CHAPTER IV

SYNTHESES AND ANTI-MICROBIAL STUDIES OF 4-OXO-2(E)-ALKENOIC ACIDS
4.1 INTRODUCTION

4-Oxo-2(E)-nonenoic acid 1 (Fig 1), a natural antibiotic,\(^1\) was isolated by Pfefferle \textit{et al.}\(^2\) from the mycelium of \textit{streptomyces olivaceus} Tü 4018.

![Fig 1](image1)

Compound 1 is reported to be important for its antibacterial activity against various Gram-positive and Gram-negative strains, especially against \textit{staphylococcus aureus} ATCC 11632.\(^2\) It is also found to be an inhibitor for human mitochondrial aldehyde dehydrogenase.\(^3\) When literature search for this and several other similar compounds was done, we found that 4-oxo-2(E)-alkenoic acids in general are highly useful and important molecules.

4-Phenyl-4-oxo-2(E)-butenoic acid 2 (Fig 2) and its derivatives act as inhibitors of kynurenine-3-hydroxylase (KYN-3-OHase), an enzyme involved in the metabolism of kynurenine.\(^4\)

![Fig 2](image2)

Therefore they can be used in the prevention and/or treatment of neurodegenerative diseases, such as Huntington’s chorea, Alzheimer’s disease, dementia caused by acquired immune deficiency syndrome (AIDS), infarctual dementia, cerebral ischemia, cerebral hypoxia, Parkinson’s disease, epilepsy, head and spinal cord injury, amyotrophic lateral sclerosis, glaucoma/retinopathy, infections and inflammation of the brain.\(^4\) The amides of these acids 3 (Fig 3) are cytoprotective and promote the healing
of the stomach ulcers.\textsuperscript{5}

\begin{center}
\includegraphics[width=0.5\textwidth]{fig3}
\end{center}

Fig 3

3-Acylprop-2-enoic acid moiety is an important part in many biologically active natural products, such as pyrenophorin \textsuperscript{4,6,7} macrosphelides \textsuperscript{5,8} and A267713 B \textsuperscript{9} (Fig 4).

\begin{center}
\includegraphics[width=0.7\textwidth]{fig4}
\end{center}

Fig 4

The aroyl\textsuperscript{10} and heteroaroyl\textsuperscript{11} acids are useful systems for the preparation of $\gamma$-oxo and $\gamma$-hydroxy substituted $\alpha$-amino acids and acylacrylic subunit appears to have broad potential as a crystallization induced asymmetric transformation (CIAT) template.\textsuperscript{12,13}

4-Oxo-2($E$)-alkenoic acid derivatives are used as starting materials or intermediates in the synthesis of various bioactive compounds, for instance a series of inhibitors of glucosamine-6-phosphate synthase 7 (Fig 5) were synthesized using such acids.\textsuperscript{14}
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**Fig 5**

Compound 8 is used as precursor for the synthesis of A267713B \(^{15}\) and compound 9 (Fig 6) for pyrenophorine.\(^{16}\)

**Fig 6**

### 4.2 LITERATURE REVIEW

4-Oxo-2(E)-alkenoic acid type of compounds are being synthesized and used since late 19\(^{th}\) century, especially the aromatic compounds. The first preparation of \(\beta\)-benzoylacrylic acid 2 was reported in the year 1882\(^{17}\) by Pechmann from maleic anhydride, benzene and aluminium chloride. Then it has been also prepared by

1. The condensation of acetophenone and chloral to 1,1,1-trichloro-2-hydroxy-3-benzoylpropane, followed by hydrolysis to corresponding acid and dehydration.\(^{18}\)
2. The action of iodine, potassium iodide and sodium carbonate on \(\gamma\)-phenylisocrotonic acid.\(^{19}\)
3. Bromination of \(\beta\)-benzoylpropionic acid and subsequent dehydrohalogenation.\(^{20}\)
4. The action of phenylzinc chloride on maleic anhydride.\(^{21}\)
5. The condensation of the acid chloride of ethylhydrogen maleate with benzene in the presence of aluminum chloride followed by hydrolysis.\(^{22}\)
6. The method developed by Pechmann in 1882 was modified and is still used for the preparation of such molecules even though there are methods developed by others (Scheme I).

![Scheme I](image)

**Scheme I**

The method developed by Koenigs and Wagstaffe was also used for the synthesis of aliphatic keto-enoic acids by condensing methyl ketones with chloral followed by hydrolysis and dehydration (Scheme II).

![Scheme II](image)

**Scheme II**

Similar method of condensation was used by Jakubec et al. for the synthesis of 4-oxo-2(E)-alkenoic acids (Scheme III). However, this method could be applied to limited examples only.

