Chapter 5

Photooxygenation Studies on Furanoterpenes
Introduction

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.\(^1\)\(^-\)\(^4\) 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.\(^5\)\(^-\)\(^8\) However, a significant and related work on photochemistry of medicinally and biologically active compounds from plants is sporadic.\(^9\)\(^-\)\(^12\) It is of importance to study the photoreactivity of biologically active plant metabolite for a correlation to their possible \textit{in vivo} photoreactions and phototoxicity. Several natural plant extracts containing terpenoids are widely used in agriculture and medicine.\(^13\)\(^,\)\(^14\) 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.\(^15\) 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 photooxidation reactions of the following terpenoids:

[A] Sensitized photooxygenation of tinosponone, a clerodane diterpene from *Tinospora cordifolia*.

Sensitized photooxygenation of tinosponone, a clerodane diterpene from *Tinospora cordifolia*

Tinosponone (1), a clerodane diterpene isolated from *Tinospora cordifolia*, a plant of recognized medicinal values which is widely used as anti-bacterial, analgesic, antipyretic and also for the treatment of jaundice, skin diseases, diabetes, anaemia etc.\(^{16-20}\) Several compounds containing 3-substituted furan moiety have been isolated from this plant species.\(^{21-23}\) 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 tinosponone 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 RX1. \(^1\)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. FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using \(m\)-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. 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 analytical grade. Tinosponone was isolated from stem of *Tinospora cordifolia* according to literature procedure.\textsuperscript{24} The purity of 1 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**

An air-saturated benzene solution of tinosponone (1) (100 mg, 1.5 mM in 200mL) was irradiated for 8 hr with a medium pressure mercury vapour lamp (125 W) in presence of methylene blue (0.01 gm, 10% wt/wt of tinosponone). Complete decomposition of 1 was monitored by thin layer chromatography (ethyl acetate: hexane; 3 : 7). Removal of the solvent under reduced pressure and column chromatography of the resulting photoproduct on silica gel yielded compound 2 and 3. This photoreaction was also carried out under nitrogen atmosphere by saturating the solution with nitrogen prior to irradiation and with continuous bubbling during irradiation.

Similar experiments were carried out by using different combinations of solvents and sensitizers (Table 5.1 and 5.2). Two different sets of reactions were also carried out in similar way by using DABCO/sodium azide (10% wt/wt of tinosponone) with methylene blue as sensitizer.
Characterization of products

\((3S,4aS,4bS,8R,8aR,10aR)-8\text{-Hydroxy-3-/(5'\text{-hydroxy-2'-oxo-2',5'-dihydrofuran-3'-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[ff]isochromene-1,5(4aH,4bH)-dione (2):}\) mp 177°C; \(R_f 0.83; \left[\alpha\right]^{23}_D -93.7 \text{ (c 0.92, CHCl}_3\); HRMS: \([M^+]\) calcd for C\(_{19}\)H\(_{22}\)O\(_7\), 362.3738; found, 362.3731; IR (KBr) v 3500, 3390, 1745, 1715, 1678, 1450 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\), \(\delta, \text{ ppm): 6.94 (d, 1H, J = 7.1 Hz, H-4'), 6.67 (dd, 1H, J = 10.8, 5.2 Hz, H-7), 6.15 (d, 1H, J = 7.0 Hz, H-5'), 5.91 (d, 1H, J = 10.8 Hz, H-6), 4.91 (dd, 1H, J = 12.2, 3.6 Hz, H-3), 4.82 (brs, exch., -OH), 4.28 (d, 1H, J = 5.1 Hz, H-8), 2.42 (dd, 1H, J = 14.8, 3.4 Hz, H-4), 2.38 (brs, 1H, H-10a), 2.21 (m, 1H, H-9), 2.20 (brs, 1H, H-4b), 2.18 (m, 1H, H-10), 1.65 (dd, 1H, J = 15.4, 12.2 Hz, H-4), 1.61 (m, 1H, H-10), 1.42 (s, -CH\(_3\)), 1.1 (dt, 1H, J = 13.8, 4.5 Hz, H-9), 0.80 (s, -CH\(_3\)); \(^{13}\)C-NMR (CDCl\(_3\), \(\delta, \text{ ppm): 204.5 (C-5), 176.2 (C-1), 175.7 (C-3), 141.5 (C-1), 143.1 (C-7), 136.5 (C-2), 127.5 (C-6), 97.5 (C-10), 74.5 (C-8), 60.5 (C-3), 52.6 (C-4b), 49.2 (C-10a), 42.9 (C-8a), 40.1 (C-4), 34.8 (C-4a), 30.9 (CH\(_3\)), 29.9 (C-9), 26.6 (CH\(_3\)), 19.2 (C-10); MS m/z (relative intensity): 345 (C\(_{19}\)H\(_{21}\)O\(_6^+\), 14), 318 (C\(_{18}\)H\(_{22}\)O\(_5^+\), 100), 303 (C\(_{18}\)H\(_{23}\)O\(_4^+\), 15), 274 (C\(_{17}\)H\(_{22}\)O\(_3^+\), 10), 263 (C\(_{15}\)H\(_{19}\)O\(_2^+\), 17), 261 (C\(_{17}\)H\(_{23}\)O\(_2^+\), 9); Anal. calcd. for C\(_{19}\)H\(_{22}\)O\(_7\): C 62.97, H 6.12, O 30.91; found C 62.84, H 6.08, O 30.98.

