Chapter 3

*Photooxygenation Studies on Limonoids*
Introduction

Limonoids are phytochemicals and they are classed as tetranotriterpenes. Ongoing studies show that limonoids are highly oxygenated, modified terpenoids and have recently attracted attention because compounds belonging to this group have exhibited a wide range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans.\textsuperscript{1-4} It appears that the addition of oxygen incorporation into ring structures and side groups to the cyclohexane ring structures contribute to the ability of secondary plant compounds to damage insects. One such oxygen arrangement found on many of the limonoid groups is an epoxide. This addition of an oxygen to two carbons formerly possessing a double bond between them is a toxic metabolite often bioactivated by enzymes in humans.\textsuperscript{5} These oxygen additions are often very reactive and often cause problems by adducting to DNA or binding enzymes.

As a health conscious society increasing concern and desire for effective cancer treatments, the limonoids are extremely attractive. With over 300 similar compounds, the bioassay of known limonoids for their anticancer and antimutagenic activities will encourage much more investigation. Like many of their secondary plant metabolite cousins, limonoids appear to be a source of countless possible resources that can benefit the human race.

For over a century, natural products have served as tools and leads for the developments of new drugs, and several natural compounds from plants and animals kingdom are now useful drugs. Moreover, plenty of plant materials for their
biologically active principles have proved to be of potential medicinal value.\textsuperscript{6-9} The photoreactivity of synthetic drugs have been intensively studied in the recent past for their photosensitizing properties, phototoxicity and phototherapeutic values and also for photodegradation studies.\textsuperscript{10-13} However, a significant and related work on photochemistry of medicinally and biologically active compounds from plants is sporadic.\textsuperscript{14-17} It is of importance to study the photoreactivity of biologically active plant metabolite for a correlation to their possible in vivo photoreactions and phototoxicity. Several natural plant extracts containing terpenoids are widely used in agriculture and medicine.\textsuperscript{18,19} Photochemical study is expected to through light on improving the stability of these compounds into the biological extracts containing terpenoids. Moreover, the significance of generation and reactions of singlet oxygen with biomolecules in plants and living systems have been recognized.\textsuperscript{20} It is now generally accepted that certain secondary plant substances have a defensive role, offering protection against predators, pathogens and competitors. It is increasingly recognized that certain of these defensive chemicals are capable of photosensitizing reactions that involve the transfer of light energy to oxygen. It is thus apparent that plants may utilize these activated forms of oxygen, such as singlet oxygen, in their own defense. Other secondary plant products may have a physiological role in that they protect the plant against damaging photodynamic reactions by quenching the excited singlet state of oxygen. Within the context we have investigated the photooxidation reactions of the following terpenoids:

[A] Photooxidation of fissinolide.

[B] Sensitized photooxygenation of fraxinellone, a triterpenoid from \textit{Melia azedarach}. 
[A] Photooxidation of fissinolide

Limonoids, a novel class of tetranotriterpenoids is the constituents of several medicinal plants and is well known for their medicinal values, e.g. antifungal, bactericidal and antiviral, growth regulating and antifeedant activity. We have initially investigated photooxidation of fissinolide in its reaction with singlet oxygen ($^{1}$O$_{2}$) using different reaction media.

The dye sensitized photooxygenation of furans has been the subject of extensive study. Furan behaves as a typical 1,4-diene and undergoes [4$\pi$ + 2$\pi$] cycloaddition with dienophile ($^{1}$O$_{2}$) produced in situ by the photo-dye-sensitization. The reaction is thought to proceed by way of a cyclic peroxide formed by 1,4-addition of oxygen, which further transforms into oxygenation products. The secondary plant products are known to have a physiological role in that they protect the plant against damaging photodynamic reactions by quenching the excited singlet state of oxygen. The furan moiety in fissinolide may be susceptible to attack by $^{1}$O$_{2}$. Several limonoids are known to occur naturally along with fissinolide hence it is also of interest to study photooxygenation of fissinolide in order to have knowledge of its parallel reaction with $^{1}$O$_{2}$ in plants.
Experimental

Instrumentation and chemicals

All chemicals used were of analytical grade. Fissinolide (1) was extracted from the bark of Khaya senegalensis as described in the literature. UV spectra were recorded on a Shimadzu 160 A instrument. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RXI. \(^1\)H-NMR and \(^{13}\)C-NMR spectra were recorded on a Bruker Avance DRX-300 spectrometer using TMS as internal standard and CDCl\(_3\) as solvent. EIMS were obtained on a VG-ZAB-HS mass spectrometer. High-resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer. Gas chromatography was carried out with a Perkin Elmer model 154 (thermal chromatograph conductivity detector). Merck silica gel 60 F\(_{254}\) plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70-230 mesh).

