 2 and 3 indicate that singlet oxygen is predominantly formed by the irradiation of cacalol and its derivative, following a type II mechanism. The capacity to produce 1O_2 , coupled with their lack of toxicity and mutagenic properties, make cacalol and cacalol acetate interesting options for PDT.

Antioxidants are primarily reducing agents prone to scavenge reactive oxygen species, counteracting the effect of PDT.¹² Although known for their protective properties, antioxidants can exhibit pro-oxidant activity and a PDT-enhancing effect.¹² With the intention of generating new strategies for antioxidants to be used as practical therapeutic agents in PDT, we considered the possibility of providing evidence to demonstrate that cacalol, which is already known as antioxidant, can exhibit a pro-oxidant activity in the presence of a sensitizer (for example, hematoporphyrin). In the experiments, an ethanolic solution of hematoporphyrin (HP) in the presence of TEMP was irradiated for 20 min with $\lambda > 400$ nm generating the TEMPO-signal, which indicated the photoproduction of 1O_2 (Fig. 4). When **C** was added the intensity of the TEMPO-signal continued to increase significantly, indicating that cacalol behaves as a pro-oxidant rather than as a scavenger of 1O_2 . This effect may enhance the photodamaging activity of PDT, since cacalol acts as photo-producer of reactive oxygen species.

Antioxidant activity

The role of **C** and **CA** as scavengers of free radicals was investigated by EPR spectroscopy, using 5,5 dimethyl-1-pyrroline-*N*-oxide (DMPO) as spin-trap material, which reacts selectively

with $^{\cdot}OH$ ²² and 1,1-diphenyl-2-picrylhydrazyl (DPPH), a nitrogen-centered radical. The antioxidant capacity of cacalol compounds was also assessed using the TBARS test.

In the first instance, the $^{\cdot}OH$ scavenging properties of **C** and **CA** were evaluated; the hydroxyl free radical was formed by means of Fenton reaction, *via* decomposition of H_2O_2 by ferrous iron (reaction 1):¹³



A solution containing ferrous salt, H_2O_2 and DMPO was employed for producing the well characterized 1 : 2 : 2 : 1 pattern of DMPO- $^{\cdot}OH$ adduct (Fig. 5A), with $g = 2.0057$ and hfccs were $a_N = a_H = 14.9$ G (where a_H is the hydrogen hyperfine coupling constant).²⁰ When **C** and **CA** were added to the Fenton reaction, the intensity of the EPR signals was significantly inhibited: 30% for **C** (Fig. 5B) and 27% for **CA** (Fig. 5C). In the absence of DMPO (Fig. 5D) or Fenton reagents (Fig. 5E), no EPR signals are detected in above solution. These results show the $^{\cdot}OH$ -scavenging function of **C** that was previously demonstrated by chemical reactions.⁹ In the case of **CA**, its efficiency to trap

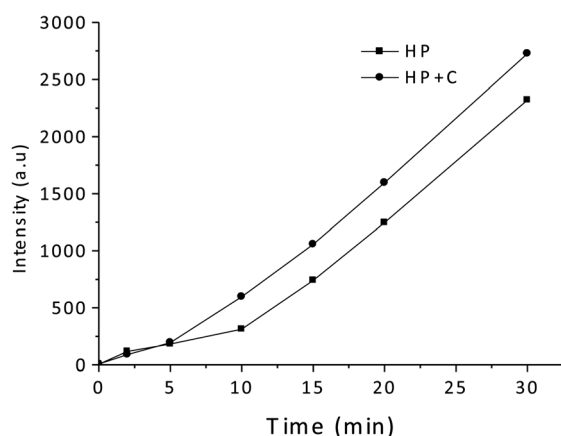


Fig. 4 Photoproduction of TEMPO as a function of irradiation time in aerated ethanol solutions, under visible light irradiation ($\lambda > 400$ nm); HP (54 μM) and HP + **C** when 70 μM of cacalol was added.