\((3S,4aS,4bS,8R,8aR,10aR)-8\text{-Hydroxy-4a,8a-dimethyl-3-/(1'\text{-oxo-4',6'-dioxabicyclo[3.1.0]hexan-1'-yl)-3,4,8,8a,9,10-hexahydro-10aH-benzo[ff]isochromene-1,5(4aH,4bH)-dione (3):}\) mp 164°C; \(R_f 0.62; \left[\alpha\right]^{23}_D -84.6 \text{ (c 0.47, CHCl}_3\); HRMS: \([M^+]\) calcd for C\(_{19}\)H\(_{22}\)O\(_7\), 362.3738; found, 362.3751;
IR (KBr) ν 3430, 1750, 1710, 1660, 1210, 950, 745 cm⁻¹; ¹H-NMR (CDCl₃, δ, ppm): 6.52 (dd, 1H, J=10.6, 4.8 Hz, H-7)), 5.75 (d, 1H, J=10.4 Hz, H-6), 5.23 (s, 1H, H-4), 4.87 (dd, 1H, J=12.4, 3.8 Hz, H-3), 4.30 (d, 1H, J=5.1 Hz, H-8), 4.12 (brs, exch., -OH), 2.28 (dd, 1H, J=14.2, 2.9 Hz, H-4), 2.22 (brs, 1H, H-10a), 2.36 (m, 1H, H-9), 2.2 (s, 1H, H-1), 2.16 (brs, 1H, H-4b), 1.98 (m, 1H, H-10), 1.71 (m, 1H, H-10), 1.58 (dd, 1H, J=15.3, 11.8 Hz, H-4), 1.38 (s, CH₃), 1.12 (dt, 1H, J=13.6, 4.4 Hz, H-9), 0.80 (s, CH₃); ¹³C-NMR (CDCl₃, δ, ppm): 202.6 (C-5), 175.2 (C-1), 171.1 (C-2), 145.2 (C-7), 130.7 (C-6), 120.4 (C-4), 75.2 (C-8), 59.2 (C-10), 56.8 (C-3), 52.5 (C-4b), 48.1 (C-10a), 44.2 (C-8a), 41.7 (C-4), 36.6 (C-4a), 36.2 (C-1), 31.3 (CH₃), 25.4 (CH₃), 18.8 (C-10), 18.6 (C-9); MS m/z (relative intensity): 318 (C₁₉H₂₂O₅⁺, 20), 290 (C₁₇H₂₂O₄⁺, 100), 278 (C₁₅H₁₈O₅⁻, 11), 276 (C₁₇H₂₄O₅⁺, 11), 263 (C₁₅H₁₉O₄⁺, 9), 221 (C₁₄H₂₁O₂⁺, 16). 84 (C₄H₄O₂⁺, 19); Anal. calcd. for C₁₉H₂₂O₅: C 62.97, H 6.12, O 30.91; found C 62.74, H 6.34, O 31.05.