Irradiation procedure

Irradiation of fissinolide in benzene

Compound 1 (100 mg, 0.195mM) was dissolved in benzene (250 ml) and the solution was irradiated, under continuous bubbling of air, with a light from a 400W medium pressure mercury lamp housed in a water cooled immersion well quartz photo-reactor. The Progress of reaction was monitored by thin layer chromatography (TLC), which indicated gradual disappearance of starting material. When the rate of product formation became negligible, solvent was removed and the residue was purified by
TLC on silica gel, eluting with 50% ether-hexane, where it yielded hydroxybutenolide (4) and epoxylactone (6) as the products (Scheme 3.1).

**23-hydroxy-fissinolide (4):** Yield: 34.24 mg; mp 292 °C; HRMS calcd. for (M+) C_{29}H_{36}O_{10} 544.5988 found 544.5980; IR ν_{max}^{cm^{-1}}: 3620, 1765, 1732, 875; ¹H-NMR (CDCl₃) δ 1.11 (s,3H, H-28, H-29 & OOCCH₃), 1.26 (s,3H, H-18 & H-19), 1.70;1.45 (t,2H, H-12), 1.74;1.49 (t, 2H, H-11), 2.0 (s, 1H,OH), 2.16 (t, 1H, H-9), 2.33 (d, J= 10.5 Hz, 1H, H-2), 2.33;2.08 (d, J=15 Hz, 2H, H-30), 2.34;2.09 (d, 2H, H-6), 2.39 (s, 3H, OOCCH₃), 2.97;2.87 (s, 2H, H-15), 3.50 (d, J= 10.5 Hz, 1H, H-3), 3.67 (t, 1H, H-5), 4.66 (s, 1H, H-17), 6.65 (d, j=1.8 Hz, 1H, H-23), 7.62 (d, 1H, H-22); ¹³C-NMR (CDCl₃) δ 11.5 (OOCCH₃), 17.5 (C-19),19.5 (C-28, C-29 & C-18), 20.1 (C-11), 26.4 (C-30), 29.6 (C-6), 29.8 (C-12), 33.3 (C-15), 38.3 (C-13), 39.2 (OOCCH₃), 41.5 (C-4), 44.3 (C-9), 44.8 (C-10), 45.3 (C-2), 51.9 (C-5), 64.0 (OOCCH₃), 84.7 (C-3), 85.8 (C-17), 130.8 (C-8), 136.7 (C-14), 136.9 (C-20), 142.9 (C-22), 169.5 (C-16), 170.0 (C-21), 173.1 (OOCCH₃), 216.7 (C-1). Anal. Calc. for C_{29}H_{36}O_{10} : C 63.96, H 6.66, found: C 63.92, H 6.64. m/z 544 (M+).

**20, 22-epoxy-fissinolide (6):** Yield: 17.45 mg; mp 288 °C; HRMS calcd. for (M+) C_{29}H_{36}O_{10} 544.5988 found 544.5982; IR ν_{max}^{cm^{-1}}: 1765, 1732, 875; ¹H-NMR (CDCl₃) δ 1.11 (s,3H, H-28, H-29 & OOCCH₃), 1.26 (s,3H, H-18 & H-19), 1.70;1.45 (t,2H, H-12), 1.74;1.49 (t, 2H, H-11), 2.16 (t, 1H, H-9), 2.33 (d, J= 10.5 Hz, 1H, H-2), 2.33;2.08 (d, J=15 Hz, 2H, H-30), 2.34;2.09 (d, 2H, H-6), 2.39 (s, 3H, OOCCH₃), 2.97;2.87 (s, 2H, H-15), 3.50 (d, J= 10.5 Hz, 1H, H-3), 3.67 (t, 1H, H-5), 3.75 (t, 1H, H-22), 4.55;4.30 (d, 2H, H-23),4.92 (s, 1H, H-17); ¹³C-NMR (CDCl₃) δ 11.5 (OOCCH₃), 17.5 (C-19),19.1 (C-18), 19.5(C-28 & C-29), 20.0 (C-11), 26.4 (C-30),
29.6 (C-6), 29.4 (C-12), 33.2 (C-15), 34.7 (C-13), 39.2 (OOCCH₃), 41.5 (C-4), 43.5 (C-22), 44.3 (C-9), 44.8 (C-10), 45.3 (C-2), 51.9 (C-5), 55.3 (C-20), 64.0 (OOCCH₃), 69.9 (C-23), 84.5 (C-17), 84.7 (C-3), 130.8 (C-8), 136.7 (C-14), 169.5 (C-16), 173.1 (OOCCH₃), 174.0 (C-21), 216.7 (C-1). Anal. Calc. for C₃₀H₄₀O₁₀: C 63.96, H 6.66, found: C 63.93, H 6.65. m/z 544 (M⁺).

**Photosensitized oxygenation of fissinolide (1) in methanol**

Compound 1 (100 mg, 0.195 mM), was dissolved in 250 ml MeOH containing 100 mg of rose bengal. The solution was irradiated with a 400W medium pressure mercury lamp in a water-cooled immersion well type quartz photo reactor with continuous supply of O₂. The progress of the reaction was monitored by TLC (silica gel, ether-hexane). When the rate of product formation became negligible solvent was evaporated in a rotary evaporator, and the residue taken up in ether, the ether was washed with water, treated with activated charcoal, dried and evaporated to yield 7 as colorless oil (Scheme 3.2).