![Scheme III](image)

**Scheme III**

Walton reported synthesis of alkyl-4-oxo-2-alkenoates by the retrogressive Diels-Alder reaction of the cyclopentadiene adduct. The corresponding adducts were in turn prepared through the interaction of alkylzinc chlorides and half ester chloride of bicyclo[2,2,1] 5-heptene-2,3-bicarboxylic acids (Scheme IV).

![Scheme IV](image)
Kawashima et al.\textsuperscript{15} reported a synthesis of 4-oxo-2-alkenoic acids via 4-chloro-3-alkenoic acids \textsuperscript{12} which was obtained when $\beta$-(1-chlorovinyl)-$\beta$-propiolactone \textsuperscript{13} was reacted with organocopper reagent (Scheme V).

Bonete and Najera\textsuperscript{16} reported a synthesis of precursor of pyrenophorin by dilithiation of $\beta$-tosylated acids \textsuperscript{14} to give lithium-3-lithio-3-tosylalkanoate \textsuperscript{15}, acylation followed by esterification and treatment with DBU afforded 4-ketoesters from \textsuperscript{15} (Scheme VI).
There are several reports of preparation of such compounds or their ester derivatives by opening of furan rings. Asaoka et al.\textsuperscript{26} reported the synthesis of 3-acylacrylic acids by reaction of 2-(trimethylsiloxy)furans \textbf{16} with lead(IV) acetate to give \(\alpha,\beta\)-unsaturated-\(\gamma\)-acetoxy-\(\gamma\)-lactones \textbf{17} followed by acid hydrolysis (Scheme VII).

\textbf{Scheme VII}

Finlay et al.\textsuperscript{27} used methyltrioxorhenium (MTO) as a catalyst for the oxidative ring opening of substituted furans (Scheme VIII).

\textbf{Scheme VIII}

Generation of 4-oxo-2-alkenoic acids from 2-alkyl furans has also been accomplished previously through a variety of multistep procedures. Rao et al.\textsuperscript{28} reported the synthesis
of acids using 2-alkylfurans by bromination followed by oxidation (Scheme IX). This method was specifically used for the synthesis of ethyl-7-acetoxy-4-oxo-2(E)-octenoate.

\[
\begin{array}{ccc}
\text{Br}_2, \text{Py}, \text{acetone}, & \text{R} & \text{NaClO}_2, \text{NaH}_2\text{PO}_4, \text{2-Methyl-2-Butene} \\
\text{H}_2\text{O}, 85\% & \text{CHO} & 75\%
\end{array}
\]

Scheme IX

Kobayashi et al.\(^{29}\) reported a two step synthesis of \(\gamma\)-oxo-\(\alpha,\beta\)-unsaturated carboxylic acids by initially oxidation of the aldehyde group to acid using \(\text{NaClO}_2\) (Scheme X).

\[
\begin{array}{ccc}
\text{NBS, Py,} & \text{R} & \text{NaClO}_2, \text{t-BuOH, Buffer} \\
\text{Acetone:THF:H}_2\text{O} 5:4:2, 20^\circ\text{C}, 1\text{h}, \text{RT, 4h}, 73\% & \text{CHO} & \text{pH-3.6, 85\%}
\end{array}
\]

Scheme X

Synthesis of specifically 4-oxo-2(E)-nonenoic acid was published by Ballini and Bosica,\(^1\) wherein, monoalkylation of furan was performed. The ring was then opened by PCC and oxidized to acid using Jone's reagent (Scheme XI).

\[
\begin{array}{ccc}
\text{C}_5\text{H}_4\text{I} & \text{Pentane, THF} & \text{PCC, CH}_2\text{Cl}_2 \\
\text{73\%} & 70\% & \\
\text{H}_{11}\text{C}_5 & \text{CHO} & \text{H}_{11}\text{C}_5
\end{array}
\]

Scheme XI

Saldabol et al.\(^{30}\) while studying the nitration of 2-substituted 4-(2-furyl)thiazoles found, nitration followed by acidic oxidation of furan gives \(\gamma\)-oxo-\(\alpha,\beta\)-unsaturated acids (Scheme XII).
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The synthesis of 4-oxo-2-alkenoic acids was achieved by Annangudi et al.\textsuperscript{31} by the reaction of 2-alkylfurans with sodium chlorite in acidic aqueous solution (Scheme XIII).

Scheme XIII

Recently the synthesis of 4-oxo-2(E)-nonenoic acid was reported by Maras et al.\textsuperscript{32} from 2-methoxyfuran by its photooxygenation (Scheme XIV).