(3S,4aS,4bS,8R,8aR,10aR)-8-Hydroxy-3-(5'-hydroperoxy-2'-methoxy-2',5'-dihydrofuran-3'-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[f]
isochromene-1,5(4aH,4bH)-dione (4): mp 169°C; [α]²³D -77.8 (c 0.34, CHCl₃); HRMS: [M⁺] calcd for C₂₀H₂₅O₈, 394.4156; found, 394.4152; IR (KBr) ν 3520, 3500, 1670, 1250 cm⁻¹; ¹H-NMR (CDCl₃, δ, ppm): 8.12 (brs, exch., OOH), 6.61 (dd, 1H, J=10.7, 5.2 Hz, H-7), 6.21 (d, 1H, J=7.4 Hz, H-10), 5.84 (s, 1H, H-3), 5.72 (d, 1H, J=7.2 Hz, H-1), 5.62 (d, 1H, J=10.8 Hz, H-6), 4.94 (dd, 1H, J=12.1, 3.2 Hz, H-3), 4.64 (brs, exch., -OH) 4.26 (d, 1H, J=4.8
Hz, H-8), 3.22 (s, 3H, -OCH3), 2.48 (dd, 1H, J=13.8, 3.2 Hz, H-4), 2.30 (brs, 1H, H-10a), 2.22 (m, 1H, H-9), 2.20 (brs, 1H, H-4b), 2.12 (m, 1H, H-10), 1.68 (dd, 1H, J=15.2, 12.8 Hz, H-4), 1.65 (m, 1H, H-10), 1.48 (s, 3H, CH3), 1.1 (dt, 1H, J=13.8, 4.0 Hz, H-9), 0.82 (s, 3H); ¹³C-NMR (CDCl₃, δ, ppm): 200.5 (C-5), 173.4 (C-1), 143.7 (C-7), 141.4 (C-2), 129.4 (C-6), 114.7 (C-1), 114.7 (C-1), 112.2 (C-10), 98.4 (C-3), 73.8 (C-8), 58.1 (C-3), 53.8 (OCH3), 51.3 (C-4b), 47.6 (C-10a), 43.1 (C-8a), 39.2 (C-4a), 33.7(C-CH3), 26.1 (C-9), 25.2 (CH3), 18.4 (C-10); MS m/z (relative intensity): 301 (C₁₈H₂₁O₄⁺, 100), 363 (C₁₉H₂₃O₇⁺, 18), 361 (C₂₀H₂₅O₆⁺, 21), 331 (C₁₉H₂₃O₅⁺, 13), 288 (C₁₇H₂₀O₄⁺, 11), 206 (C₁₄H₂₂O⁺, 15), 84 (C₅H₄O₂⁺, 21); Anal. calcd. for C₂₀H₂₆O₈: C 60.90, H 6.45, O 32.52.

**Results and discussion**

Irradiation of air-saturated benzene solution of tinosponone 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 2 (epimeric mixture) and 3. When tinosponone 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 (2 and 3), rather a new product 4 (epimeric mixture) was observed (Scheme 5.1). 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 the 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 yields of products were determined in these cases. For this purpose, different reaction mixtures were irradiated under standard condition 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 5.1). This observation may be attributed to longer lifetime of $^1\text{O}_2$ in polar solvents.\textsuperscript{25,26} Owing to the solubility problem, the concentration of 1 was not same in all the solutions, as it was in the case of methylene blue therefore, 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 tinosponone in different solvents. Similar pattern of product formation was also obtained in these cases, 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 yields of the 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 1 (Table 5.2). This may be due to the fact that rose bengal and methylene blue, with lower triplet energies, produce singlet oxygen in large amount\textsuperscript{27,28} by type II mechanism.\textsuperscript{29}
Scheme 5.1
Solvent Lifetime of $^1\text{O}_2$ (a) Yields of products (%b) (2+3)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Lifetime (μs)</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>24</td>
<td>32.3 (19.6+12.7)</td>
</tr>
<tr>
<td>Acetone</td>
<td>26</td>
<td>31.6 (18.9+12.7)</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>30</td>
<td>35.2 (22.8+12.4)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>60</td>
<td>40.4 (27.1+13.3)</td>
</tr>
</tbody>
</table>

Concentration of tinosponone =100 mg/200mL, 1.5 mM. Concentration of methylene blue = 10% wt/wt of tinosponone. Time of irradiation = 4 hours. aSee refs. [25, 26]. bYields of the products were determined after isolation according to experimental part.