**23-methoxy-21-hydroperoxy-dihydrofissinolide (7):** Yield: 33.20 mg; mp 280 °C; HRMS calcd. for (M⁺) C₃₀H₄₀O₁₁ 576.6410 found 576.6400; IR ν_max cm⁻¹: 3515, 2135, 1835, 1716, 1650, 1250; ¹H-NMR (CDCl₃) δ 1.11 (s,3H, H-28, H-29 & OOCCH₃), 1.26 (s,3H, H-18 & H-19), 1.70;1.45 (t,2H, H-12), 1.74;1.49 (t, 2H, H-11), 2.16 (t, 1H, H-9), 2.33 (d, J= 10.5 Hz, 1H, H-2), 2.33;2.08 (d, J=15 Hz, 2H, H-30), 2.34;2.09 (d, 2H, H-6), 2.39 (s, 3H, OOCCH₃), 2.97;2.87 (s, 2H, H-15), 3.24 (OCH₃), 3.50 (d, J= 10.5 Hz, 1H, H-3), 3.67 (t, 1H, H-5), 4.66 (s, 1H, H-17), 5.77 (d, J= 1.8 Hz, 1H, H-22), 5.78 (d, J= 1.8 Hz, 1H, H-23), 6.16 (s,1H, H-21), 8.14 (s,1H, -OOH); ¹³C-NMR (CDCl₃) δ 11.5 (OOCCH₃), 17.5 (C-19), 19.5(C-28 & C-29), 19.8 (C-18), 20.1 (C-
Rearrangement of 7 under acidic condition

Compound 7 (0.2 mM) was taken in MeOH to which 5% HCl was added until the solution became cloudy. The mixture was refluxed for 2 hr, cooled, diluted with water, and extracted with ether to yield a compound identified as 4. All the spectral values in IR, $^1$H-NMR, $^{13}$C-NMR and mass spectra were found to correspond to that of 4 (Scheme 3.3).

Pyrolysis of Photoproduct 7

A sample of 7 (0.2 mM) was taken in benzene and injected into the gas chromatograph (column, 200$^\circ$, injection block 250$^\circ$). A single product as 8 was formed. The product was collected from the gas chromatograph (Scheme 3.3).

23-methoxy-fissinolide (8): Yield: 28.15 mg; UV $\lambda_{\text{max}}$ 210 nm; mp 282$^\circ$C; HRMS calcd. for (M$^+$) C$_{30}$H$_{38}$O$_{10}$ 558.6257 found 558.6250; IR $\nu_{\text{max}}$ cm$^{-1}$: 3460, 2955, 1765, 1225, 876; $^1$H-NMR (CDCl$_3$) $\delta$ 1.11 (s,3H, H-28, H-29 & OOCCH$_3$), 1.26 (s,3H, H-18 & H-19), 1.70;1.45 (t, 2H, H-12), 1.74;1.49 (t, 2H, H-11), 2.16 (t, 1H, H-9), 2.33 (d, J= 10.5 Hz, 1H, H-2), 2.33;2.08 (d, J=15 Hz, 2H, H-30), 2.34;2.09 (d, 2H, H-6), 2.39 (s, 3H, OOCCH$_3$), 2.97;2.87 (s, 2H, H-15), 3.24 (OCH$_3$), 3.50 (d, J= 10.5 Hz, 1H, H-3), 3.67 (t, 1H, H-5), 4.66 (s, 1H, H-17), 6.27 (d, J= 1.8 Hz, 1H, H-23), 7.62 (d,
J= 1.8 Hz, 1H, H-22); $^{13}$C-NMR (CDCl$_3$) δ 11.5 (OOCCH$_3$), 17.5 (C-19), 19.5 (C-28 & C-29 & C-18), 20.1 (C-11), 26.4 (C-30), 29.6 (C-6), 29.8 (C-12), 33.3 (C-15), 38.3 (C-13), 39.2 (OOCCH$_3$), 41.5 (C-4), 44.3 (C-9), 44.8 (C-10), 45.3 (C-2), 51.9 (C-5), 53.4 (OCH$_3$), 64.0 (OOCCH$_3$), 84.7 (C-3), 85.8 (C-17), 102.3 (C-23), 130.8 (C-8), 136.7 (C-14), 136.9 (C-20), 142.9 (C-22), 169.5 (C-16), 170.0 (C-21), 173.1 (OOCCH$_3$), 216.7 (C-1). Anal. Calc. for C$_{30}$H$_{38}$O$_{10}$: C 64.50, H 6.86, found: C 64.48, H 6.84. m/z 558 (M$^+$.)

Reduction of 7 with triphenylphosphine

A solution of 7 (0.2 mM) in ether was added drop wise to a refluxing solution of triphenylphosphine in 30 ml ether during 1.5 h. The solution was refluxed 1 h, chilled to -5°C and filtered to remove the triphenylphosphine oxide. The ether was removed with a rotary evaporator, and the residue was chromatographed on silica gel column to give 11 (Scheme 3.3).

