CHAPTER -2

Intra molecular Cyclisation and Elimination Reactions of Acyloxy benzalacetophenones upon Protonation: A Tandem Mass Spectrometric Study
2.1. Introduction

The EI mass spectra of aromatic compounds exhibit peaks corresponding to fragment ions, due to extensive rearrangements [1-14, 38-41]. Many of these fragment ions have heterocyclic structures due to intramolecular cyclisation taking place within the molecular radical cations [4, 15, 16]. Some of these gas–phase cyclisations have been utilized for synthesis [17, 18] of heterocyclic compounds. However, rearrangements of protonated molecules generated by ionization methods such as FAB, CI and ESI are not common. This is probably due to the lack of rearrangement in the [M+H]+ ions of low energy in comparison with the M+ ions of high energy produced by EI.

Acid catalyzed reactions/rearrangements are common in synthetic organic chemistry but mechanisms need not be unimolecular [19, 20, 21, 22]. However, the mechanisms of unimolecular rearrangements of protonated organic molecules in gas-phase can be investigated by using mass spectrometric methods [5, 9, 23, 24]. The investigation of the mechanism for the elimination of benzoic acid from protonated N-[2-benzoyloxy phenyl] benzamide [25] provides a typical example. This rearrangement is analogous to the acid catalyzed rearrangement of the same molecule in solution-phase. The cyclisation of protonated aromatic nitro compounds has been extensively studied in the gas-phase [9, 24, 26, 27]. The mechanism involves initial protonation of the nitro group followed by electrophilic cyclisation as established by both experimental data and ab initio molecular orbital calculations [28, 29].

Upon protonation by FAB, 1, 2-diacetoxy biphenyl eliminates a molecule of acetic acid, Scheme 1, leading to a cyclic product ion [5]. The protonation occurs on one of the carbonyl oxygen of the acetyl group converting it to a leaving group and the carbonyl oxygen of the second acetyl group acts as a nucleophile. Similar nucleophilic displacement of acetic acid occurs in protonated 4,5-diacetoxyphenanthrene [5].
similar nucleophilic displacement of acetic acid from the [M+H]$^+$ ion of acetylated hyperacine, a marine natural product, has lead to the establishment of its structure [30].

![Scheme 1](image_url)

**Scheme: 1**

2-Acetoxybenzal acetophenone is a compound containing acetoxy group and another carbonyl group that can act as a nucleophile. The molecular radical cation of 2-acetoxybenzalacetophenone dissociates via elimination of acetoxy radical to yield flavylium cation [4]. The CI mass spectrometric study of nitro-substituted benzalacetophenones has been reported [9]. But there is no report on the gas-phase rearrangements of protonated acetoxybenzalacetophenone. In this project the investigation of the possible rearrangements in the gas-phase, upon protonation by FAB, CI and ESI, of 2-acetoxy and 2-benzoyloxy benzalacetophenones is under taken to determine whether the carbonyl oxygen can act as a nucleophile and displace the acyloxy group. The compounds 2.1-2.4 are the acyloxybenzal acetophenones and 2.5 and 2.6 are the reference compounds selected for this project. Compounds 2.1–2.5 were synthesised and 2.6 was purchased from Aldrich Chemical Co. and used with out further purification.
2.2. Results and Discussion

2.2.1. Mass spectra of 2-acetoxybenzal acetophenone

The FAB mass spectrum, Fig.2.1, of 2-acetoxybenzal acetophenone (2.1) shows [M+H]$^+$ ion of m/z 267 and abundant fragment ions of m/z 225 and 207. The fragment ion of m/z 225 corresponds to a loss of ketene from the molecular ion (loss of mass 42 a.m.u), a fragmentation characteristic of acetates. High-resolution mass measurements revealed that the elemental composition of m/z 207 corresponds to C$_{15}$H$_{11}$O (measured mass = 207.0815; calculated mass = 207.0810). Hence the formation of the ions of m/z 207 involves the elimination of the elements of acetic acid from the protonated molecule. The FAB metastable ions (MI) mass spectrum, Fig.2.2a, of [M+H]$^+$ of compound 2.1 exhibit peaks corresponding to the ions of m/z 225, 207 and 105 indicating that these fragment ions are formed by the direct dissociation of the molecular ion. But a step-wise process forms the ion of m/z 147 present in the spectrum. The ion of m/z 105, benzoyl cation, is formed by a simple cleavage reaction.
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Upon collision activation the abundance of the benzoyl cation increases compared to that of the ions of m/z 225 and 207 as indicated by the CAD mass spectrum, Fig. 2.2b. This suggests that the ions of m/z 225 and 207 (low energy processes) are formed as a result of rearrangement and the formation of benzoyl cation (high energy process) does not involve rearrangement.