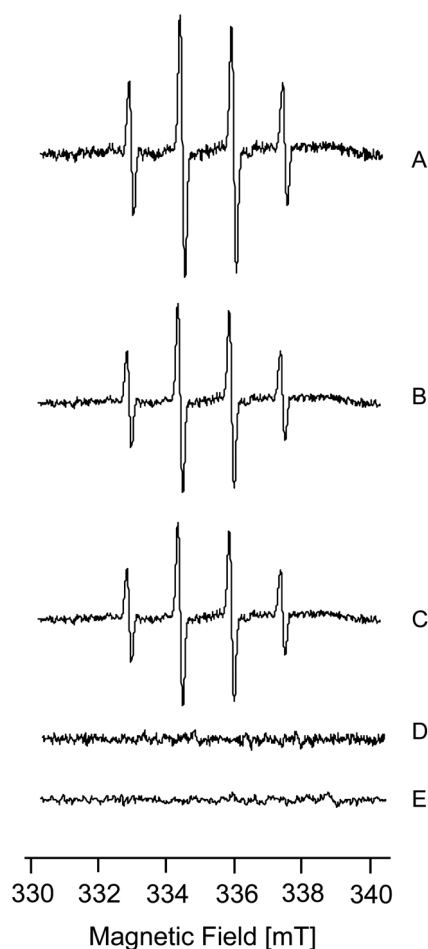


Fig. 5 EPR spectra at room temperature of (A) DMPO- $^{\cdot}OH$ adduct formation by Fenton reaction. (B) Effect of **C** in the formation of DMPO- $^{\cdot}OH$ adduct. (C) Same experimental conditions as B but with **CA**. Control experiments: (D) in absence of DMPO, (E) in absence of $FeSO_4$ and H_2O_2 .

OH was similar to C and was not affected by the acetylation of the cacalol structure.

As a second step, we examined C and CA reactivity and their ability to scavenge nitrogen-centered radicals. For this, DPPH was used, which is characterized by a quintet with $g = 2.0036$ and $a_N = 9$ G (see inset in Fig. 6). This EPR signal was drastically affected in the presence of C, obtaining an exponential decrease with $IC_{50} = 27.88$ μ M (Fig. 6). By contrast, no scavenging activity of DPPH by CA was observed. This behavior was confirmed by the spectrophotometric method (Table 1), where IC_{50} for C was 24.09 μ M (more active than the BHT positive standard with 74.91 μ M), while no trapping activity of DPPH was found for CA. These results indicate the DPPH-scavenging function of cacalol and the effect of acetylation on the ability to trap nitrogen-centered radicals.

We took phenolic compounds as the basis to explain the observed behavior of C as a scavenger of nitrogen-centered radicals. The ability of phenolic compounds to trap DPPH²³ has been explained according to their low redox potentials.²⁴ Cyclic voltammetry measurements for C showed anodic peaks at 1.19 and 1.37 V versus normal hydrogen electrode (NHE), corresponding to the oxidation of the phenolic group –OH, and at 2.34 V vs. NHE, corresponding to the oxidation of the furan ring. C showed a greater ability to trap DPPH, possibly due to its low potential values, close to phenol potentials. In comparison, CA exhibited higher potential values (1.74 and 2.19, V vs. NHE), due to the electron-withdrawing effect of the acetyl group on the structure of cacalol. Furthermore, CA and DPPH are known as bulky groups; they make the approach between them difficult because of steric hindrance, resulting in the incapacity of CA to trap DPPH.

TBARS test

The antioxidant response of the C and CA compounds was also assessed by the TBARS test. Lipid peroxidation was initiated

Table 1 Cacalol and cacalol acetate activities on DPPH radical reduction measured at 515 nm, and inhibition of TBARS production in brain homogenate induced by FeSO₄ and AAPH. The values of inhibitory concentration 50% (IC_{50}) are in μ M