Table 5.1 Yields of products, by methylene blue photosensitized reaction of tinosponone with different solvents
<table>
<thead>
<tr>
<th>Sensitizer</th>
<th>Triplet energy$^a$ (Kcal/mole)</th>
<th>Yields of products (%)$^b$ (2+3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td>33.5 – 34.0</td>
<td>31.7 (19.1+12.6)</td>
</tr>
<tr>
<td>Rose bengal</td>
<td>39.2 – 42.2</td>
<td>30.3 (17.1+13.2)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>57.8</td>
<td>21.2 (11.3+9.9)</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>68.6 – 69.1</td>
<td>19.9 (9.9+10.0)</td>
</tr>
</tbody>
</table>

Concentration of tinosponone = 100 mg/200mL, 1.5 mM. Concentration of dye = 10% wt/wt of tinosponone. Time of irradiation = 4 hours. $^a$See ref. [35]. $^b$Yields of the products were determined after isolation according to experimental part. Benzene was used as solvent.

**Table 5.2** 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 1 photosensitized photooxidation, do not produce significant amount of $^1O_2$. The participation of $^1O_2$ in the reaction was confirmed by studying the effect of some scavengers on the yield of this photooxidation reaction. The drastic lowering of the yields of the products in presence of scavengers (DABCO-17%; sodium azide-14%) confirms that $^1O_2$ is active oxidizing species in this photoreaction. Also no reaction was observed on conducting experiments under nitrogen atmosphere. 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, mass spectral and elemental analysis studies. The spectral data of photoproducts 2, 3 and 4 were found to be similar with that of 1 except for the furan signals. The furan ring has 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 studies suggested that product 2 posses $\delta$-hydroxy butenolide moiety instead of furan moiety. The additional IR bands at 3390 cm$^{-1}$ (hydroxy group), 1670 (\(\alpha, \beta\)-unsaturated ketone) and extra carbonyl $^{13}$C- resonance at $\delta$ 175.7 suggested an extra lactone carbonyl compared to that of parent compound. The $^{13}$C-NMR signal for C-5' at $\delta$ 97.5ppm (attached to proton at a downfield value of $\delta$ 6.15 ppm) indicated that carbon is attached to two oxygen atoms. This proton is in split
with the proton of $\alpha, \beta$-unsaturated carbon at $\delta$ 6.94, suggesting that carbon attached to two oxygen atoms must be adjacent to $\alpha, \beta$-unsaturated carbon. The change of furan ring to $\delta$-hydroxy butenolide moiety is also evident from the two carbon signals at $\delta$ 136.5 and 175.7 instead of the olefinic signals of furan at $\delta$ 121.5 and 139.7.

The spectral data for compound 3 was also found to be similar with that of 1 except for the furan signals. On the basis of following spectral data we conclude the presence of epoxy lactone in 3. The $^{13}$C-NMR spectrum indicated an additional lactone carbonyl resonance at $\delta$ 171.1. A signal at $\delta$ 120.4 was assigned to a dioxygenated carbon of the epoxylactone ring, with additional support from its $^1$H-NMR signal at $\delta$ 5.23. Of the other carbons of the lactone ring, a carbon resonance value at $\delta$ 36.2 along with a proton resonance at $\delta$ 2.2 was assigned to the methylene carbon and at $\delta$ 59.2 assigned to the quaternary carbon. The formation of lactone ring gets additional support from the IR spectrum of 3, 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 compound 4 was having a comparably similar spectral data to 1, 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 $^1$H-NMR spectrum recorded a highly deshielded signal at $\delta$ 8.1 (brs, exch., 1H) and a three proton singlet at $\delta$ 3.32, which were assigned to the allylic $-\text{OOH}$
group (at C-5') and -OMe group (at C-2') respectively. This regiostructure gets support from the \(^1\)H-NMR signals as a singlet at a low value of \(\delta 5.84\) for C-2' proton and a doublet at a high value of \(\delta 6.21\) for C-5' proton. Of the olefinic carbons, a carbon resonance at \(\delta 114.7\) was assigned to C-4' and at \(\delta 141.4\) was assigned to C-3'. The signals for the protons at C-4' and -OCH\(_3\) were appropriately observed at \(\delta 5.72\) and 3.32, respectively. Additional structural information for the compound 4 was inferred from its following chemical properties: 1) with Pb(OAc)\(_4\), gas was evolved, which is characteristic of compounds containing -OOH group; 2) with potassium iodide-acetic acid solution it liberated iodine, indicating presence of O-O bond.\(^{32}\)

