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Photooxygenation of Cyclohexenone Derivatives and Their Effect on DNA

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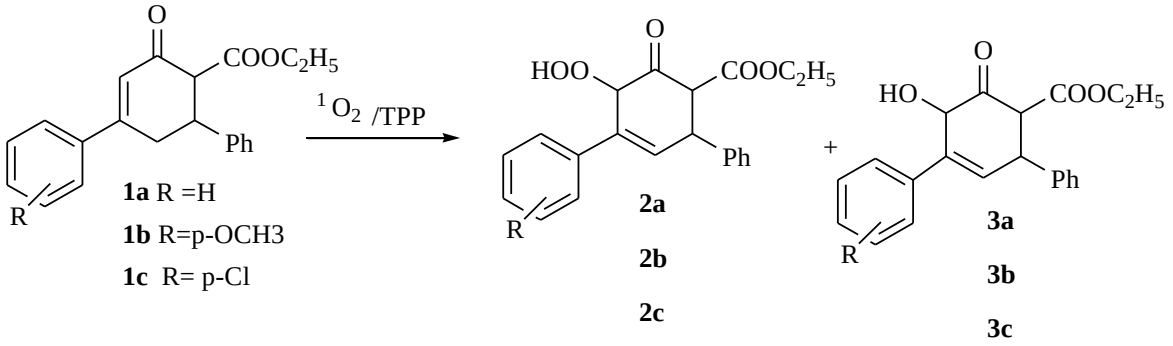
Abstract The photoxygenation reaction of cyclohexenone derivatives **1a-c** using sodium and mercury lamp afforded the hydroperoxides **2a-c** and hydroxyl derivatives **3a-c**. Similarly, the photooxygenation of derivative **4** gave the hydroperoxide **5** as a sole product . On the other hand, the photooxygenation of cyclohexenone derivative **7** afforded the hydroxyl derivatives **10**, while cyclohexenone derivatives **11** yielded two photoproducts **12** and **13**. Epoxidation of cyclohexenone derivative **1b** gave epoxide derivative **14**. Hydroperoxide derivatives **2a** and **5** showed good cleaving agent for DNA.cyclohexenone.

Introduction

Hydroperoxides are known to produce singlet ($^1O_2$) by photosensitization reactions using ultraviolet or visible light (*Khan et al., 1963, Wasserman et al., 1979*, Foote *et al., 1979, Bellus.,* 1978), which are caused relatively little oxidative DNA damage (Zheng *et al., 2011) via* the formation of hydroxyl radical which is formed either by ionizing radiation or by UV radiation of some hydroperoxide derivatives. On the other hand, The epoxide derivatives can be used as an efficient DNA-alkylating agent, (*Stables et al., 1995, DeRosa, et al.,* 2002). Hydrogen peroxides is always used as epoxidizing agent but only in the presence of transition metal salts or other complexes (Lane *et al.,* 2002) as catalyst. In view of the therapeutic interest of hydroperoxide derivatives. (*Carballal, et al., 2003).* It has been investigated in details the photooxygenation and epoxidaton of theses cyclohexenone derivatives.

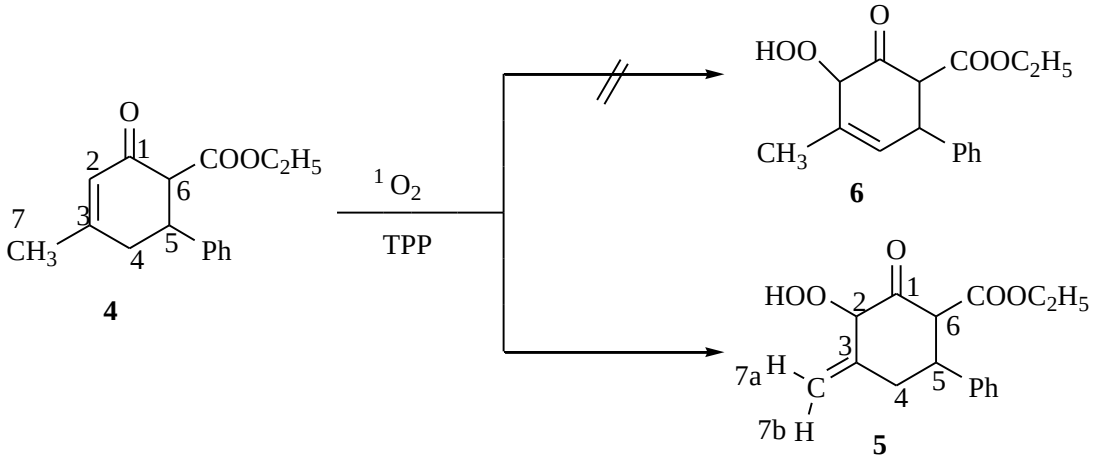
Result and Discussion

The photooxygenation reaction of cyclohexenone derivatives (*Muathen et al., 2007, Abou-Elzahab, et al., 1994*) 1a-c in the presence of 5,10,15,20-tetraphenyl-21H-porphyrine (TPP) as a singlet oxygen sensitizer in chloroform solution using sodium and mercury lamps gave via ene mechanism a mixture of two products 6-carboethoxy-3,5-disubstituted -2-hydroperoxide derivatives **2a-c** in yield 15, 17 and 19% respectively, in addition to hydroxyl derivatives **3a-c** in yield 40, 35 and 35 % respectively **(Scheme 1)**.