23-dihydrofissinolide (11): Yield: 28.80 mg; UV $\lambda_{\text{max}}$ 211 nm; $\text{mp}$ 276°C; HRMS calcd. for (M$^+$) C$_{29}$H$_{36}$O$_{11}$ 528.5994 found 528.5982; IR $\nu_{\text{max}}$ cm$^{-1}$: 3465, 2952, 1765, 1715, 1650, 874; $^1$H-NMR (CDCl$_3$) δ 1.11 (s, 3H, H-28, H-29 & OOCCH$_3$), 1.26 (s, 3H, H-28 & H-19), 1.70; 1.45 (t, 2H, H-12), 1.74; 1.49 (t, 2H, H-11), 2.16 (t, 1H, H-9), 2.33 (d, J= 10.5 Hz, 1H, H-2), 2.33; 2.08 (d, J= 15 Hz, 2H, H-30), 2.34; 2.09 (d, 2H, H-6), 2.39 (s, 3H, OOCCH$_3$), 2.97; 2.87 (s, 2H, H-15), 3.50 (d, J= 10.5 Hz, 1H, H-3), 3.67 (t, 1H, H-5), 4.66 (s, 1H, H-17), 4.92 (d, J= 1.8 Hz, 1H, H-23), 7.62 (d, J= 1.8 Hz, 1H, H-22); $^{13}$C-NMR (CDCl$_3$) δ 11.5 (OOCCH$_3$), 17.5 (C-19), 19.5 (C-18), 20.1 (C-11), 26.4 (C-30), 29.6 (C-6), 29.8 (C-12), 33.3 (C-15), 38.3 (C-13), 39.2 (OOCCH$_3$), 41.5 (C-4), 44.3 (C-9), 44.8 (C-10), 45.3 (C-2), 51.9 (C-5), 64.0
(OOCCH₃), 70.2 (C-23) 84.7 (C-3), 85.5 (C-17), 130.8 (C-8), 136.7 (C-14), 136.9 (C-20), 144.0 (C-22), 169.5 (C-16), 170.0 (C-21), 173.1 (OOCCH₃), 216.7 (C-1).

Anal. Calc. for C₂₉H₃₆O₁₁: C 65.89, H 6.86, found: C 65.87, H 6.88. m/z 528 (M⁺).

Irradiation of 1 in Silica gel bound Rose bengal

Compound 1 (100 mg, 0.195 mM), [P₄]–rose bengal²⁵ (200 mg, 6.5 mg/g) and 100 ml of methanol were placed in the photochemical reactor and irradiated at 10°C in the presence of bubbling oxygen. The progress of reaction was monitored by TLC. After 10 h of irradiation, the reaction mixture was removed, washed with methanol and chromatographed on silica gel to give two products, identified to be same as 4 and 6 by comparison of their spectral data. It was found that upon standing the reaction mixture and so also on addition of dil HCl in the reaction mixture, the product 6 (epoxy lactone) converted into hydroxy butenolide 4 (Scheme 3.2).

Result and Discussion

Irradiation of fissinolide (1) in benzene under continuous air bubbling with quartz filtered light from a medium pressure mercury lamp, and purification of the crude product by silica gel column chromatography afforded compound 4 and 6, identified as 23-hydroxy-fissinolide and 20,22-epoxy-fissinolide, respectively. Both the fissinolide (1) and hydroxybutenolide (4) have been isolated from the same plant species and it has been indicated that 1 is probable natural artefact of 4.²⁴

The mechanism of formation of γ-hydroxybutenolide (4) and epoxylactone (6) is depicted in Scheme 3.1. [4π+2π] Cycloaddition of O₂ to furan moiety of 1 gives an unstable ozonide peroxide intermediate (2), which by homolytic cleavage of O-O
bond produces diradical intermediate 3. Intermediate 3 on epoxycyclization followed by 1,2–hydrogen shift gives compound 6. In an alternative competitive path a 1,4-hydrogen migration in the intermediate 3 gives product 4 (Scheme 3.1). Cyclic peroxides are generally unstable, however in some cases stable peroxides have been isolated.\[^{26}\] The participation of \(^1\)O\(_2\) in this reaction was confirmed by studying the effect of DABCO (singlet oxygen scavenger) on the yields of photooxidation products. The drastic lowering of the yield of products in presence of DABCO confirms that \(^1\)O\(_2\) is active oxidizing species in this photoreaction. Also no reaction was observed on conducting experiments under nitrogen atmosphere.

The \(^1\)H-NMR and \(^{13}\)C-NMR spectrum of compound 4 were similar to those of 1 except for the furan signals. The extra carbonyl resonance at \(\delta\) 170.0 ppm indicated an additional lactone carbonyl compared to that of parent compound. The absence of C/H NMR signals due to furan moiety indicated that the furan ring had been the site of attack. \(^{13}\)C-NMR signals at \(\delta\) 173.1 ppm (carbon having no proton), indicated that the carbon must be attached to two oxygen atoms.