Fig. 2.1. FAB mass spectrum of compound 2.1

Fig. 2.2a FAB - MI spectrum of [M+H]^+ of compound 2.1.
It is envisaged that protonation occurs at the carbonyl oxygen of the acetyl group converting it to a leaving group, **Scheme 2.1**. The oxygen atom of the keto group then displaces a molecule of acetic acid in a nucleophilic mechanism to afford a heterocyclic fragment ion, **2.1a**, flavylium cation. The generation of the fragment ion of m/z 225 involving the elimination of ketene is the usual fragmentation process for acetates. The structure of the ion of m/z 225 is proposed to be that of protonated 2-hydroxybenzalacetophenone, **2.1b**. This fragment ion may have a cyclic structure **2.1c** in equilibrium with **2.1b**.

**Scheme: 2.1.** Proposed mechanism for elimination of acetic acid and ketene
Further, FAB – CAD spectrum of the fragment ion of m/z 207, Fig.2.3a, from compound 2.1 was compared with that of flavylium cation, Fig.2.3b, from its chloride (compound 2.5) taken as reference standard. The two spectra are closely similar indicating that the [M+H- CH₃ COOH]⁺ ion has indeed flavylium cation structure, 2.1a. This supports the proposed mechanism for the elimination of acetic acid from [M+H]⁺ of compound 2.1.

For further understanding of the mechanism of fragmentation of the protonated 2-acetoxybenzal acetophenone the chemical ionization (CI) mass spectrum, Fig.2.4, was also examined. The CI mass spectrum of compound 2.1 showed an abundant molecular ion, [M+H]⁺ of m/z 267 and fragment ions of m/z 225, 207, 147 and 105.
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**Fig. 2.4.** CI (methane) mass spectrum of compound 2.1

**Fig. 2.5.** The CI- MI (a) and CI-CAD (b) mass spectra of [M+H]+ of compound 2.1
The ions of m/z 225 and 207 correspond to the loss of ketene and acetic acid respectively from the molecular ion. The CI-MI and CAD spectra (Fig. 2.5) of compound 2.1 exhibit the peaks corresponding to the ions of m/z 225 and 207 similar to the FAB-MI and CAD mass spectra, Fig.2.2. The elimination of ketene (m/z 225) and acetic acid (m/z 207) from the molecular ion of compound 2.1 can be explained by the mechanism already proposed in Scheme: 2.1. The structure of the ion of m/z 207 produced by CI ionization of compound 2.1 was confirmed to be that of flavylium cation as in the case of FAB ionization. The CAD mass spectra of flavylium cation and that of the ion of m/z 207 from compound 2.1 were compared. The two mass spectra are closely similar as shown in Fig.2.6

![Fig.2.6](image)

The CAD mass spectra of (a) CI produced ion of m/z 207 from 2.1 and (b) that of flavylium cation
Moreover, in the mechanism proposed in Scheme: 2.1 for the elimination of ketene from the protonated molecule, the ion of m/z 225 is envisaged to be having the structure of protonated 2-hydroxybenzal acetophenone. Therefore the MI mass spectra of the fragment ion of m/z 225, Fig.2.7a, was compared with that of the ion of [M+H]⁺ ion of 2-hydroxybenzal acetophenone obtained by chemical ionization, Fig.2.7b. The two spectra are closely similar indicating that the fragment ion obtained by the ketene elimination from compound 2.1 has the same fragmentations and hence possesses the same structure.