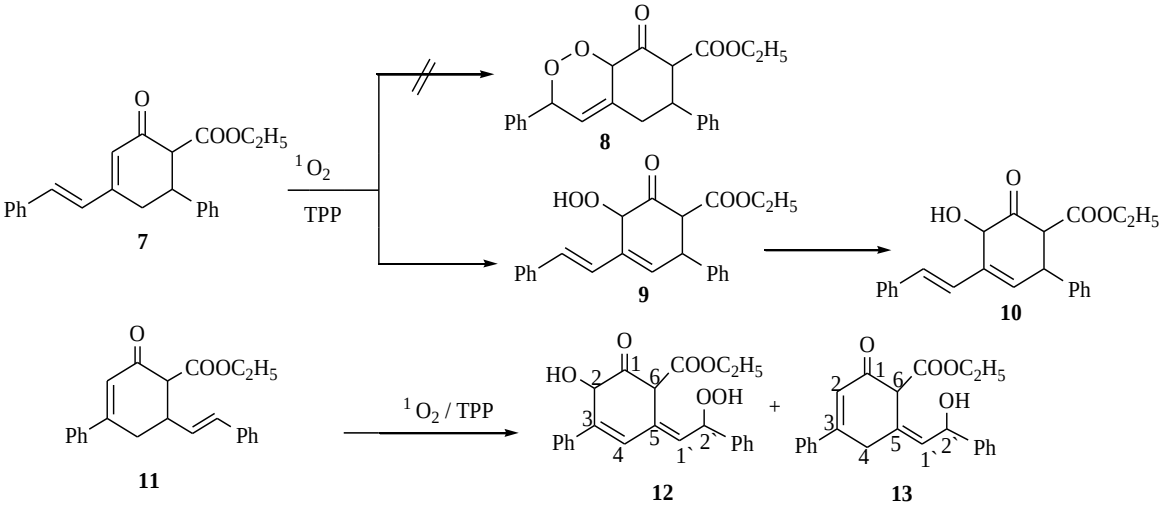
(Scheme 1)

Similarly, the photoxygenation of 6-carboethoxy-3-methyl-5- cyclohexenone derivative **4** in chloroform was carried out using TPP in the presence of sodium and mercury lamps leading to the hydroperoxide derivatives **5** and not hydroperoxide derivative **6** as a sole product, in a quantitative yield through the ene mechanism (*Linker et al., 2007)* (**Scheme 2**).



(Scheme 2)

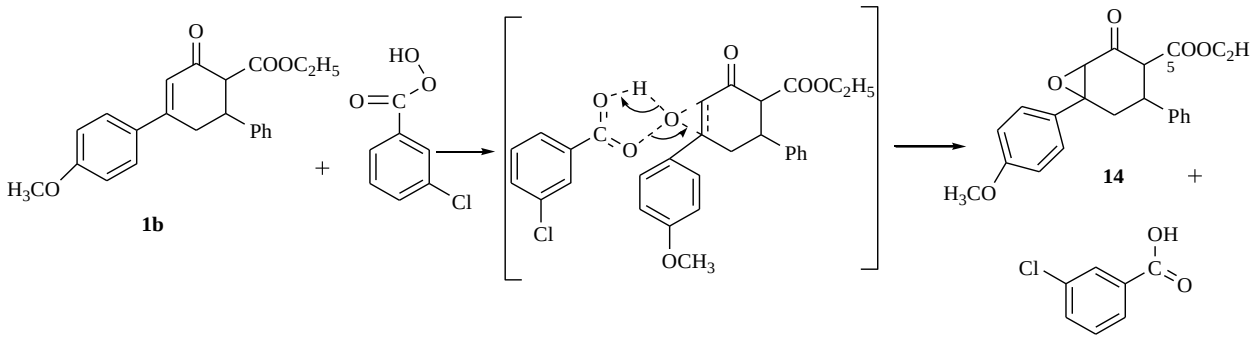
The photooxygenation of 6-carboethoxy-3-styryl-5-phenyl-2-cyclohexenone **7** which is adiene in the presence of TPP as a singlet oxygen sensitizer to form of the endoperoxy derivative **8** was unsuccessful through [4+2] cycloaddition reaction Diels Alder type; however the isolated photoproduct **10** was obtained instead of the anticipated endoperoxy **8** derivative **8**. The hydroperoxide derivatives **9** showed difficulty for isolation due to its instability (it gave brown colour with spraying agent potassium iodide solution). ^{1}H-NMR spectrum of **9** showed peak at δ 8.85 ppm characteristic for hydroperoxide group. Compound **9** was decomposed to give the new product after short time, which was characterized as the corresponding hydroxide derivative **10** (**Scheme 3**). Finally, photooxygenation of 6-carboethoxy-3-phenyl-5-styryl-2-cyclohexen-1-one **11** in chloroform using TPP for 2 hrs yielded two photoproducts **12** and **13**.



(Scheme 3)

Epoxidation of cyclohexenone derivative 1b

In view of the well known that the epoxides can be efficient DNA-alkylating agents (*Knobler et al., 1988, Sharifi et al., 1999)*, little to known about such activity for the cyclohexenone derivatives. To make such novel cyclohexenone epoxide available for genotoxic testing, We have investigated the epoxidation of cyclohexenone derivative **1b** using meta-chloroperbenzoic acid (m-CPBA) to obtain the corresponding epoxide **14**, as the potentially DNA alkylating agent. The mechanism of the formation of **14** was believed to be as the following mechanism (*Singer et al.1983)*.