The formation of photoproducts 2, 3 and 4 can be envisaged to occur from unstable cyclic peroxide \(1\)a, which initially results by a \([4\pi+2\pi]\) cycloaddition of \(^1\)O\(_2\) to furan ring (Scheme 5.1). This unstable cyclic peroxide (\(1\)a) undergoes homolytic cleavage of O-O bond to give a diradical which affords products 2 and 3 by following two competing processes:\(^{33,34}\) in one way the diradical intermediate formed undergoes epoxycyclization followed by 1,2-hydrogen shift gives compound 3. In an alternative competitive path a 1,4-hydrogen migration in the diradical intermediate gives product 2 (Scheme 5.2). In the presence of polar methanol solvent, the solvolysis induced transformation of intermediate \(1\)a leads to the formation of product 4.
Scheme 5.2
Photooxidation of 2β-angeloyloxy-10β-H-furanoeremophilane

Furanoeremophilanes, a novel class of sesquiterpenes is the constituents of several medicinal plants and is well known for their medicinal values e.g. antioxidant and antiradical property, toxicity and antifeedant activity. Herein we have investigated photooxidation of 2β-angeloyloxy-10β-H-furanoeremophilane (5) in its reaction with singlet oxygen \( (^1O_2) \) using different reaction media.

Experimental

Instrumentation and chemicals

Same as in section [A]

Irradiation procedure

\textit{Irradiation of 2β-angeloyloxy-10β-H-furanoeremophilane (5) in benzene}

2β-Angeloyloxy-10β-H-furanoeremophilane was isolated as described in the literature. Compound 5 (100 mg, 0.316 mM) 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 (8) and epoxylactone (10) as the products (Scheme 5.3).

\[2\beta\text{-Angeloyloxy-8-hydroxy-10\beta-H-eremophilanolide (8):}\]
Yield: 38.48 mg; mp 210° C; HRMS calcd. for \(\text{C}_{20}\text{H}_{28}\text{O}_{5}\) 348.198 found 348.199; IR \(v_{\text{max}}^{\text{cm}^{-1}}:\)
3620 (-OH), 1765 (Lactone), 1715, 1650 (C=\text{C}-\text{COOR}); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\)
1.06 (d, \(J=6.8\) Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.16 (s, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.55 (dd, \(J=17\) & 9 Hz, 1H, H-9), 1.59 (m, 1H, H-4), 1.71 (d, \(J=1.5\) Hz, 3H, H-4'), 1.75 (s, 1H, H-6), 1.76 (m, 2H, 1-H & 3-H), 1.80 (d, \(J=17\) & 9 Hz, 1H, H-9), 1.93 (s, 6H, H-5' & H-13), 2.00 (s, 1H, H-6), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\)
11.6 (C-13), 12.1 (C-4'), 16.4 (C-15), 17.5 (C-5'), 20.5 (C-14), 25.4 (C-10), 26.5 (C-6), 31.1 (C-4), 35.1 (C-1), 37.3 (C-3), 40.6 (C-9), 50.8 (C-5), 71.8 (C-2), 110.9 (C-8), 125 (C-11), 128.3 (C-2'), 138.6 (C-3'), 156.3 (C-7), 167.2 (C-1') 176.0 (C-12); El-MS m/z (rel. int.%): 349 (M+1, 25), 245 (M+1-RCOOH, 42), 218 (100).

\[2\beta\text{-Angeloyloxy-7,8-epoxy-10\beta-H-eremophilanolide (10):}\]
Yield: 19.75 mg; mp 206 °C; HRMS calcd. for \(\text{C}_{20}\text{H}_{28}\text{O}_{5}\) 348.198 found 348.197; IR \(v_{\text{max}}^{\text{cm}^{-1}}:\)
1765 (Lactone), 1715, 1650 (C=\text{C}-\text{COOR}) 1420, 1380, 1330, 1293, 1171, 964, 816, 733, 562; \(^1\)H-NMR (CDCl\(_3\)) \(\delta\)
1.06 (d, \(J=6.8\) Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.24 (d, \(J=7\) Hz, 3H, H-13), 1.33 (dd, \(J=15\) & 7 Hz, 1H, H-6), 1.41 (s, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.58 (dd, \(J=15\) & 7 Hz, 1H, H-6), 1.59 (m, 1H, H-4), 1.64 (dd, \(J=15\) & 7 Hz, 1H, H-9), 1.71 (d, \(J=1.5\) Hz, 3H, H-4'), 1.76 (m, 2H, H-1 & H-3), 1.89 (dd, \(J=15\) & 7, 1H, H-9) 1.93 (s, 3H, H-5'), 2.78
(q, J=7 Hz, 1H, H-11), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); $^{13}$C-NMR (CDCl$_3$) δ 10.3 (C-13), 12.1 (C-4'), 16.3 (C-15), 17.5 (C-5'), 20.1 (C-5), 27.4(C-10), 30.5 (C-9), 30.7(C-4), 34.4 (C-1), 36.7 (C-6), 37.2 (C-3), 71.8 (C-2), 93.3 (C-8), 128.3 (C-2'), 138.6 (C-3'), 167.2 (C-1'), 177.4 (C-12); EI-MS m/z (rel. int.%) : 347 ( M$^+$, 1), 329 (M$^+$-H$_2$O, 2), 247 (M$^+$-C$_4$H$_7$COOH, 12), 83 (C$_4$H$_7$CO$^+$, 100), 55 (83-CO, 44).