Scheme XIV

Obrecht and Weiss\textsuperscript{33} developed a method for the preparation of 3-acylprop-2(E)-enoic acids via acid-catalyzed isomerization of the corresponding acylprop-2-ynal acetals\textsuperscript{18} (Scheme XV).
A procedure for synthesis of 4-oxo-2(E)-nonenoic acid was reported from our laboratory by Shet et al.\textsuperscript{34} as an application of domino oxidation-Wittig reaction (Scheme XVI).

A methodology for oxidation of allylic carbon catalyzed by dirhodium caprolactamate via aqueous tert-butylhydroperoxide was developed by Mclaughlin et al.\textsuperscript{35} This was applied for the synthesis of 4-oxo-2(E)-nonenoic acid (Scheme XVII).
4.3 RESULTS AND DISCUSSION

The presence of 4-oxo-2(E)-nonenoic acid as an antibiotic in nature and its broad spectrum antibacterial activity coupled with, it also being a lipid peroxidation product attracted us as a synthetic target. The main functional part of it being γ-oxo-nonenoic acid, after going through the literature methods, we realized that there is a need to develop a general and simple route which would be adaptable to make libraries of such compounds. Earlier we had developed a route (Chapter I) for the synthesis of 4-ONE, a lipid peroxidation product, we thought of extending the same for the synthesis of γ-oxo-enoic acid by carrying out the Wittig reaction on glyoxalic acid as shown in the scheme XVIII below.

![Scheme XVIII](image)

The phosphorane 19 was prepared by acylating the stable phosphorane, ethyl(triphenylphosphoranylidene) acetate 20, using hexanoyl chloride followed by decarboxylative hydrolysis of 21 (Scheme XIX) as described in chapter I.

![Scheme XIX](image)

Thus, 1-(triphenylphosphoranylidene)-2-heptanone 19 was reacted with glyoxylic acid in a mixture of chloroform: methanol (1:1) as a solvent, at room temperature to get an acid. (Scheme XX)
The acid 1 in its IR spectrum (KBr) showed bands at 1712.79 cm\(^{-1}\) and 1660.71 cm\(^{-1}\), indicating the presence of two carbonyl groups. The acid group was confirmed by the broad band at 2505.19-3382.91 cm\(^{-1}\).

Its \(^1\)H NMR (CDCl\(_3\), 300 MHz, \(\delta\) ppm), spectrum (Fig 7a) showed triplet at \(\delta\) 0.85 \((J = 7.1\) Hz) for three protons indicated the presence of \(-\text{CH}_2\text{CH}_3\) group. A multiplet at \(\delta\) 1.26 integrating for four protons can be attributed for two methylene groups. A multiplet at \(\delta\) 1.62 for two protons indicated another methylene group. A triplet at \(\delta\) 2.60 \((J = 7.2\) Hz) integrating for two protons can be attributed for \(-\text{CH}_2\text{CO}\) group. Two doublets at \(\delta\) 6.62 \((J = 15.9\) Hz) and 7.19 \((J = 15.9\) Hz) integrating for one proton each indicated the ethylene group \((-\text{CH=CH-})\) and their J values indicated \(E\) configuration of the double bond.

Its \(^{13}\)C NMR (CDCl\(_3\), 75 MHz, \(\delta\) ppm) spectrum (Fig 7b) showed \(\delta\) 13.8\(\downarrow\) (-CH\(_3\)), 22.3\(\uparrow\) (-CH\(_2\)-), 23.2\(\uparrow\) (-CH\(_2\)), 31.2\(\uparrow\) (-CH\(_2\)), 41.6\(\uparrow\) (-CH\(_2\)), 129.4\(\downarrow\) (-CH=), 140.9\(\downarrow\) (-CH=), 169.0 (Cq-COOH), 199.6 (Cq-CO).

The multiplicities of the carbon signals were obtained from DEPT 135 experiment.

The high resolution mass spectrum (HRMS) of the compound 1 displayed a strong peak at \(m/z\) 193.0844 presumably due to \((M + Na)^+\) pseudo ions. Thus elemental composition was determined to be C\(_9\)H\(_{14}\)O\(_3\). HRMS; \(m/z\) calculated for C\(_9\)H\(_{14}\)O\(_3\)Na \([\text{(M + Na)}^+\] was 193.0841 and found = 193.0844.

Melting point of 1 was found to be 104-105°C, which was matching well with the literature\(^{40}\) value (105-106°C).
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Fig 7a

Fig 7b
Thus based on the mode of formation, spectral analysis and comparison with literature data, \( {\text{1}} \) was assayed to be the expected natural acid, 4-oxo-2(\( E \))-nonenoic acid.