Induction of DNA damage by photooxygentaed product 2a and 5

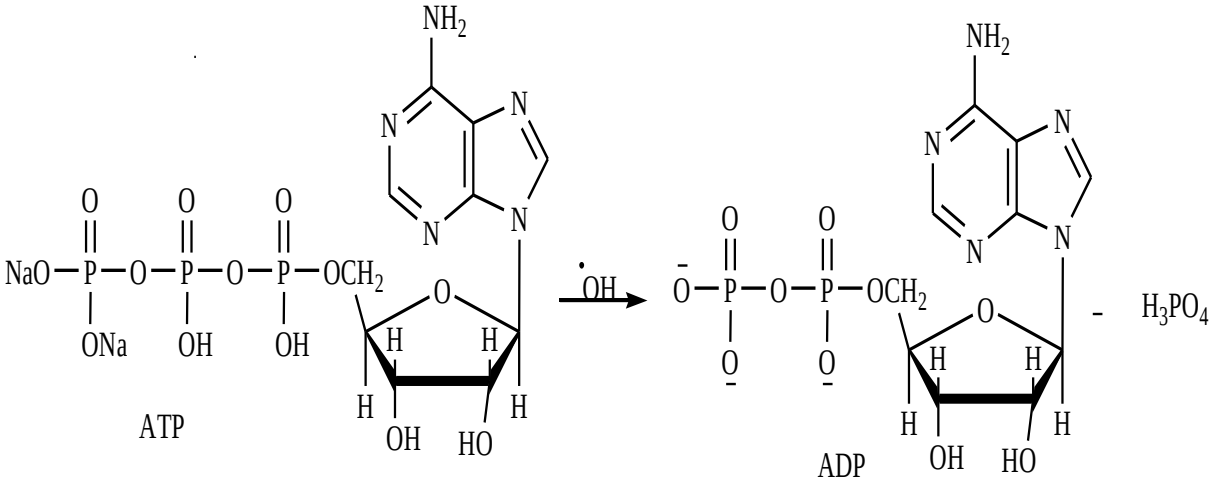
A solution of photooxygentaed products **2a** and **5** (6 mg in 2 ml DMF) and DNA (30 mg in 30 ml saline solution) was irradiated respectively at 0°C using sodium and mercury lamp as illustrated in Experimental Section. Samples at different time was taken to determine the effect of these photooxygenated product on DNA damage, it has been found that the photooxygenated product **2a** gave positive results when the irradiation time was prolonged more than 7 hrs as shown in fig. 1. While photooxygenation product **5** gave positive result when irradiation time is 10 hrs as shown in figure 2. Upon excitation by visible and UV light radiation, many sensitizer induce oxidative DNA damage either indirectly *via* singlet oxygen ($^1O_2$)
(type II reaction) or directly *via* hydrogen abstraction or electron transfer (type I reaction) with DNA (*Foot et. al., 1968, Kochevar et al., 1990, Epe et al., 1993).* hydroperoxides have

been shown to generate hydroxyl radicals (˙OH) which showed efficiently produce a type of DNA damage photochemically (*Epe et al., 1994, Saito et al., 1990, Matsugo et al., 1991).*

The results presented above indicated that the DNA damaging properties of photo-excited cyclohexenone derivatives **2a** and **5** gave efficiently to DNA stand breaks, probably *via* hydroxyl radical formation as illustrated in Experimental Section.

Mechanism of ATP degradation

A stock solution of adenosine triphosphate disodium salt (ATP) in saline solution and the photooxygenated products **2a, 2b** and **2c** was mixed then illuminated for 1 hr by fluorescent lamps. The molybdate followed by $SnCl_2$ solution were added dropwise to the reaction mixture, deep blue colour was appeared indicating phosphoric acid formation. Hydroperoxides formed by photooxygenation have short life time; it decomposed after some time and in order to determine the mechanism of decomposition. Hydroperoxide derivative was dissolved in benzene, then irradiated it using UV lamp for 1 hr. The resulting solution tested for phenol using $FeCl_3$ solution, it has been found that deep violet colour is appeared due to the formation of phenol as shown in equation below