Further, \(\delta\) 7.23 and 6.13, the H-23 and H-22 signal in compound 1 changes to \(\delta\) 6.65 and 7.62 ppm, suggesting that double bond between C\(_{22}\)-C\(_{23}\) in 1 is shifted to C\(_{20}\)-C\(_{22}\) in 4, and the carbon connected to two oxygen atoms must be adjacent to \(\beta\) carbon of the \(\alpha\), \(\beta\)-unsaturated ketone system. The presence of other carbon signals at 170.0, 142.9, 136.9 and 96.7 ppm indicated that the furan ring has been modified to a \(\gamma\)-hydroxybutenolide moiety. The compound was thus assigned structure as 4 with a molecular formula C\(_{29}\)H\(_{36}\)O\(_{11}\) (M\(^+\), 544).
Scheme 3.1
The spectral data of photoproduct 6 was almost identical to that of starting compound 1, except for the values corresponding to an epoxide at C_{20}-C_{22} and an epoxy lactone in place of furan ring. This is evidenced by the following changes in the furan moiety at δ 7.23, 6.13, ppm (C-23 & C-22) in 1 changed to δ 4.55, 4.30 (C-23), 3.75 ppm (C-22) of methylene carbons in 6, and signal at δ 7.11 disappear in the compound 6, suggesting that the change has occurred at C_{22}-C_{23}. Further $^{13}$C-NMR value at δ 55.3 ppm (C-20) suggested that initially sp$^2$ hybridized carbon changed to quaternary carbon. This suggested that both the double bonds of furan ring were utilized in epoxide and lactone formation. $^{13}$C-NMR exhibited signal due to lactone carbonyl at δ 174.0 ppm which is supported by the IR bands at 1765 cm$^{-1}$ (lactone) and 1732 cm$^{-1}$ (carbonyl group).

Photooxygenation of 1 in methanol gave a compound identified as a crystalline hydroperoxide whose properties require that it should have structure 7. The compound has absorption bands at 3515 cm$^{-1}$ (-OOH) and 1250 cm$^{-1}$ (C-O) but none in –C=O region indicating it to be a hydroperoxide. Its $^1$H-NMR spectrum has significant signals at δ 8.14 (1H, OOH, exch.) and δ 3.24 (3H, OCH$_3$) consistent with structure 7. A quantitative Zeisel determination indicated the presence of one OMe group and the result of quantitative peroxide and active hydrogen determinations were consistent with the presence of one O-O and one OH group. When 1 was irradiated in presence of [P$_2$]-rose bengal$^{25}$ in methanol under bubbling oxygen a mixture of products 4 and 6 was obtained. It was found that upon standing the reaction mixture and so also on addition of dil HCl in the reaction mixture, the product 6 (epoxy lactone) converted into hydroxy butenolide 4 (Scheme 3.2).
Treatment of methoxy hydroperoxide 7 with methanolic HCl gave a product identified as 4. Whereas pyrolysis of 7 produced 8 (Scheme 3.3). The structure of 8 was readily established by its spectral and chemical properties. In the IR, the compound absorbs at 1765 cm\(^{-1}\), characteristics of \(\gamma\)-oxygenated \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone functionality. In the UV as well, the absorption characteristics of this chromophore occurred at \(\lambda_{\text{max}}\) 210 nm. The NMR spectrum clearly showed the presence of a –OMe group at \(\delta\) 3.24 (3H). The presence of the OMe group was confirmed by a quantitative zeisel determination. Reduction of 7 with triphenylphosphine in ether gave a product which was identified as 11. Its formation could be realized via unstable hemiacetal 10 (Scheme 3.3). Its IR absorption at 1765 cm\(^{-1}\) and UV \(\lambda_{\text{max}}\) 211 nm are characteristic of an \(\alpha, \beta\)-unsaturated \(\gamma\)-lactone. The compound was identified as 23-dihydrofissinolide (11).
Scheme 3.2
Scheme 3.3
[B] Sensitized photooxygenation of fraxinellone, a triterpenoid from *Melia azedarach*.

Fraxinellone (12), a triterpenoid isolated from *Melia azedarach*, a plant of recognized medicinal values which is widely used as antiplatelet-aggregation and vascular relaxing activities.\(^\text{27}\) In spite of immense medicinal use of this plant extract the photochemical sensitivity of their bioactive constituents has not been described in the literature. The 3-substituted furan moiety is quite susceptible to attack by biological oxygens; we therefore, have investigated photooxygenation of fraxinellone under different combinations of sensitizer dyes and solvents.

**Experimental**

**Apparatus and chemicals**

Irradiations were carried out in a photoreactor equipped with medium pressure mercury vapour lamp inserted in a water-cooled immersion well with continuous supply of water. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RXI. \(^1\)H-NMR and \(^{13}\)C-NMR spectra were recorded on a Bruker Avance DRX-300 spectrometer using SiMe\(_4\) as internal standard and CDCl\(_3\) as solvent. Elemental analyses were carried on a Carlo Erba model 1108 Elemental analyzer. High-resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer. All solvents and chemicals used were of HPLC and pharmaceutical grade. Fraxinellone was isolated from root bark of *Melia azedarach* according to literature procedure.\(^\text{28}\) The purity of 12 was determined by comparison of its melting point and \(^1\)H-NMR with that of literature value. Merck silica gel 60 F\(_{254}\) plates were used for
analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 mesh).