<table>
<thead>
<tr>
<th>(^1)H NMR observed</th>
<th>(^1)H NMR reported</th>
<th>(^1)H NMR observed</th>
<th>(^1)H NMR reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta 0.85 ) (t, 3H, ( J = 7.1 ) Hz)</td>
<td>( \delta 0.90 ) (t, 3H, ( J = 6.9 ) Hz)</td>
<td>( \delta 2.60 ) (t, 2H, ( J = 7.2 ) Hz)</td>
<td>( \delta 2.62 ) (t, 2H, ( J = 7.5 ) Hz)</td>
</tr>
<tr>
<td>( \delta 1.26 ) (m, 4H)</td>
<td>( \delta 1.31 ) (m, 4H)</td>
<td>( \delta 6.62 ) (d, 1H, ( J = 15.9 ) Hz)</td>
<td>( \delta 6.68 ) (d, 1H, ( J = 16.2 ) Hz)</td>
</tr>
<tr>
<td>( \delta 1.62 ) (m, 2H)</td>
<td>( \delta 1.65 ) (m, 2H)</td>
<td>( \delta 7.19 ) (d, 1H, ( J = 15.9 ) Hz)</td>
<td>( \delta 7.15 ) (d, 1H, ( J = 16.2 ) Hz)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(^{13})C NMR observed</th>
<th>(^{13})C NMR reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta 13.8 ) (C(_9)), 22.3 (C(_8)), 23.2 (C(_7)), 31.2 (C(_6)), 41.6 (C(_5)), 129.4 (C(_3)), 140.9 (C(_2)), 169.0 (C(_1)), 199.6 (C(_4)).</td>
<td>( \delta 13.8 ) (C(_9)), 22.4 (C(_8)), 23.3 (C(_7)), 31.2 (C(_6)), 41.7 (C(_3)), 129.5 (C(_3)), 141.2 (C(_2)), 170.9 (C(_1)), 199.7 (C(_4)).</td>
</tr>
</tbody>
</table>

The yield of acid \( {\text{1}} \) was found to be 67%.

Having successfully synthesized the natural acid \( {\text{1}} \), we next decided to make different analogues of acid \( {\text{1}} \) as mentioned earlier. Towards this end the required phosphorane \( {\text{23a-e}} \) were similarly prepared by acylating the stable phosphorane ethyl(triphenylphosphoranylidene) acetate \( {\text{20}} \) to get keto-ester-phosphoranes \( {\text{22a-e}} \), which were subsequently decarboxylated to get keto-phosphoranes \( {\text{23a-e}} \). The keto-phosphoranes \( {\text{23a-e}} \) were then condensed with glyoxalic acid to get \( \gamma \)-keto-enoic acid \( {\text{24a-e}} \) in good yields. (Scheme XXI).
Here R = a) - Ethyl, b) - Propyl, c) - n-Butyl, d) - Isobutyl, e) - Heptyl

For aromatic compound, acetophenone phosphorane 25 was prepared by reaction of triphenylphosphine with α-bromoacetophenone 26 in dry benzene. The salt obtained was treated with 20% NaOH to get the free base (Scheme XXII).

The yellow solid obtained was in 68.5% with mp 178°C – 179°C, (lit41 177-178°C). Keto-phosphorane 25 was then reacted with glyoxylic acid in chloroform:methanol (1:1) as a solvent to give the desired acid 27 as shown in the Scheme XXI above.

Once we had sufficient amount of these compounds in our hands, the anti-microbial activity was studied against various gram-positive and gram-negative bacteria.

The antimicrobial activity of the compound was assessed against fourteen microbial strains viz Salmonella typhimurium, Salmonella paratyphi A, Proteus mirabilis,
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*Klebsiella pneumoniae, Staphylococcus citreus, Escherichia coli, Staphylococcus aureus MRSA, Escherichia coli K-12 (MTCC 1302), Bacillus subtilis (MTCC 121), Staphylococcus ATCC, Candida albidans, Aspergillus niger, Pencilliunn sp. Saccharomyces cerevisiae.* Bacterial cultures were generated by inoculating a loopful of cultures in separate 100 mL nutrient broths and incubating at 37°C for 24 h. The cells were harvested by centrifuging at 4000 rpm for 5 min again and diluted in normal saline to obtain a density of 5 X 10⁵ cfu/mL.

Conventional disc diffusion method⁴² was employed for the assessment of antimicrobial potential of the compounds. Sterile 6.0 mm diameter blank discs were impregnated with the test substances at a dose of 15 μg/disc. These discs, along with the control disc (4-oxo-2E-nonenolic acid, 1, 15 μg/disc) were placed on petridishes containing a suitable agar medium seeded with the test organisms using sterile forceps and kept at 4°C to facilitate maximum diffusion. The plates were kept in incubator (37°C) to allow the growth of the bacteria. The antimicrobial activity of the test agents were determined by measuring the diameter of the zone of inhibition in terms of millimeters. The results obtained are summarized in table I.