Compound	IC_{50} (μ M)		
	DPPH	TBARS FeSO ₄	TBARS AAPH
Cacalol acetate (CA)	No activity	0.40 ± 0.02	25.95 ± 3.56
Cacalol (C)	24.09 ± 0.78	0.41 ± 0.03	29.69 ± 1.16
BHT	74.91 ± 5.76	1.22 ± 0.44	14.19 ± 1.89

using FeSO₄ and AAPH as inducers. Iron can initiate lipid peroxidation through the generation of OH, the formation of Fe–O complexes or by interacting with pre-existing lipid peroxides.²⁵ AAPH is a water-soluble azo compound used extensively as a free radical generator; the azo compound decomposes without involving enzymes or biotransformation, yielding molecular nitrogen and two carbon radicals R. The carbon radicals may combine to produce stable products or react rapidly with molecular oxygen to give peroxy radicals, ROO.^{28b}

The results are summarized in Table 1 and BHT was used as reference standard. The compounds showed that the percentage of lipid peroxidation needed for inhibition depends on the intermediates formed from both inducers. When lipid peroxidation induction was done with FeSO₄, both compounds showed the same antioxidant activity (Fig. 7). The IC_{50} value was 0.4 μ M that is higher than BHT standard with $IC_{50} = 1.22$ μ M ($p \leq 0.05$ values). Induction with AAPH produced an antioxidant activity with $IC_{50} = 29.6$ μ M for CA and $IC_{50} = 25.9$ μ M for C (Fig. 8); this slight difference was not statistically significant ($p > 0.05$), and both compounds were less active than BHT with $IC_{50} = 14.9$ μ M ($p \leq 0.05$ values).

Both lipid peroxidation tests showed similar trends. In the case of lipid peroxidation induced by Fenton reaction, where

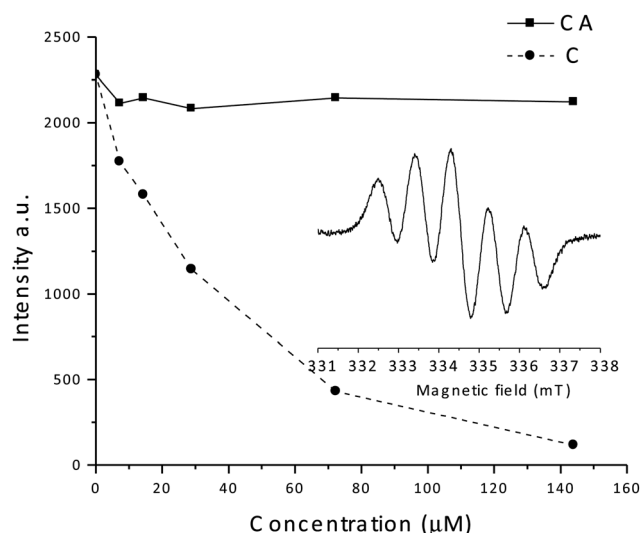


Fig. 6 Intensity of DPPH-signal as a function of ethanol solutions of C and CA. The inset shows the EPR spectrum of the observed DPPH adduct with $g = 2.0036$ and $a_N = 9$ G.

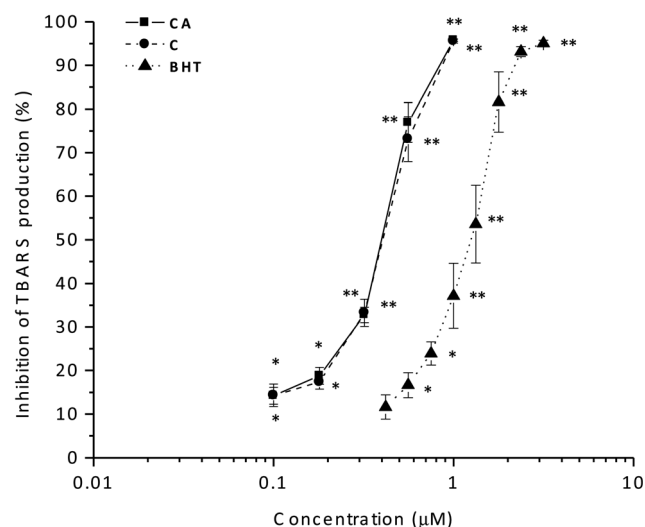


Fig. 7 Effect of C and CA concentrations on TBARS formation induced with Fe²⁺, with statistical significance for $p \leq 0.05$ (*) and $p \leq 0.01$ (**) values. BHT was used as standard.