**Irradiation procedure**

Irradiation of air-saturated solution of fraxinellone (12) (100mg/200mL, 1.5 mM) in benzene with methylene blue (0.01 gm, 10% wt/wt of fraxinellone) as sensitizer was carried out with medium pressure mercury vapour lamp (125 W) for 8 hours. Complete decomposition of 12 was monitored by thin layer chromatography (ethyl acetate: hexane; 3 : 7). Removal of solvent under reduced pressure and column chromatography of the resulting photoproduct on silica gel yielded compound 13 and 14. This photoreaction was also carried out under nitrogen atmosphere. The solutions to be irradiated were saturated with air/nitrogen prior to irradiation and were continuously bubbled during irradiation.

Similar experiments were carried out by using different combinations of solvents and sensitizers (Table 3.1 and 3.2). Two different sets of reactions were also carried out in similar way by using DABCO/sodium azide (10% wt/wt of fraxinellone) with methylene blue as sensitizer.

**Characterization of products**

(3R,3aR)-3-(5-hydroxy-2-oxo-2,5-dihydrofuran-3-yl)-3a,7-dimethyl-3a,4,5,6-tetrahydroisobenzofuran-1(3H)-one (13): mp 118°C; Rf 0.52; [α]$_D$ $^2$ - 45 (c 0.1, EtOH); HRMS: [M$^+$] calcd. for C$_{14}$H$_{16}$O$_5$, 264.278 found, 264.272; IR (KBr) ν 3448, 3392, 2880, 1755, 1675, 1505 cm$^{-1}$; $^1$H-NMR (CDCl$_3$, δ, ppm): 1.24 (s, 3H, H-9), 1.68 ;1.46 (t, 2H, H-4), 1.69;1.62 (t, 2H, H-5), 1.72 (s, 3H, H-8), 2.24;2.21 (t, 2H, H-
(3R,3aR)-3a,7-dimethyl-3-[(1S)-3-oxo-4,6-dioxa-bicyclo[3.1.0]hexan-1-yl]-3a,4,5,6-tetrahydroisobenzofuran-1(3H)-one (14): mp 105°C; R$_f$ 0.60; [α]$^{22}_D$ -43.5 (c 0.4, EtOH); HRMS : [M$^+$] calcd. for C$_{14}$H$_{16}$O$_5$, 264.278 found, 264.271; IR (KBr) ν 3150, 1750, 1710, 1660, 1210, 950, 745 cm$^{-1}$; $^1$H-NMR (CDCl$_3$, δ, ppm): 1.25 (s, 3H, H-9), 1.68;1.46 (t, 2H, H-4), 1.69;1.58 (t, 2H, H-5), 1.73 (s, 3H, H-8), 2.23;2.22 (t, 2H, H-6),2.31;2.26 (s, 2H, H-4'), 4.27 (s, 1H, H-3), 5.21 (s, 1H, H-2'); $^{13}$C-NMR (CDCl$_3$, δ, ppm): 16.7 (C-8), 18.6 (C-5), 25.2 (C-9), 27.5 (C-3a), 35.2 (C-4'), 32.6 (C-6), 37.2 (C-4), 55.3 (C-3'), 78.5 (C-2'), 97.2 (C-3), 137.5 (C-7a), 146.3 (C-7), 172.5 (C-1), 176.2 (C-5'). Anal. Calc. for C$_{14}$H$_{16}$O$_5$ : C 63.63, H 6.10, found: C 63.61, H 6.11. m/z 264 (M$^+$).

(3R,3aR)-3-(5-hydroperoxy-2-methoxy-2,5-dihydrofuran-3-yl)-3a,7-dimethyl-3a,4,5,6-tetrahydroisobenzofuran-1(3H)-one (15): mp 112°C; [α]$^{22}_D$ -35.8 (c 0.5, EtOH); HRMS : [M$^+$] calcd. for C$_{15}$H$_{20}$O$_6$, 296.3202 found, 296.3200; IR (KBr) ν 3445, 3178, 2882, 1745, 1672 cm$^{-1}$; $^1$H-NMR (CDCl$_3$, δ, ppm): 1.27 (s, 3H, H-9), 1.68;1.42 (t, 2H, H-4), 1.69;1.61 (t, 2H, H-5), 1.73 (s, 3H, H-8), 2.23;2.21 (t, 2H, H-6), 8.23 (brs, exch., -OOH), 3.27 (s, 3H, OCH$_3$), 5.75 (d, J= 0.7 Hz, 1H, H-4'), 5.72 (s, 1H, H-2'), 6.22 (d, J= 1.8 Hz, 1H, H-5'); $^{13}$C-NMR (CDCl$_3$, δ, ppm): 16.7 (C-8),
18.6 (C-5), 25.3 (C-9), 31.8 (C-3a), 32.5 (C-6), 37.6 (C-4), 53.7 (OCH₃), 80.3 (C-3), 97.5 (C-2’), 110.9 (C-5’), 114.6 (C-4’), 139.3 (C-7a), 144.5 (C-7), 145.2 (C-3’), 176.2 (C-1). Anal. Calc. for C₁₅H₂₀O₆: C 60.80, H 6.80, found: C 60.77, H 6.78. m/z 296 (M⁺).