**Table I** Anti-microbial activity by agar diffusion test (Inhibition zones in mm)

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>24a</th>
<th>24b</th>
<th>24c</th>
<th>24d</th>
<th>1</th>
<th>24e</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1mg/mL</td>
<td>1mg/mL</td>
<td>1mg/mL</td>
<td>1mg/mL</td>
<td>1mg/mL</td>
<td>1mg/mL</td>
<td>1mg/mL</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>-</td>
<td>-</td>
<td>10mm</td>
<td>9mm</td>
<td>7mm</td>
<td>14mm</td>
<td>7mm</td>
</tr>
<tr>
<td><em>Salmonella paratyphi A</em></td>
<td>-</td>
<td>-</td>
<td>10mm</td>
<td>-</td>
<td>11mm</td>
<td>-</td>
<td>12mm</td>
</tr>
<tr>
<td>Organism</td>
<td>8mm</td>
<td>10mm</td>
<td>11mm</td>
<td>12mm</td>
<td>13mm</td>
<td>14mm</td>
<td>15mm</td>
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<tr>
<td><em>Proteus mirabilis</em></td>
<td></td>
<td></td>
<td>10mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus citreus</em></td>
<td>8mm</td>
<td></td>
<td>16mm</td>
<td>11mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus MRSA</em></td>
<td></td>
<td></td>
<td></td>
<td>7mm</td>
<td>12mm</td>
<td>15mm</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> K-12 (MTCC 1302)</td>
<td>11mm</td>
<td></td>
<td>10mm</td>
<td>14mm</td>
<td>19mm</td>
<td>15mm</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (MTCC 121)</td>
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</tr>
<tr>
<td><em>Staphylococcus ATCC</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10mm</td>
<td>19mm</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>9mm</td>
<td></td>
<td>13mm</td>
<td>13mm</td>
<td>18mm</td>
<td></td>
<td></td>
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<tr>
<td><em>Aspergillus niger</em></td>
<td></td>
<td></td>
<td>10mm</td>
<td></td>
<td>16mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium sp.</em></td>
<td></td>
<td></td>
<td>11mm</td>
<td></td>
<td>11mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
The results of this anti-microbial activity test showed that the acids with lower alkyl derivative (R = ethyl, propyl) were either active against very few microbes or not active at all. Whereas higher derivatives (R = butyl) was as active as that of lead compound (R = pentyl). Its inhibition zones were smaller than the lead compound but was active against almost all the microbes. But the results were not encouraging in the case of higher derivatives of acid than the lead compound. The heptyl exhibited good inhibition zone but it was active against only four microbes. This indicates it to be quite selective. The branched alkyl derivative (R = isobutyl) showed activity against very few microbes compared to its n-alkyl isomer as well as the lead compound. The aromatic compound, known to be the antiulcer agent, was active against almost all the microbes with higher inhibition zone than the lead compound.
4.4 CONCLUSION

1. We have successfully applied Wittig reaction for the synthesis of various derivatives of $\gamma$-keto-$\alpha,\beta$-unsaturated acids, thus we have developed a general method for the synthesis of such compounds in good yields.

2. We have studied antimicrobial activity of $\gamma$-keto-$\alpha,\beta$-unsaturated acids over a broad spectrum of gram-positive and gram-negative bacteria and some fungi. These compounds can effectively act as potent antibiotics if relevant studies are conducted in future.
4.5 EXPERIMENTAL

4.5.1 Preparation of Keto-phosphorane (23)

\[
\text{RCOOH} \xrightarrow{\text{SOCl}_2} \text{RCOCI}
\]

Thionyl chloride (1.2 equivalents) was slowly added to the cooled acid (1 equivalent) and refluxed it for 4.0 h. It was then removed by distillation under vacuum. The crude acid chlorides were purified by distilling under vacuum to obtain light yellow liquid and were used for the next reaction.

Ethyl(triphenylphosphoranylidene) acetate 20 (2 equivalent) was added to the solution of freshly prepared acid chloride (1 equivalent) in 15 mL of toluene. The solid obtained after stirring for 5.0 h at room temperature, was filtered. The keto-ester-phosphorane 22 obtained after concentration of the filtrate under reduced pressure was in 82 – 96% yield with respect to the acid chloride. The melting ranges of these compounds found are given in table II. Without further analysis these phosphoranes were used for the next reaction.