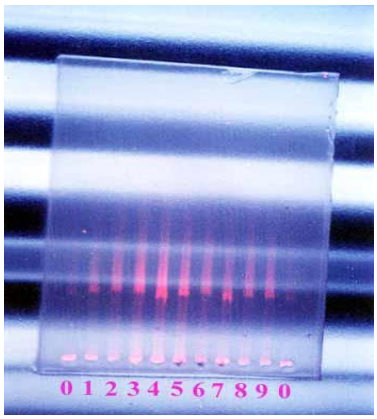


Fig. 1: Degree of DNA degradation using hydroperoxide derivative 2a

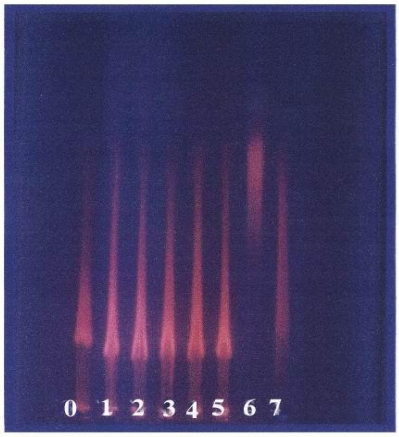


Fig. 2: Degree of DNA degradation using hydroperoxide derivative 5

Experiments

Selected 2-Cyclohexenones derivatives **1a-c, 4, 7 and 11** were prepared according to the literature procedures (*Sammour et. al., 1971, Salem et al., 1981,* Khachatryan *et al., 1981, El-Sadany et al., 1981).* Melting points (ºC) are uncorrected and were measured on Gallenkamp electric melting point apparatus. Elemental analysis of the stable photoproducts was in good agreement with the proposed structures. It was carried out at Microanalytical Unit, Faculty of Science, Cairo University. Infrared spectra (IR) were measured using KBr disc on a Mattson 5000 FTIR spectrometer. ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with a Brucker 200 MHz apparatus and the ^{1}H-NMR chemical shifts were reported in (δ) ppm using residual $CHCl_3$ (delta 7.24) in the predueterated solvent as the internal standard and coupling constants were expressed in Hz. ^{13}C-NMR spectra were reported on Brucker AC 300 (75.5 MHz). ^{13}C-NMR chemical shifts were reported in delta relative to internal standard $CDCl_3$ (δ 77). Mass spectra (MS) were carried out on Shimadzu GC-MS QP 1000 EX spectrometer. Thin layer chromatography (TLC). Polygram SIL G/UV 254. Silica gel 60 GF 254 used in TLC was from Merck and silica gel 0.063-0.2 nm for column chromatography purchased from Fluka Co. For the removal of the solvents a rotatory evaporator (at 20 ºC/15 Torr) was used. Hydroperoxide derivatives were detected by KI spray reagent. For the photolysis, a sodium lamp (Phillips G/5812 SON) and mercury lamp (HRI-T250 W) were used. Biological screens were carried out at Biochemistry lab. Faculty of Science, Mansoura University.

General Procedure for Photoocygenation of 2-Cyclohexenones derivatives 1a-c

A solution of 2-Cyclohexenones derivatives (1 mmol) in $CHCl_3$ (20 ml) and tetraphenylporphyrine (TPP) (2 mg) was irradiated using sodium and mercury lamps at -20ºC for 15, 8 11 hrs respectively during the irradiation, a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a very slow rate to avoid solvent evaporization and oxygen consumption. Then the solvent was evaporated at 20°C/15 Torr to give a gummy material which was purified by column chromatography, the eluent was a mixture of petroleum ether 60-80ºC and ethyl acetate (4:1)

6-carboethoxy-3, 5-diphenyl-2-hydroperoxy-3-cyclohexen-1-one(2a).

Yield 15%; (white crystals); m.p 130ºC; R_f= 0.17[pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3603, 3527, 3505-3121(br, OOH), 2860(CH, CH_2 Str). 1728($COOC_2H_5$), 1695(C=O), 1614(C=C); ^{1}H-NMR($CDCl_3$) 1.1(t, 3H, CH_3), 3.1(dd, J=6.96, J=26.9Hz, 1H, H-6), 3.95(dd, J=3.6, J=26.5Hz, 1H, H-5), 4.1(dd, J=7.02, J=17.5Hz, 1H, H-4), 4.15(q, 2H, OCH_2), 6.65(s, 1H, H-2), 7.2-7.8(m, 10H, Aromatic protons) and 10.05(s, 1H, OOH); MS, m/z 352 [M^+,$C_{21}H_{20}O_5$] (0.4), 318 (2.0), 279 (9.0), 119 (11.0), 118 (100, base peak) and 114 (15.0).

6-carboethoxy-3, 5-diphenyl-2-hydroxy-3-cyclohexen-1-one (3a).
Yield 40%; (white crystals); m.p 65ºC; R_f= 0.345 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3456 (OH), 3062, 2978 (CH,CH_2 str), 1730 ($COOC_2H_5$), 1651 (C=O), 1612 (C=C) and 1275 (C-O); ^{1}H-NMR ($CDCl_3$) 1.1(t, 3H, CH_3), 3.1(dd, 1H, H-6), 3.5(dd, 1H, H-5), 3.6 (dd, 1H, H-4), 3.95(q, 2H, OCH_2), 4.0 (s, 1H, OH), 6.65(s, 1H, H-2), 7.2-7.5(m, 10H, Aromatic protons); ^{13}C-NMR ($CDCl_3$) δ 13.96 (CH_3), 33.01(C-5), 49.66(OCH_2), 62.22(C-6), 80.37(C-2), 121.7(C-4), 126.3(C-Ar), 127.9(C-Ar), 128.47(C-3), 128.6(C-Ar), 128.9(C-Ar), 130.77(C-Ar), 137.78(C-Ar), 162.2(C-Ar), 169.12(COO), 195.03 (C-1); MS, m/z 336[M^+, $C_{21}H_{20}O_4$] (0.94), 318 (3.61), 290 (2.6), 263 (4.7), 245 (1.75), 235(6.13), 215(3.99), 118 (100, base peak), and 115 (33.28); Analysis calculated for Mol. Formula $C_{21}H_{20}O_4$ (336.39): C, 74.98; H, 5.99. Found: C, 75.31; H, 6.22.