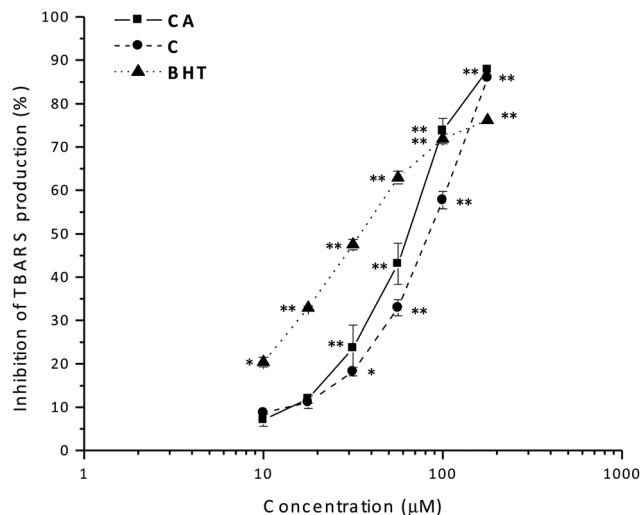


Fig. 8 Effect of C and CA concentrations on TBARS formation induced with AAPH, with statistical significance for $p \leq 0.05$ (*) and $p \leq 0.01$ (**) values. BHT was used as standard.

the reaction is induced by OH , cacalol compounds showed the same antioxidant activity. This behavior was also observed in the DMPO-EPR experiments, where C and CA were able to trap hydroxyl radicals with similar efficiency. It is worth remembering that OH are characterized by their reactivity, instability and high oxidation potential (2.8 V vs. NHE).²⁶ In the peroxidation reaction induced by AAPH that produces as intermediates R $\cdot$ and $\text{OOR}\cdot$, the differences between C and CA were not statistically significant. The results suggest that the capacity of cacalol to trap OH , R $\cdot$ and $\text{OOR}\cdot$ was not affected by the introduction of the acetate group into its structure. In spite of the fact that cacalol acetate is a non-phenolic compound, it showed important antioxidant properties, capturing hydroxyl radicals and the alkyl and peroxy radicals, which are responsible for propagating oxidation in organic matter.

Materials and methods

Materials

Cacalol (C) was isolated and purified from *Psacalium decompositum* (matarique complex) according to Anaya *et al.*⁴ Cacalol acetate (CA) was prepared as reported previously.²⁷ The complete characterization of these compounds is provided in the ESI,[†] Section A2. 5,5-dimethyl-1-pyrroline-*N*-oxide, DMPO, of ultra high purity was purchased from Dojindo; 2,2,6,6-tetramethylpiperidine TEMP (99%), 2,2,6,6-tetramethylpiperidine-1-oxyl TEMPO (99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hematoporphyrin HP (50%) from Sigma-Aldrich; 2-tiobarbituric acid TBA (99%) from ICN Biomedical, Inc. (Ohio); trichloroacetic acid, TCA (99%) from Fluka; iron(II) sulfate heptahydrate (99%), ethylenediaminetetraacetic acid disodium salt dehydrate EDTA (98.5%), 2,2'-azobis(2-amidinopropane) dihydrochloride AAPH (97%), sodium potassium tartrate tetrahydrate, butylated hydroxytoluene BHT (99%), copper(II) sulfate 99% and (+)- α -tocopherol from Sigma Chemical Co.; acetonitrile (99.8%),

dichloromethane (99%), ethyl alcohol absolute (99.95%) and dimethyl sulfoxide 99.9% from J.T.Baker; and Milli Q water.

Optical spectroscopy

The steady-state absorption spectra of both cacalol molecules were taken in a Cary-50 spectrophotometer, using 1 cm quartz cells.