Table II Yields and melting points of the ester-keto-phosphoranes

<table>
<thead>
<tr>
<th>Code</th>
<th>Product</th>
<th>Yield</th>
<th>mp</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td><img src="image.png" alt="Image" /></td>
<td>93.5%</td>
<td>76 - 78°C</td>
</tr>
</tbody>
</table>
A solution containing keto ester phosphorane 22 (10 mmol) in a mixture of trifluoroacetic acid (15 mL) and water (2.5 mL) was heated under reflux for 6.0 h. The reaction mixture was then poured onto ice and basified with 2% sodium bicarbonate solution. This was followed by extraction with diethyl ether (3 X 10 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by column chromatography using ethyl acetate/petroleum ether (1/5, vol/vol) to obtain 50 – 68% of the targeted keto-phosphorane 23.

<table>
<thead>
<tr>
<th></th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22a</td>
<td><img src="image" alt="Structure" /></td>
<td>88.0%</td>
<td>123-124°C (lit.43 123-125°C)</td>
</tr>
<tr>
<td>22b</td>
<td><img src="image" alt="Structure" /></td>
<td>96.2%</td>
<td>133-134°C (lit.44 130-131°C)</td>
</tr>
<tr>
<td>22c</td>
<td><img src="image" alt="Structure" /></td>
<td>82.7%</td>
<td>97-98°C</td>
</tr>
<tr>
<td>22d</td>
<td><img src="image" alt="Structure" /></td>
<td>98.4%</td>
<td>80-81°C</td>
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<tr>
<td>22e</td>
<td><img src="image" alt="Structure" /></td>
<td>95.5%</td>
<td>111-112°C</td>
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### Table III Yields and Melting points of the Keto-phosphoranes

<table>
<thead>
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<th>product</th>
<th>yield</th>
<th>melting range</th>
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<tbody>
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<td>19</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>50.0%</td>
<td>oil</td>
</tr>
<tr>
<td>23a</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>74.0%</td>
<td>215-216°C (lit. 215-218°C)</td>
</tr>
<tr>
<td>23b</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>71.4%</td>
<td>144-145°C (lit. 144-145°C)</td>
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<tr>
<td>23c</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>69.0%</td>
<td>108-110°C (lit. 109-111°C)</td>
</tr>
<tr>
<td>23d</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>67.0%</td>
<td>120-122°C (lit. 120-121°C)</td>
</tr>
<tr>
<td>23e</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>73.3%</td>
<td>light yellow oil</td>
</tr>
</tbody>
</table>

**4.5.2 Synthesis of α-bromoacetophenone (26)**

![Chemical Reaction](image)

Bromine (1.25 mL, 25 mmol) was added dropwise to the solution of acetophenone (3...
mL, 25 mmol) in 10 mL glacial acetic acid at 0°C such that the temperature does not exceed 20°C. The mixture was stirred for 1 h at room temperature before quenching it into water. The solution was neutralized using solid NaHCO₃ and extracted with CHCl₃ (4 X 20 mL). All the combined organic extracts were dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to obtain a solid product. Recrystallization using absolute alcohol yielded 3.3 g (66.7%) of white crystalline solid 26; mp 49-51°C (lit. 50-51°C).

4.5.3 Synthesis of acetophenone phosphorane (25)

α-Bromoacetophenone 26 (1.4 g, 7 mmol) was added dropwise to the stirred solution of triphenylphosphine (1.86 g, 7 mmol) in dry benzene (15 mL). Initially exotherm was observed with formation of the salt, the mixture was stirred further for 10 h. The salt formed was dissolved in water and the unreacted starting was washed with benzene (3 X 10 mL). The aqueous layer was then basified with 20% NaOH to phenolphthalein pink and extracted the product in benzene (5 X 20 mL). The combined organic extracts were dried over anhydrous Na₂SO₄, benzene was distilled out first on water bath and then under vacuum. The product obtained as a yellow solid was 1.84 g (68.5%); mp 178-180°C (lit. 177-178°C).

4.5.4 Synthesis of 4-oxo-2(E)-alkenoic acid (24, 27)

To the solution of keto-phophorane (5 mmol) in 20 mL mixture of chloroform: methanol (1:1), 7.5 mmol of glyoxalic acid was added dropwise while stirring it vigorously. The reaction mixture was stirred at room temperature until the
disappearrence of starting material (monitored by TLC). The solvent was removed under vacuum and the solid obtained was dissolved in benzene. Benzene layer was washed well with saturated NaHCO₃ solution (7 X 15 mL). The basic aqueous layer was then acidified using dilute HCl to pH 2-3 and the product was extracted in chloroform (4 X 15 mL). All the organic extracts were combined, dried over anhydrous Na₂SO₄, filtered and concentrated the filtrate to obtain crude acids which were then recrystallized using the mixture of ethyl acetate/petroleum ether to obtain the product in 59-68% of yield. The melting range of the products is mentioned in table IV. All the acids displayed cabonyl bands in IR spectrum (KBr if solids and neat if liquids) at around 1720. Also band at 1660 cm⁻¹ and a broad band from 2500 to 3100 cm⁻¹, confirmed the presence of COOH group.