6-carboethoxy-3-(4`-methoxyphenyl) -5-phenyl-2-hydroperoxy-3-hexen-1-one (3b)
Yield 17%; (white crystals); m.p 140ºC; R_f= 0.15 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3534, 3446, 3413-3405(br, OOH), 2981, 2931(CH, CH_2 Str.), 1739($COOC_2H_5$), 1662(C=O) and 1610(C=C), 1251(C-O); ^{1}H-NMR ($CDCl_3$) δ 1.0(t, 3H, CH_3), 3.1(dd, 1H, H-6), 3.5(dd, 1H, H-5), 3.8(s, 3H, OCH_3), 4.1(dd, 1H, H-4), 4.2(q, 2H, OCH_2), 6.65 (s, 1H, H-2), 7.2-7.8(m, 9H, Aromatic protons) and 10.14(s, 1H, OOH); MS m/z 382[$C_{22}H_{22}O_6$, M^+] (2.1), 366 (6.7), 349 (5.3), 321 (15.7), 293 (5.0), 248 (12.88), 265 (23.4), 251 (1.9), 221 (56.9), 148 (100, base peak), 121 (43.9), 115 (27.6) and 77 (31.0).

6-carboethoxy-3-(4`-methoxyphenyl)-5-phenyl-2-hydroxy-3-cyclohexen-1-one (2b).
Yield 35%; (white crystals); m.p 110ºC; R_f= 0.37 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3459(OH), 2939(CH and CH_2 str.) 1730($COOC_2H_5$), 1650(C=O), 1613(C=C), 1450 and 1247(C-O); ^{1}H-NMR ($CDCl_3$) δ 1.1(t, 3H, CH_3), 3.1(dd, 1H, H-6), 3.5(dd, 1H, H-5), 3.75(d.d, 1H, H-4), 3.8(s, 3H, OCH_3), 4.1(s, 1H, OH), 4.3(q, 2H, OCH_2), 6.5(s, 1H, H-2) and 7-7.6(m, 9H, Aromatic protons); ^{13}C-NMR ($CDCl_3$) δ 14.012(CH_3), 33.19(C-5), 48.9(OCH_3), 55.23(OCH_2), 62.25(C-6), 80.55(C-2), 113.86(C-4), 121.74(C-3), 126.32(C-Ar), 128.9(C-Ar), 129.65(C-Ar), 129.8(C-Ar), 130.75(C-Ar), 137.8(C-Ar), 159.2(C-Ar), 162.32(C-Ar), 182.69(COO) and 195.18(C-1); MS m/z 366 [$C_{22}H_{22}O_5$, M^+] (2.5), 348 (1.1), 320 (0.2), 293 (2.4), 275 (0.9), 265 (9.5), 251 (1.9), 247 (1.3), 221 (37.0), 216 (1.3), 148 (100, base peak), 115 (35.0) and 77 (22.0); Analysis calculated for Mol. Formula $C_{22}H_{22}O_5$ (366.42): C, 72.11; H, 6.05. Found: C, 72.16; H, 6.30.

6-carboethoxy-3-(4`-chlorophenyl)-5-phenyl-2-hydroperoxy-3- cyclohexen-1-one (2c).
Yield 19%; (white crystals); m.p 123ºC; R_f=0.17 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3619, 3438, 3417-3367 (br, OOH), 2975, 2929, 2896, (CH_2, CH_3 str.) 1733($COOC_2H_5$), 1662(C=O), 1611(C=C) and 1216(C-O); ^{1}H-NMR($CDCl_3$) δ 1.2(t, 3H, CH_3), 3.2(dd, 1H, H-6), 3.7(d.d, 1H, H-5), 4.1(dd, 1H, H-4), 4.2(q, 2H, OCH_2), 6.7(s, 1H, H-2), 7.3-7.7(m, 9H, Aromatic protons) and 10.13(s,1H, OOH); MS, m/z 388 [M^+ +2] (1.03), 386[M^+, $C_{21}H_{19}ClO_5$] (0.98), 371 (5.0) 370 (4.4), 353 (0.9), 352 (2.7), 324.5 (14.0), 298 (2.3), 297 (8.4), 256 (2.1), 255, (2.2), 145 (32.9), 144 (100, base peak), 116 (25.6), 115 (47.0), and 77 (14.0).

6-carboethoxy-3-(4`-chlorophenyl) 5-phenyl-2-hydroxy-3-cyclohexen-1-one (3c).
Yield 35%; (white crystals); m.p 110ºC; R_f=0.35 [pet. ether 40-60ºC/ ethylacetate (4:1)];IR (KBr) cm^{-1} 3570(OH), 2923(CH, CH_2 str.), 1736($COOC_2H_5$), 1661(C=O), 1608(C=C); ^{1}H-NMR ($CDCl_3$) δ 1.0 (t, 3H, CH_3), 3.0 (dd, 1H, H-6), 3.9(dd, 1H, H-5), 4.07(dd, 1H, H-4), 4.1(s, 1H, OH), 4.2(q, 2H, OCH_2), 6.7(s, 1H, H-2), 7.4-7.8(m, 9H, Aromatic protons); ^{13}C-NMR ($CDCl_3$) δ 13.9(CH_3), 32.9(C-5), 48.9(OCH_2), 62.3(C-6), 80.2(C-2), 121(C-4), 126(C-3), 128(C-Ar), 128.6(C-Ar), 129(C-Ar), 130(C-Ar), 130.8(C-Ar), 133.7(C-Ar), 136.2(C-Ar), 137.6(C-Ar), 168.9(COO), 194.7(C-1); MS, m/z 372 [M^++2] (0.13), 370 [M^+,$C_{21}H_{19}ClO_4$](0.4), 353(0.4), 352 (0.7), 308(0.9), 307.5(1.3), 298(0.7), 297.5 (3.0), 223(0.2), 222 (0.1), 151 (100, base peak), 115(44.0); Analysis calculated for Mol.