Photosensitized oxygenation of 2β-angeloyloxy-10β-H-furanoeremophilane (5) in methanol

Compound 5 (100 mg, 0.316 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 O$_2$. 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 11 as colorless oil (Scheme 5.4).

2β-Angeloyloxy-10β-H-8-methoxy-12-hydroperoxy dihydrofuranoeremophilane (11): Yield: 37.20 mg; mp 198° C; HRMS calcd. for (M$^+$) C$_{21}$H$_{32}$O$_6$ 380.219 found 380.220; IR $\nu_{\text{max}}$ cm$^{-1}$: 3514, 2130, 1830, 1715, 1650, 1250; $^1$H-NMR (CDCl$_3$) δ 1.06 (d, J=6.8 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.41 (m, 1H, H-10), 1.42 (dd, J= 15 & 7 Hz, 1H, H-9), 1.57 (m, 2H, 3H, H-14), 1.41 (m, 1H, H-10), 1.42 (dd, J= 15 & 7 Hz, 1H, H-9), 1.57 (m, 2H,
H-1). 1.51 (m, 2H, H-3), 1.59 (m, 1H, H-4), 1.67 (dd, J= 15 & 7 Hz, 3H, H-9), 1.71 (d, J=1.5 Hz, 3H, H-4'), 1.75 (dd, J=15 & 7 Hz, 1H, H-6), 1.76 (m, 2H, H-3), 1.81 (d, J=1.5 Hz, 3H, H-13), 1.93 (s, 3H, H-5'), 3.24 (s, -OCH3), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'), 8.16 (s, 1H, H-12); 13C-NMR (CDCl3) δ 7.6 (C-13), 12.1 (C-4'), 16.3 (C-15), 17.5 (C-5'), 20.5 (C-14), 21.2 (C-6), 25.8 (C-10), 35.4 (C-1), 37.3 (C-3), 39.5 (C-9), 51.4 (C-5), 51.6 (-OCH3), 71.8 (C-2), 110.3 (C-12), 112.0 (C-8), 128.3 (C-2'), 130.8 (C-11), 138.6 (C-3'), 141.7 (C-7), 167.2 (C-1'); EI-MS m/z (rel. int.%): 381 (M+ 7), 363 (M-H2O, 13), 209 (15), 180.15 (3), 83 (100).

**Rearrangement of 11 under acidic condition**

Compound 11 (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 8. All the spectral values in IR, 1H-NMR, 13C-NMR and mass spectra were found to correspond to that of 8 (Scheme 5.5).