Table IV Yields and Melting points of the Acids

<table>
<thead>
<tr>
<th>code</th>
<th>product</th>
<th>yield</th>
<th>mp</th>
</tr>
</thead>
<tbody>
<tr>
<td>24a</td>
<td>4-oxo-2(E)-hexenoic acid</td>
<td>68.0%</td>
<td>105-106°C (lit.¹⁴ 105-108°C)</td>
</tr>
<tr>
<td>24b</td>
<td>4-oxo-(2E)-heptenoic acid</td>
<td>61.0%</td>
<td>108-109°C (lit.¹³ 107-109°C)</td>
</tr>
<tr>
<td>24c</td>
<td>4-oxo-2(E)-octenoic acid</td>
<td>60.0%</td>
<td>111-112°C (lit.¹⁴ 98-100°C)</td>
</tr>
<tr>
<td>24d</td>
<td>6-methyl-4-oxo-(2E)-heptenoic acid</td>
<td>59.0%</td>
<td>98-99°C (lit.⁴⁰ 91.5-92.5°C)</td>
</tr>
<tr>
<td>Code</td>
<td>酸</td>
<td>产率</td>
<td>熔点 (°C)</td>
</tr>
<tr>
<td>------</td>
<td>-----------------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
<td>4-oxo-2(E)-nonenoic acid</td>
<td>67.0%</td>
<td>104-105°C (lit. 40-105-106°C)</td>
</tr>
<tr>
<td>24e</td>
<td>4-oxo-(2E)-undecenoic acid</td>
<td>60.0%</td>
<td>Colourless oily liquid</td>
</tr>
<tr>
<td>27</td>
<td>4-oxo-4-phenyl-(2E)-butenoic acid</td>
<td>66.3%</td>
<td>95-96°C (lit. 23° 94-96°C)</td>
</tr>
</tbody>
</table>

**Table V** The $^1$H NMR and $^{13}$C NMR of the acids

<table>
<thead>
<tr>
<th>Code</th>
<th>Spectral details in δ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>24a</td>
<td>$^1$H NMR (CDCl$_3$, 300 MHz, Fig 8a) δ 1.17 (t, 3H, $J = 7.2$ Hz), 2.71 (q, 2H, $J = 7.2$ Hz), 6.70 (d, 1H, $J = 15.9$ Hz), 7.17 (d, 1H, $J = 15.0$ Hz)</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C NMR (CDCl$_3$, 75 MHz, Fig 8b) δ 7.49 (t, CH$_3$CH$_2$), 35.35 (CH$_3$CH$_2$), 129.39 (CH), 140.97 (CH), 170.35 (Cq-COOH), 199.63 (Cq-CO).</td>
</tr>
<tr>
<td>24b</td>
<td>$^1$H NMR (CDCl$_3$, 300 MHz, Fig 9a) δ 0.98 (t, 3H, $J = 7.3$ Hz), 1.71 (m, 2H), 2.66 (t, 2H, $J = 7.2$ Hz), 6.69 (d, 1H, $J = 15.9$ Hz), 7.15 (d, 1H, $J = 15.9$ Hz).</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C NMR (CDCl$_3$, 75 MHz, Fig 9b) δ 13.57 (t, CH$_3$), 17.09 (CH$_2$-), 43.54 (CH$_2$-), 129.43 (CH), 141.15 (CH), 170.29 (Cq-COOH), 199.47 (Cq-CO).</td>
</tr>
</tbody>
</table>
Chapter IV