**Results and discussion**

Irradiation of air-saturated benzene solution of f raxinellone with methylene blue as sensitizer in a water-cooled immersion well type photoreactor equipped with medium pressure mercury vapour lamp and purification of the crude product by silica gel column chromatography afforded two compound 13 and 14. When fraxinellone was irradiated with methylene blue in methanol, the chromatographic analysis (TLC) of irradiated mixture did not show the presence of any of the previously identified products (13 and 14), rather a new product 15 was observed (Scheme 3.4). When these photoreactions were carried out in the absence of sensitizer same products were obtained but the reaction was observed to be slow.

The effect of nature of solvent on photooxidation was studied by using different solvents. The amount of substrate could not be kept same, as the solubility of substrate was different in different solvents. Therefore, relative yield of products was determined in these cases. For this purpose, different reaction mixtures were irradiated under standard conditions for the same time period. Then 15 ml of each solution was taken out, concentrated and subjected to preparative TLC for the isolation of the products, and correlation of their yields. Yields of products in different solvents were found to vary with the polarity of the solvent. The yield was
higher in polar solvents in comparison to non-polar solvents (Table 3.1). This observation may be attributed to longer lifetime of $^1\text{O}_2$ in polar solvents.\textsuperscript{29, 30} Owing to the solubility problem, the concentration of 12 was not same in all the solutions, as it was of methylene blue therefore, the possibility of energy transfer for different yields of products cannot be discarded. To confirm whether energy transfer or longer lifetime of $^1\text{O}_2$ is responsible for different yields of products, we conducted experiments by varying the concentration of sensitizer ($5\times10^{-3}$ to $2\times10^{-2}$ mol L$^{-1}$) to the concentration of fraxinellone in different solvents. Similar product patterns were obtained in these cases also, which supports the fact that lifetime of $^1\text{O}_2$ and in turn polarity of solvent is responsible for the observed difference in the yields. The dependence of percentage yield of products on triplet energies of various sensitizers has also been studied. It was observed that rose bengal and methylene blue was much more efficient than riboflavin and benzophenone in the photosensitized decomposition of 12 (Table 3.2). This may be due to the fact that rose bengal and methylene blue, with lower triplet energies, produce singlet oxygen in large amount\textsuperscript{31,32} by type II mechanism.\textsuperscript{33}
Scheme 3.4
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Lifetime of $^1\text{O}_2$ (^{(a)}) (µs)</th>
<th>Yields of products (%) (^{(b)}) (2+3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>24</td>
<td>32.2 (19.5+12.6)</td>
</tr>
<tr>
<td>Acetone</td>
<td>26</td>
<td>31.4 (18.7+12.5)</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>30</td>
<td>35.1 (22.7+12.3)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>60</td>
<td>40.3 (27.2+13.4)</td>
</tr>
</tbody>
</table>

Concentration of fraxinellone =100 mg/200 mL, 1.5 mM. Concentration of methylene blue = 10% wt/wt of fraxinellone. Time of irradiation = 4 hours. 

\(^{(a)}\) See refs. [29, 30]. \(^{(b)}\) Yields of the products were determined after isolation according to experimental part.

**Table 3.1** Yields of products, by methylene blue photosensitized reaction of fraxinellone with different solvents.
<table>
<thead>
<tr>
<th>Sensitizer</th>
<th>Triplet energy&lt;sup&gt;a&lt;/sup&gt; (Kcal/mole)</th>
<th>Yields of products (%)&lt;sup&gt;b&lt;/sup&gt; (2+3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td>33.5 – 34.0</td>
<td>31.6 (19.2+12.7)</td>
</tr>
<tr>
<td>Rose bengal</td>
<td>39.2 – 42.2</td>
<td>30.2 (17.2+13.3)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>57.8</td>
<td>21.1 (11.2+9.8)</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>68.6 – 69.1</td>
<td>19.8 (9.8+10.1)</td>
</tr>
</tbody>
</table>

Concentration of fraxinellone =100 mg/200mL, 1.5 mM. Concentration of dye = 10% wt/wt of fraxinellone. Time of irradiation = 4 hours. <sup>a</sup>See ref. [34].<sup>b</sup>Yields of the products were determined after isolation according to experimental part. Benzene was used as solvent.