EPR measurements

Electron Paramagnetic Resonance Spectroscopy (EPR) determinations were carried out in an EPR spectrometer (Jeol JES-TE300), operated in the X-Band mode at a modulation frequency of 100 KHz, with a cylindrical cavity (TE_{011} mode). The individual samples were placed in a quartz flat cell (synthetic quartz, Wilmad Glass Company) with a path length of 0.2 mm. The external calibration of the magnetic field was carried out using a JEOL ES-FC5 precision gaussmeter. The acquisition and manipulation of spectra were performed using the ES-IPRIT/TE program. The experiments for measuring the photogeneration of $^1\text{O}_2$ were carried out with a 1000 W Hg lamp (ES-USH10) and an optic filter N-WG320 SCHOTT ($\lambda_{\text{transmitted}} > 300$ nm). The incident light flux I_0 was measured by actinometry, using potassium ferrioxalate¹² (see ESI[†] for details on determination of I_0).

$^1\text{O}_2$ formation

The detection of $^1\text{O}_2$ is based on the specific reaction between $^1\text{O}_2$ and TEMP that yields a stable TEMP- $^1\text{O}_2$ radical adduct (known as TEMPO). The detection of $^1\text{O}_2$ was carried out according to the following procedure: samples of C and CA (0.8–1.45 mM) in an air-equilibrated ethanol solution with an amount of TEMP (30 mM) were irradiated for up to 20 min with UV-vis light ($\lambda > 300$ nm), generating a TEMPO signal that indicated the photoproduction of $^1\text{O}_2$. The EPR parameters were as follows: center field, 334.5 ± 4 mT; microwave frequency, 9.43 GHz; modulation width, 0.79×0.1 mT; time constant, 0.1 s; amplitude, 200. In each case, the EPR parameters were held constant, as was the concentration of TEMP; the samples were irradiated directly within the EPR cavity.

The amount of TEMPO produced by photochemical reaction was determined by comparing the integrated intensity of the EPR spectrum with that of the known concentration of commercial TEMPO. To compare the production of $^1\text{O}_2$ by C and by its acetate derivative CA, the ethanolic solution concentrations employed were normalized to the same number absorbed photons ($I_a/I_0 = 1$; Concentration of C = 1.0 mM, Concentration of CA = 1.4 mM, see ESI[†], Section A1.2). All experiments were repeated at least three times, and the data were obtained with errors of less than 5%.

On the other hand, to determine the role of cacalol compounds as scavengers of $^1\text{O}_2$, this oxygen intermediate was generated in presence of hematoporphyrin (a photosensitizer) under visible light irradiation: a sample of hematoporphyrin (HP) in an air-equilibrated ethanol solution (54 μM) and in presence of TEMP (30 mM) was irradiated for up to 20 min with $\lambda > 400$ nm, generating a TEMPO signal. Then, an amount of C

(1.0 mM) was added to HP solution and was irradiated directly within the EPR cavity, following the same procedure.

$O_2^{\cdot -}$ formation

The spin trapping EPR with DMPO was used to determine the generation of superoxide anion radicals by **C** and **CA**. The formation of superoxide anion radicals was measured in an aerated solution (0.8–1.45 mM) of **C** and **CA**, which were added to a 30 mM solution of DMPO in DMSO. Samples were irradiated for up to 20 min with $\lambda > 300$ nm, directly within the EPR cavity, and EPR signals were monitored.

OH measurements

After the formation of OH by a Fenton-type reaction, we determined the ability of **C** and **CA** to trap hydroxyl radicals through the EPR method, using DMPO as spin-trap material. The procedure was as follows: the reaction mixture composed by 30 mM DMPO, 50 mM $FeSO_4$, 25 mM H_2O_2 was prepared in a PBS solution at pH = 7.4. An amount of **C** or **CA** (0.34 mM) dissolved in acetonitrile was added to iron solution. Then, it was transferred to the flat cell and the EPR signal was measured under the following experimental conditions: center field, 334.5 ± 4 mT; microwave frequency, 9.43 GHz; modulation width, 0.79×0.1 mT; time constant, 0.1 s; amplitude, 200. In each case, the EPR parameters were held constant. The concentration of DMPO-OH was evaluated using the peak-height intensity of the second peak. The control samples were carried out: with/without iron salt, H_2O_2 or DMPO.