Formula $C_{21}H_{19}ClO_4$ (370.84); C, 68.01; H, 5.16. Found: C, 68.40; H, 5.15.

Photooxygenation reaction of 6-carboethoxy-3-methyl-5-phenyl-2-cyclohexen-1-one (4).

A solution of **4** (0.26 g, 1 mmol) and tetraphenylporphyrine (TPP) (5 mg) in $CHCl_3$ (80 ml) was photooxygenated using sodium and mercury lamps at –20ºC for 9 hrs, during the irradiation, a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a very slow rate to avoid solvent evaporization and oxygen consumption. The solvent was evaporated at 20°C/15 Torr to give a gummy material which was separated and purified using preparative thin layer chromatography using petroleum ether 60-80°C and ethyl acetate (4:1) as eluent to give **5**.

6-carboethoxy-3-methylenyl-5-phenyl-2-hydroperoxy-cyclohexan-1-one (5).
Yield 20%; (an oil); R_f=0.12; R_f = 0.23 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3523, 3428, 3405-3343 (br, OOH), 3025, 2978(CH_2, CH_3 str.), 1739($COOC_2H_5$), 1664(C=O) and 1626(C=C); ^{1}H-NMR ($CDCl_3$) δ 0.8(t, 3H, CH_3), 2.1(m, 1H, H-5), 2.6(m, 2H, H-4), 3.3(m, 1H, H-6), 3.8(d, 1H, J= 2.5 Hz, H-7a), 3.9(d, 1H, J = 2.52 Hz, H-7b) 4.1(q, 2H, OCH_2), 6.15(S, 1H, H-2), 7.15(s, 5H, Aromatic protons) and 10.11(s, 1H, OOH); MS, m/z 290[M^+, $C_{16}H_{18}O_5$] (1.0), 204 (1.5), 203(2.4), 192(54.5), 145(27.4), 144(52.2), 118(100, base peak) 116(17.3).

Photooxygenation of 6-carboethoxy-3-styryl-5-phenyl-2-cyclohexen-1- one (7).
A solution of **7** (0.35 g, 1 mmol) in $CHCl_3$ (20 ml) and tetraphenylporphyrine (TPP) (2 mg) was photooxygenated using sodium and mercury lamps for 10 hrs at -20ºC, during the irradiation, a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a very slow rate to avoid solvent evaporization and oxygen consumption. The reaction mixture was tested using TLC, gave one product which gave brown color, when tested using KI solution. Prolonged irradiation of the reaction mixture for 20 hours, then the solvent was evaporated at 20°C/15 Torr to dryness, giving a gummy material, which was separated and purified using preparative thin layer chromatography using petroleum ether 60-80ºC to ethyl acetate (3:2) as eluent to give **10**

6-carboethoxy-3-styryl-5-phenyl-2-hydroxyl-3-cyclohexen-1-one (10).
Yield 50%; (An oil product); R_f = 0.25 [pet. ether 40-60ºC/ ethylacetate (3:2)]; IR (KBr) cm^{-1} 3454(OH), 3040, 2980(CH_2, CH_3 str.), 1739($COOC_2H_5$), 1659(C=O), 1614(C=C); ^{1}H-NMR ($CDCl_3$) δ 1.2(t, 3H, CH_3), 2.7(dd, 1H, H-6), 3.8(m, 1H, H-5), 4.05(q, 2H, OCH_2), 4.2 (s, 1H,OH), 4.25(dd, 1H, H-4), 6.2(s, 1H, H-2), 7.05(d, 1H, H-2`), 7.25-7.45(m, 10H, Aromatic protons), 7.5(d, 1H, H-1`); MS, m/z 362[M^+, $C_{23}H_{22}O_4$] (0.4), 345 (1.16), 344 (4.5), 289 (3.3), 261 (5.9), 247 (0.4), 186 (0.2), 184 (0.5), 170 (100, base peak), 141 (90.0), 144 (2.5), 142 (81.3), 114 (35.0) and 81 (7.0), 77 (30.5); Analysis calculated for Mol. Formula $C_{23}H_{22}O_4$ (362.43): C, 76.22; H, 6.12. Found: C, 76.11; H, 6.20.

Photooxygenation of 6-carboethoxy-3-phenyl-5-styryl-2-cyclohexen-1-one (11).
A solution of **11** (0.346 g, 1 mmol) and tetraphenylporphyrine (TPP) (2 mg) in $CHCl_3$ (20 ml) was irradiated using sodium and mercury lamps at –20ºC for 2 hrs, during the irradiation, a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a very slow rate to avoid solvent evaporization and oxygen consumption. The solvent was evaporated at 20°C/15 Torr to give a gummy material, which was purified using preparative thin layer chromatography using petroleum ether and ethyl acetate (4:1) as eluent to give two products **12** and **13**.