**Pyrolysis of Photoproduc 11**

A sample of 11 (0.2 mM) was taken in benzene and injected into the gas chromatograph (column, 200°, injection block 250°). A single product as 12 was formed. The product was collected from the gas chromatograph (Scheme 5.5).
2β-Angeloyloxy-10β-H-8-methoxy-eremophilanolide (12): Yield: 31.15 mg; UV
λ<sub>max</sub> 216 nm; mp 200° C; HRMS calcd. for (M<sup>+</sup>) C<sub>21</sub>H<sub>29</sub>O<sub>5</sub> 362.208 found 362.209; IR ν<sub>max</sub> cm<sup>-1</sup>: 1779, 1760, 1698, 1650, 1533; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.06 (d, J=6.8 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.41 (m, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.53 (dd, J=15 & 7 Hz, 1H, H-9), 1.59 (m, 1H, H-4), 1.71 (d, J=15 Hz, 3H, H-4'), 1.75 (dd, J=15 & 7 Hz, 1H, H-6), 1.76 (m, 2H, H-1 & H-3), 1.78 (dd, J=15 & 7 Hz, 1H, H-9), 2.00 (dd, J=15 & 7, 1H, H-6), 3.24 (s, -OCH<sub>3</sub>), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ 7.6 (C-13), 12.1 (4'-C), 16.4 (C-15), 17.5 (C-5'), 20.5 (C-14), 21.2 (C-6), 25.8 (C-10), 31.1 (C-4), 35.4 (C-1), 39.5 (C-9), 51.4 (C-5), 51.6 (-OCH<sub>3</sub>), 71.8 (C-2), 110.3 (C-12), 112.0 (C-8), 128.3 (C-2'), 130.8 (C-11), 138.6 (C-3'), 141.7 (C-7), 167.2 (C-1'); El-MS m/z (rel. int.%): 363 (M+, 34), 332 (M<sup>+</sup>-OCH<sub>3</sub>, 13), 263 (M<sup>+</sup>-C<sub>4</sub>H<sub>7</sub>COOH, 11), 83 (C<sub>4</sub>H<sub>7</sub>CO<sup>+</sup>, 93), 55 (C<sub>4</sub>H<sub>7</sub>CO<sup>+</sup>-CO, 40).

Reduction of 11 with triphenylphosphine

A solution of 11 (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 16 (Scheme 5.5).

2β-Angeloyloxy-10β-H-eremophilanolide (16): Yield: 31.85 mg; UV λ<sub>max</sub> 217 nm; mp 195° C; HRMS calcd. for (M<sup>+</sup>) C<sub>20</sub>H<sub>28</sub>O<sub>4</sub> 332.198 found 332.199; IR ν<sub>max</sub> cm<sup>-1</sup>: 1808, 1800, 1765, 1715, 1650, 1000; <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.06 (dd,
J=15 & 7 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.40 (dd, J=15 & 7 Hz, 1H, H-9),
1.41 (m, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.59 (m, 1H, H-4), 1.65 (dd,
J=15 & 7 Hz, 1H, H-9), 1.75 (d, J=17 Hz, 1H, H-6), 1.76 (m, 2H, 1H & H-3),
1.93 (s, 3H, H-5'), 1.93 (s, 3H, H-13), 3.91 (m, 1H, H-2), 4.91 (dd br, J=6.5 &
10 Hz, 1H, H-8), 6.03 (m, 1H, H-3'); ^13C-NMR (CDCl3): δ 11.3 (C-13), 12.1
(C-4'), 16.4 (C-15), 17.5 (C-5'), 20.5 (C-14), 31.1 (C-4), 31.6 (C-10), 32.7 (C-
6), 34.0 (C-9), 34.8 (C-1), 37.3 (C-3), 50.5 (C-5), 71.8 (C-2), 81.2 (C-8), 125.9
(C-11), 128.3 (C-2'), 138.6 (C-3'), 164.6 (C-7), 167.2 (C-1'), 176.0 (C-12); EI-
MS m/z (rel. int. %): 333 (M^+, 37), 277 (M^+-C4H7, 24), 233 (M^+-C4H7COOH,
31), 83 (C4H7CO^+, 91), 55 (C4H3CO^+-CO, 13).

Irradiation of 5 in Silica gel bound Rose bengal

Compound 5 (100 mg, 0.316 mM), [Psi]-rose bengal^31 (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 8 and 10 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 10 (epoxy lactone) converted into hydroxy
butenolide 8 (Scheme 5.4).
Result and Discussion

Irradiation of furanoeremophilane (5) 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 8 and 10, identified as 2β-angeloyloxy-8-hydroxy-10β-H-eremophilanolide and 2β-angeloyloxy-7,8-epoxy-10β-H-eremophilanolide, respectively. Both the 2β-angeloyloxy-10β-H-furanoeremophilane (5) and hydroxybutenolide (8) have been isolated from the same plant species and it has been indicated that 5 is probable natural artefact of 8.45

The mechanism of formation of γ-hydroxybutenolide (8) and epoxylactone (10) is depicted in Scheme 5.3. [4π+2π] Cycloaddition of O2 to furan moiety of 5 gives an unstable ozonide peroxide intermediate (6), which by homolytic cleavage of O-O bond produces diradical intermediate 7. Intermediate 7 on epoxycyclization followed by 1,2 – hydrogen shift gives compound 10. In an alternative competitive path a 1,4-hydrogen migration in the intermediate 7 gives product 8 (Scheme 5.3). Cyclic peroxides are generally unstable, however in some cases stable peroxides have been isolated.36 The participation of O2 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 O2 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 8 were similar to those of 5 except for the furan signals. The extra carbonyl resonance at $\delta$ 177.4 ppm indicated an additional lactone carbonyl compared to that of parent compound. This was confirmed by the presence of IR bands at 1765, 1715 and 1650 cm$^{-1}$. 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 $\delta$ 110. ppm (carbon having no proton), indicated that the carbon must be attached to two oxygen atoms.