DPPH assay by EPR spectroscopy and UV-vis spectrophotometry

An ethanolic solution of DPPH at a final concentration 84 μ M was mixed with different concentrations of **C** and **CA** (0–144 μ M); then it was transferred to the flat cell and the EPR signal was measured under the following experimental conditions: power = 8 mW; center field, 334.5 ± 4 ; modulation width, 0.1×0.1 mT; amplitude 250; time, 2 min. The concentration of DPPH was evaluated from EPR spectrum, using the peak-height intensity of the central peak.

The reduction of DPPH was monitored by UV-vis spectrophotometry using the following procedure: the test was carried out on 96 well microplates, 50 μ L of the solution of the test compound were mixed with 150 μ L of the ethanolic solution of DPPH (final concentration, 100 μ M). After incubation at 37 °C for 30 min, the absorbance of DPPH solutions was measured at 515 nm in a microplate reader ELx 808. The inhibition percentage of each compound was determined by comparison with 100 μ M blank ethanolic solution of DPPH.

TBARS assay

The Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), provided adult male Wistar rats (200–250 g). Procedures and care of animals were conducted in

conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). They were maintained at 23 ± 2 °C under a 12/12 h light–dark cycle with free access to food and water. The TBARS assay was carried out as described in previous reports.²⁸ Butylated hydroxytoluene (BHT) was used as reference standard. Briefly, supernatants of homogenates from rat brain (375 μ L) were mixed with 50 μ L of 10 μ M EDTA, 25 μ L of **C**, **CA** or BHT and were incubated for 30 min at 37 °C. In order to induce lipid peroxidation, 50 μ L of 100 μ M of $FeSO_4$ or 200 mM AAPH were added and the mixture was incubated at 37 °C for 60 min or 180 min, respectively. Then, 500 μ L of thiobarbituric acid (TBA) solution (0.5% TBA in 0.05 N NaOH and 30% trichloroacetic acid in 1 : 1 proportion) were added. The mixture was centrifugated at 12 879g for 5 min and incubated for 30 min at 80 °C. After cooling at room temperature, the absorbance of 200 μ L of supernatant was measured at 540 nm in a Bio-Tek Microplate reader ELx808; the data obtained were interpolated into a malondialdehyde (MDA) standard curve, and the final results were expressed as nmol of thiobarbituric reactive substances per mg of protein (nmol TBARS/mg protein). The inhibition percentage (%) of lipid peroxidation was calculated using the following expression: $\% = (F - E) \times 100 / F$, where F represents TBARS production with $FeSO_4$ or AAPH, and E represents the TBARS production in the presence of sample.

All data were presented as mean $\pm$ standard error (SEM). The data were analyzed by one-way ANOVA followed by a Dunnett's test for comparison against control. Values of $p \leq 0.05$ (*) and $p \leq 0.01$ (**) were considered statistically significant. The inhibitory concentration of 50 (IC_{50}) was estimated by means of a linear regression.

Conclusions

In this work we have studied the abilities of cacalol and cacalol acetate as photoproducers of singlet oxygen and as scavengers of free radicals. We found that **C** and **CA** were able to produce singlet oxygen under UV-vis irradiation. With respect to their behavior as scavengers, both **CA** and **C** showed a similar capacity to trap hydroxyl, alkyl and peroxy radicals indicating that the capacity of cacalol to trap these radicals was not affected by the introduction of the acetate group into its structure. However, the ability to trap DPPH (a nitrogen-centered radical) was drastically different between the two compounds. We found that **CA** is an effective antioxidant in *in vitro* systems. The dual role showed by cacalol and cacalol acetate (a non phenolic compound) as producers/inhibitors makes them interesting options for photodynamic therapy applications and other treatments of diseases involving uncontrolled cell proliferation.

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