5-(2`-hydroperoxy-2`-phenyl-ethylidene)-2-hydroxy-3-phenyl-2-cyclohexenone-6-carboxylic acid ester (12).
Yield 30%; (oil product); R_f = 0.16 [pet. ether 40-60ºC/ ethylacetate (5:2)]; IR (KBr) cm^{-1} 3570, 3524, 3487-3381 (br, OOH and OH),

3092, 2978, 2924 (CH, CH_2 str.), 1734($COOC_2H_5$), 1660 (C=O), 1609(C=C) and 1261(C-O); ^{1}H-NMR ($CDCl_3$) δ 1.2 (t, 3H, CH_3), 2.7 (d, 1H, H-6), 4.05 (q, 2H, OCH_2), 4.1 (s, 1H,OH), 4.25(dd, 1H, H-4), 6.2(s, 1H, H-2), 7.05(d, 1H, H-2`), 7.25-7.45 (m, 10H, Aromatic protons), 7.5(d, 1H, H-1`), 10.1 (s,1H, OOH); MS m/z 392 [M^+-2, $C_{23}H_{22}O_6$] (0.5), 344 (4.3), 271 (2.9), 243 (3.7), 218 (25.4), 144 (100, base peak), 115 (64.9), 91(31.0), 77(22.9).

5-(2`-hydroxy-2`-phenyl-ethylidene)3-phenyl -2-cyclohexenone-6-carboxylic acid ester (13).

Yield 30% (An oil product); R_f = 0.24 [pet. ether 40-60ºC/ ethylacetate (5:2)]; IR (KBr) cm^{-1} 3501(OH), 3020, 2932(CH, CH_2 and CH_3 str.) 1734($COOC_2H_5$), 1665(C=O), 1608(C=C), 1250, 1216, 1135(C-O); ^{1}H-NMR ($CDCl_3$) δ 1.16 (t, 3H, CH_3), 2.95 (d, 1H, J = 18.32 Hz, H-4e), 3.15 (m, 1H, H-6), 3.35 (d, 1H, J = 18.06 Hz, H-4a), 6.32 (d, 1H, J = 7.5 Hz, H-2`), 6.53 (d, 1H, J = 2.2 Hz, H-1`), 6.55 (s, 1H, OH), 6.95 (s, 1H, H-2), 7.16-7.55 (m, 10H, Aromatic protons); MS, m/z 362 [M^+, $C_{23}H_{22}O_4$] (1.4), 344 (4.2), 289 (3.4), 261 (4.1), 218(43.9), 171(2.4), 144(100, base peak), 115(63.0) and 77(19.8); Analysis calculated for Mol. Formula $C_{23}H_{22}O_4$ (362.43): C, 76.22; H, 6.12. Found: C, 76.10; H, 6.11.

Epoxidation of 6-carboethoxy-3-(-4`-methoxyphenyl)-5-phenyl-2-cyclohexen-1-one (1b).

A soln of cyclohexenone derivative **1b** (0.5 g, 1.4×10^{-3} mol) in $CHCl_3$ (25 ml) was added dropwise to m-chloroperbenzoic acid (m-CPBA) (3.2×10^{-3} mol, 0.55 g 80%) at 0ºC. The reaction mixture was stirred at room temperature overnight. It was washed with saturated aqueous soln of sodium bicarbonate (3×10 ml) then with distilled water (3×10 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness to give a gummy material, which was purified by preparative thin layer chromatography using pet. ether and ethyl acetate (4:1) as eluent to give **14.**

6-carboethoxy-3-(4`-methoxyphenyl)-5-phenyl-2,3-epoxy-cyclohexan-1-one (14) :

Yield 40%; (pale yellow crystals); m.p 110ºC; R_f = 0.18 [pet. ether 40-60ºC/ ethylacetate (4:1)]; IR (KBr) cm^{-1} 3448 (enolic OH), 2939(CH, CH_2 str.), 1723($COOC_2H_5$), 1664(C=O), 1615(C=C), 1250(C-O); ^{1}H-NMR ($CDCl_3$) δ 1.1(t, 3H, CH_3), 3.1(dd, 1H, H-6), 3.5(dd, 1H, H-5), 3.75(m, 1H, H-4e), 3.8(s, 3H, OCH_3), 4.1(m, 1H, H-4a), 4.3(q, 2H, OCH_2), 6.5(s, 1H, H-2) and 7-7.6(m, 9H, Aromatic protons); ^{13}C-NMR ($CDCl_3$) δ 14.01 (CH_3), 33.19(C-4), 48.9(OCH_3), 55.25(OCH_2), 62.25(C-2), 80.55(C-5), 113.86(C-6), 121.74(C-3), 126.316(C-Ar), 128.9(C-Ar), 129.65(C-Ar), 129.8(C-Ar), 130.75(C-Ar), 137.8(C-Ar), 159.2(C-Ar), 162.32(C-Ar), 182.69(COO), 195.178 (C-1); MS m/z 366[M^+, $C_{22}H_{22}O_5$] (2.2), 348 (2.22), 293 (2.2), 265 (9.87), 148 (100, base peak), 115 (40) and 77 (28.1); Analysis calculated for Mol. Formula $C_{22}H_{22}O_5$ (366.42): C, 72.11; H, 6.05. Found: C, 72.16; H, 6.06.