Further, $\delta$ 2.48 and 2.23, the H-9 signal in compound 5 changes to $\delta$ 1.80 and 1.55 ppm, suggesting that double bond between C$_7$-C$_8$ in 5 is shifted to C$_7$-C$_{11}$ in 8, 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 $\delta$ 177.4, 156.3, 125.9 and 11.6 ppm along with an IR band at 3620 cm$^{-1}$ indicated that the furan ring has been modified to a $\gamma$-hydroxybutenolide moiety. The compound was thus assigned structure as 8 with a molecular formula C$_{20}$H$_{28}$O$_5$ (M$^+$, 349).

The spectral data of photoproduct 10 was almost identical to that of starting compound 5, except for the values corresponding to an epoxide at C$_7$-C$_8$ and an epoxylactone in place of furan ring. This is evidenced by the following changes in the methylene carbon signals: $\delta$ 2.60, 2.35 ppm (C-6) and $\delta$ 2.48, 2.23 ppm (C-9), changed to $\delta$ 1.58, 133 ppm (C-6) and $\delta$ 1.89, 1.64 ppm (C-9) suggesting that the change has occurred at C$_7$-C$_8$. Further $^{13}$C-NMR value at $\delta$ 59.3 ppm
(C-7) and 93.3 ppm (C-8), suggested that initially sp² hybridized carbon changed to quaternary carbon. The compound 10 showed a proton singlet at δ 2.78 ppm, attached to C-11 (δ 47.8 ppm), which was not present in the in starting compound. This suggested that both the double bonds of furan ring were utilized in epoxide and lactone formation. ¹³C-NMR exhibited signal due to lactone carbonyl at δ 177.4 ppm which is supported by the IR bands at 1765 cm⁻¹ (lactone) and 1715 cm⁻¹ (α, β-unsaturated ester).
Scheme 5.3
Photooxygenation of 5 in methanol gave a compound identified as a crystalline hydroperoxide whose properties require that it should have structure 11. The compound has absorption bands at 3514 cm⁻¹ (-OOH) and 1250 cm⁻¹ (C-O) but none in -C=O region indicating it to be a hydroperoxide. Its \(^{1}\text{H}\)-NMR spectrum has significant signals at \(\delta\) 8.16 (1H, OOH, exch.) and \(\delta\) 3.24 (3H, OCH\(_3\)) consistent with structure 11. 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 5 was irradiated in presence of \([\text{P}_{5}]-\text{rose bengal}\) in methanol under bubbling oxygen a mixture of products 8 and 10 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 10 (epoxy lactone) converted into hydroxy butenolide 8 (Scheme 5.4).

Treatment of methoxy hydroperoxide 11 with methanolic HCl gave a product identified as 8. Whereas pyrolysis of 11 produced 12 (Scheme 5.5). The structure of 12 was readily established by its spectral and chemical properties. In the IR, the compound absorbs at 1779 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 \(\lambda_{\text{max}}\) 216 nm. The NMR spectrum clearly showed the presence of a -OMe group \(\delta\) 3.24 (3H) and an allylic Me \(\delta\) 1.71 (3H). The presence of the OMe group was confirmed by a quantitative zeisel determination. Reduction of 11 with triphenylphosphine in
ether gave a product which was identified as 16. Its formation could be realized via unstable hemiacetal 14 (Scheme 5.5). Its IR absorption at 1779 cm⁻¹ and UV λₘₐₓ 217 nm are characteristic of an α, β-unsaturated γ-lactone. The NMR spectrum showed the presence of an allylic Me group, δ 1.93 (3H, t); the single proton in the lactone ring appears as the quartet centered at δ 4.91 (1H, J=6 and 11 Hz). The compound was identified as 2β-angeloyloxy -10β-H-emophilanolide (16).
Scheme 5.4
Scheme 5.5
References