**Table 3.2** Effect of triplet energies of different sensitizers on the yields of products.
On other hand riboflavin and benzophenone (higher triplet energies) act mainly by type I photosensitized photooxidation, do not produce significant amount of $^1\text{O}_2$. The participation of $^1\text{O}_2$ in the reaction was confirmed by studying the effect of some scavengers on the yield of this photooxidation reaction. The drastic lowering of the yield of products in presence of scavengers (DABCO-17%; sodium azide-14%) confirms that $^1\text{O}_2$ is active oxidizing species in this photoreaction. Also no reaction was observed on conducting experiments under nitrogen atmosphere, which further support the involvement of $^1\text{O}_2$ in this photoreaction. When irradiations were carried out by using silica bound rose bengal, same products were obtained but the reaction was observed to be slow.

The structure of the photoproducts was assigned on the basis of IR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis studies. The spectral data of photoproducts 13, 14 and 15 were found to be similar with that of 12 except for the furan signals. The furan ring had been site of attack is evident from absence of carbon/hydrogen signals due to furan moiety in the spectral data of all the identified photoproducts. The spectral study suggested that product 13 now posses δ-hydroxy butenolide moiety instead of furan moiety. The additional IR bands at 3392 cm$^{-1}$ (hydroxy group), 1675 cm$^{-1}$ ($\alpha,\beta$-unsaturated ketone) and extra carbonyl resonance at $\delta$ 175.7 suggested an extra lactone carbonyl compared to that of parent compound. The $^{13}$C-NMR signal at $\delta$ 97.6 indicate that carbon must be attached to two oxygen atoms. This proton is in split with the proton of $\alpha,\beta$-unsaturated carbon at $\delta$ 6.67 suggested that carbon attached to two oxygen atoms must be adjacent to $\alpha,\beta$-unsaturated carbon. The change of furan ring to δ-hydroxy butenolide moiety is also evident from the two carbon signals at $\delta$ 136.7.
and 172.2 instead of the olefinic signals of furan at δ 124.5 and 139.5. The appearance of double signals for C-5’ indicated that compound 13 to be an epimeric mixture at C-5’.

The spectral data for compound 14 was also found to be similar with that of 12 except for the furan signals. On the basis of following spectral data we conclude the presence of epoxy lactone in 14. The 13C-NMR spectrum indicated an additional lactone carbonyl resonance at δ 172.5. A signal at δ 78.5 was assigned to a dioxygenated carbon of the epoxylactone ring, with additional support from its 1H-NMR signal at δ 5.21. Of the other carbons of the lactone ring, a carbon resonance value at δ 35.2 along with a proton resonance at δ 2.3 was assigned to the methylene carbon and at δ 55.3 assigned to the quaternary carbon. The formation of lactone ring gets additional support from the IR spectrum of 14, which shows characteristic absorption for two lactone carbonyl at 1750 and 1710 cm⁻¹ and for the epoxide ring at 3150, 1210, 950 and 745 cm⁻¹. The appearance of double signals for C-5’ indicated compound 14 to be an epimeric mixture at C-5’.

The compound 15 was having a comparably similar spectral data to 12, with a basic difference in furan ring values. It was shown to contain a 2,5-dihydrofuran ring with an allylic hydroperoxy and a methoxy group. The 1H-NMR spectrum recorded a highly deshielded signal at δ 8.2 (brs, exch., 1H) and a three proton singlet at δ 3.27, which were assigned to the allylic -OOH group (at C-5’) and -OMe group (at C-2’) respectively. This regiostructure gets support from the 1H NMR signals as a singlet at a low value of δ 5.72 for C-2’ proton and a doublet at a high value of δ 6.22 for C-5’ proton. Of the olefinic carbons a carbon resonance at δ 114.6 was assigned to C-4’
and at δ 145.2 was assigned to C-3’. The signals for the protons at C-4’ and OCH$_3$ were appropriately observed at δ 5.75 and 3.27 respectively. Additional structural information for the compound 15 was inferred from its following chemical properties: 1) with Pb(OAc)$_4$, gas was evolved, as it is characteristic of compounds containing -OOH group; 2) With potassium iodide-acetic acid solution it liberate iodine (suggested presence of O-O bond).$^{36}$

The formation of photoproducts 13, 14 and 15 can be envisaged to occur from unstable cyclic peroxide 12a, which initially results by the [4π + 2π] cycloaddition of $^1$O$_2$ to furan ring (Scheme 3.4). This unstable cyclic peroxide (12a) undergoes homolytic cleavage of O-O bond and afforded product 13 and 14 by following two competing processes:$^{37,38}$ elimination of proton from bridgehead position and a subsequent rearrangement gives product 13; and 1,2 hydrogen shift gives product 14, as mechanistically rationalized in Scheme 3.5. In the presence of polar methanol solvent, the solvolysis induced transformation of intermediate 12a leads to the formation of product 15.
Scheme 3.5
References


4. Bagge D., Available at: 


Academic, New York, 1979, p 120.


34. J. D. Coyle, Photochemistry in Organic Synthesis, Burlington house, London,
1986, p 191.

420.

36. C. S. Foote, M. T. Wuesthoff, S. Wexler, I. G. Binstain, R. Denny,