Degradation of ATP using photooxygenated products 2a and 5:

Reagents

1- Stock solution of adenosine triphosphate disodium salt (ATP) containing ATP (5mg) in saline solution (5 ml) (BDH-AR garde).

2- Molybdate solution

Reagent–grade ammonium molybdate (25 gm) was dissolved in about 200 ml of water. In a 1-liter measuring flask, (300 ml, 10 N) sulphuric acid were placed, the molybdate solution was added and diluted with washing to 1 liter with water. (10 N) Sulphuric acid (BDH- Analar): Concentrated sulphuric acid (450 ml) was carefully added to (1300 ml) of water, to check, the normality a 10 ml of this solution were diluted to (100 ml) in a volumetric flask mixed, and titrated a (10 ml portion) with standard 1N sodium hydroxide. From the titration results, the original solution was adjusted if necessary to make it exactly (10 N).

3- Stannous chloride $SnCl_2$ 1% soln.

4- Stock solution of new prepared hydroperoxide **2a** and **5**.

5- Light source: - two flurorescent lamps (40 watt, wave length: 300-750 nm, Phillips).

Procedure

1) A solution mixture of (0.0019 g) of ATP on (3 ml) of NaCl (saline solution) and hydroperoxide **2a** and **5** (0.001 g) in (1 ml) of DMF was irradiated using sodium lamp at 25-30ºC for 1 hrs respectively. The molybdate 2) Phosphoric acid formation in each case.

Induction of DNA damage by the photooxygenated products 2a and 5:

Reagents

1- Saline solution: Sodium chloride solution 0.9% was prepared by dissolved (0.9 g) of sodium chloride in (100 ml) of bidistilled water.

2- DNA solution: DNA (Calf thymus) (30 mg); was dissolved in saline solution (30 ml).

3- Absolute ethanol (BDH).

4- Agarose gel.

5- Tris borate buffer (TBF) solution:

The 20 fold concentrated stock buffer solution (20x) was prepared as following (10.8 g) tris base, (5.5 g) boric acid and (0.83 g) ethylene diamine tetracetic acid disodium salt (EDTA) pH (8.0) were dissolved in (100 ml) of bidistilled water.

6- Ethedium bromide solution.

7- Loading dye:

8- Radiation source

Sodium and mercury lamps.
formation.

solution (1 ml) followed by $SnCl_2$ 1% solution were added dropwise to the reaction mixture, The blue color was appeared indicating H_3PO_4. It was prepared by mixing of 0.25% bromo phenol blue, 70%, sucrose in bidistilled water and 0.1M (EDTA) pH (8.0).

Procedure

A solution of hydroperoxide **2a** and **5** (0.006 g) in DMF (2 ml) was mixed with DNA solution. The reaction mixture was irradiated at 0ºC using sodium and mercury lamps for 17 hrs.
The photodegradation of DNA was measured at different time intervals as indicated in table (1) and table (2) respectively. The photodegradation of DNA was indicated using gel electrophoresis technique in trisborate buffer (pH= 8.0).
In presence of ethedium bromide as colouring indicator, The electrophoresis running time was about 2 hrs, the photographs of the gels were taken under UV light (365 nm)

Irradiation of 2a in benzene

A solution of hydroperoxide derivative **2a** (500 mg, 1.4×10^3 mmol) and 10 ml benzene was irradiated using (UV + IR) lamp at room temperature for three hrs, then the resulting solution, tested using $FeCl_3$ which gave very intensive violet colour, due to phenol

Table 1 Degree of DNA degradation by using hydroperoxide derivative 2a.

Lanes	Sample	Time (hr)	Degree of DNA degradation
0	Control	0	-
1	H + DNA	0	None
2	H + DNA	4	None
3	H + DNA	7	Moderate
4	H + DNA	12	Moderate
5	H + DNA	16	Moderate
6	H + DNA	20	Moderate
7	H + DNA	24	Moderate

H = hydroperoxide derivative 2a

Table 2: Degree of DNA degradation by using hydroperoxide derivative 5.

Lanes	Sample	Time (hr)	Degree of DNA degradation
0	Control	0	-
1	H` + DNA	0	None
2	H` + DNA	2	None
3	H` + DNA	4	Moderate
4	H` + DNA	6	Moderate
5	H` + DNA	8	Moderate
6	H` + DNA	10	High
7	H` + DNA	12	Slight

H` = hydroperoxide derivative 5

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الأكسدة الضوئية لمشتقات السيكلوهكسينون و تأثيرها علي الحمض النووي

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الأكسدة الضوئية لمشتقات السيكلوهكسينون **1a-c** باستخدام لمبة الصوديوم و الزئبق أعطت مركبات الهيدروبيوكسيد **2a-c** و مشتقات الهيدروكسيد **3a-c**. أيضا الأكسدة الضوئية المشتق **4** أعطت ناتج واحد فقط وهو مركب الهيدروبيوكسيد **5** . من ناحية أخرى الأكسدة الضوئية لمشتق السيكلوهكسينون **7** أعطت مشتق الهيدروكسيل. **10** . بينما أكسدة مشتق السيكلوهكسينون **11** أسفرت عن مركبين **12** و**13**. عند تكوين الأيبوكسيد لمشتق السيكلوهكسينون **1b** أعطي مشتق الأيبوكسيد **14**. أظهرت مشتقات الهيدروبيوكسيد **2a** و **5** تأثير قوي علي تكسير حمض النووي الريبوزي.