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>24c</td>
<td>$^1$H NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 300 MHz, Fig 10a) $\delta$ 0.95 (t, 3H, $J = 7.3$ Hz), 1.38 (m, 2H), 1.66 (m, 2H), 2.67 (t, 2H, $J = 7.2$ Hz), 6.69 (d, 1H, $J = 15.9$ Hz), 7.16 (d, 1H, $J = 15.9$ Hz).</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 75 MHz, Fig 10b) $\delta$ 13.74$\uparrow$ (-CH&lt;sub&gt;3&lt;/sub&gt;), 22.18$\downarrow$ (-CH&lt;sub&gt;2&lt;/sub&gt;), 22.18$\downarrow$ (-CH&lt;sub&gt;2&lt;/sub&gt;), 25.68$\downarrow$ (-CH&lt;sub&gt;2&lt;/sub&gt;), 41.39$\downarrow$ (-CH&lt;sub&gt;2&lt;/sub&gt;) 129.66$\uparrow$ (=CH), 141.03$\uparrow$ (=CH), 170.46 (Cq-COOH), 199.57 (Cq-CO).</td>
</tr>
<tr>
<td></td>
<td>HRMS; m/z calculated for C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; [(M + H)&lt;sup&gt;+&lt;/sup&gt;] = 157.0864, found = 157.0858.</td>
</tr>
<tr>
<td>24d</td>
<td>$^1$H NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 300 MHz, (Fig 11a) $\delta$ 0.98 (d, 6H, $J = 6.6$ Hz), 2.22 (m, 1H), 2.55 (d, 2H, $J = 6.9$ Hz), 6.68 (d, 1H, $J = 15.9$ Hz), 7.15 (d, 1H, $J = 15.9$ Hz).</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 75 MHz, Fig 11b) $\delta$ 22.48$\uparrow$ (-CH&lt;sub&gt;3&lt;/sub&gt;), 24.72$\uparrow$ (-CH-), 50.55$\downarrow$ (-CH&lt;sub&gt;2&lt;/sub&gt;), 129.51$\uparrow$ (=CH), 141.36$\uparrow$ (=CH), 170.45 (Cq-COOH), 199.57 (Cq-CO).</td>
</tr>
<tr>
<td></td>
<td>HRMS; m/z calculated for C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; [(M + H)&lt;sup&gt;+&lt;/sup&gt;] = 157.0864, found =157.0860.</td>
</tr>
<tr>
<td>24e</td>
<td>$^1$H NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 400 MHz, Fig 12a) $\delta$ 0.88 (m, 5H), 1.28 (m, 4H), 1.56 (m, 2H), 1.68 (m, 2H), 2.66 (m, 2H), 6.73 (d, 1H, $J = 15.9$ Hz), 7.23 (d, 1H, $J = 15.9$ Hz).</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 75 MHz, Fig 12b) $\delta$ 11.66 (-CH&lt;sub&gt;3&lt;/sub&gt;), 13.83 (-CH&lt;sub&gt;2&lt;/sub&gt;), 22.73 (-CH&lt;sub&gt;2&lt;/sub&gt;), 24.19 (-CH&lt;sub&gt;2&lt;/sub&gt;), 29.48 (-CH&lt;sub&gt;2&lt;/sub&gt;), 30.49 (-CH&lt;sub&gt;2&lt;/sub&gt;), 52.83 (-CH&lt;sub&gt;2&lt;/sub&gt;), 129.69 (=CH), 140.40$\uparrow$ (=CH), 182.76 (Cq-COOH), 202.92 (Cq-CO).</td>
</tr>
<tr>
<td>27</td>
<td>$^1$H NMR (CDCl&lt;sub&gt;3&lt;/sub&gt;, 300 MHz, Fig 13a) $\delta$ 6.92 (d, 1H, $J = 15.6$ Hz), 7.55 (t, 2H, $J = 7.5$ Hz), 7.66 (t, 1H, $J = 7.5$ Hz), 8.02 (m, 1H).</td>
</tr>
</tbody>
</table>
|    | $^{13}$C NMR (CDCl<sub>3</sub>, 75 MHz, Fig 13b) $\delta$ 128.89$\uparrow$(HC<sub>m</sub>), 1218.94$\uparrow$(HC<sub>o</sub>) 131.39$\uparrow$(HC<sub>p</sub>) 134.04$\uparrow$ (=CH-), 136.38 (C<sub>aq,H</sub>), 138.44$\uparrow$ (=HC-), 170.29 (Cq-COOH),
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189.11 (Cq-CO).

HRMS; m/z calculated for C_{10}H_{8}O_{3}Na [(M + Na)^+] = 199.0379, found =199.0372.

Fig 8a
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Fig 10b

Fig 11a
Chapter IV

**Fig 12b**

![Diagram of chemical structure](image)

**Fig 13a**

![Diagram of chemical structure](image)
Chapter IV

Fig 13b
4.6 REFERENCE

17. Penchman, H. V. Ber. 1882, 15, 885.


PUBLICATIONS


CONFERENCES ATTENDED

- Advances in organic chemistry and chemical biology, conference held in January 2006, at IICT Hyderabad, India.

- Royal Society of Chemistry West India Section-Research Scholars symposium held in October 2008 at Goa University, India.

- Chemical Research Society of India meeting held in February 2009 at National Chemical Laboratory, Pune, India.