Fig.2.7a. The CI-MI of ion of m/z 225 from 2-acetoxybenzal acetophenone

Fig.2.7b. The CI-MI of [M+H]⁺ ion of (m/z 225) 2-hydroxybenzal acetophenone.
The MI mass spectrum of the fragment ion of m/z 225, Fig.2.7a, show a peak corresponding to the ion of m/z 207, 2.1a (Scheme: 2.2) indicating that the formation of this fragment ion from the [M+H]$^+$ of compound 2.1, may involve a step-wise process in which ketene is eliminated first followed by H$_2$O, a minor process that compete with the acetic acid elimination by nucleophilic displacement, Scheme: 2.1. In addition, Fig.2.7a shows that the ion of m/z 225 can fragment by eliminating a molecule of benzene to afford ions of m/z 147. This suggest that the peak corresponding to the ion of m/z 147 present in the MI and CAD mass spectra of compound 2.1 both in the FAB (Fig.2.2) and CI (Fig.2.5) mode is generated by a step-wise process. This is further supported by the fact that the ESI mass spectrum, Fig.2.8a, and the ESI-CAD (low energy in comparison with the FAB and CI-CAD) mass spectrum Fig.2.8b, do not show peak corresponding to the ion of m/z 147. The measured accurate masses for the ions of m/z 225 and 207, 225.0896 (calculated for C$_{15}$H$_{13}$O$_2$ is 225.0915) and 207.0796 (calculated for C$_{15}$H$_{11}$O, 207.0810) obtained from the ESI – CAD mass spectrum, are in agreement with the proposed eliminations of ketene and acetic acid from compound 2.1.

A second mechanism is proposed for the formation of the ions of m/z 225, 207 and 147 from protonated 2-acetoxybenzal acetophenone as shown in Scheme: 2.2. It is envisaged that the initial protonation occurs on the oxygen atom of the keto group leading to a cyclic structure, 2.1d, for the protonated molecule. The fragment ion of m/z 225 is
formed by the elimination of ketene from 2.1d. There are three pathways for the
formation of flavylium cation 2.1a. The first is the nucleophilic displacement of a
molecule of acetic acid by the oxygen atom of keto group. The second is 1,2-elimination
of the elements of acetic acid from 2.1d and third pathway involves the elimination of
H₂O from 2.1e, [M+H-ketene]⁺ fragment. In fact, flavylium chloride can be prepared by
treating 2-hydroxychalcone with concentrated hydrochloric acid. Subsequent elimination
of a molecule of benzene from the ion of m/z 225, 2.1c, produces the fragment ion of m/z
147 having the cyclic structure of protonated coumarin, 2.1f.

\[
\text{O} \quad \text{O} \quad \text{C} \quad \text{H}_3 \quad \text{O} \quad \text{O} \quad \text{H} \\
\text{H} \quad \text{O} \quad \text{+} \quad \text{O} \quad \text{+} \quad \text{O} \quad \text{H} \\
\text{2.1} \quad \text{m/z 267} \\
\text{O} \quad \text{O} \quad \text{C} \quad \text{H}_3 \quad \text{H} \quad \text{O} \quad \text{+} \quad \text{O} \quad \text{H} \\
\text{2.1f: m/z 147} \\
\text{O} \quad \text{O} \quad \text{C} \quad \text{H}_3 \quad \text{H} \quad \text{O} \quad \text{+} \quad \text{O} \quad \text{H} \\
\text{2.1c: m/z 225} \\
\text{2.1e: m/z 225} \\
\text{H} \quad \text{2.1d: m/z 267} \\
\text{O} \quad \text{1,2 - elimination} \\
\text{Nucleophilic displacement} \\
\text{2.1a: m/z 207} \\
\text{-CH}_2\text{CO} \\
\text{-H}_2\text{O} \\
\text{m/z 103 and 118 respectively.}
\]

**Scheme: 2.2.** Mechanism for the formation of the ions of m/z 225, 207 and 147
from protonated 2-acetoxybenzal acetophenone.

To confirm the proposed structure, 2.1f, for the fragment ion of m/z 147, its CI-
CAD mass spectrum, Fig.2.9a, was compared with that of protonated coumarin, Fig.2.9b.
The two spectra are closely similar indicating that ion of m/z 147 has indeed protonated
coumarin structure. Note that, the proposed structure 2.1f can easily explain the
expulsions of CO₂ (44 amu) and CHO (29 amu) to produce the characteristic fragment
ions of m/z 103 and 118 respectively.
To further confirm the proposed mechanism in Scheme: 2.2 the CI-CAD of [M+D]$^+$ of compound 2.1 was recorded. The CI-CAD mass spectrum, Fig.2.10, of [M+D]$^+$, (m/z 268), shows peak corresponding to the ion of m/z 226. This shows that the deuterium is retained in the product ion of m/z 226 during the elimination of ketene from the molecular ion (Scheme: 2.3). However, the formation of flavylium cation involves both retention and loss of D to afford fragment ions of m/z 208 and 207 in the ratio 1:3 respectively, Fig.2.10. This is probably due to the exchange of D with the H of the olefinic carbon or aromatic ring. According to the mechanism in Scheme: 2.2 reversible 1,3-hydrogen shift is possible in the cyclic structure 2.1c (m/z 226) and hence an H/D exchange may take place between the two OH groups. Hence, eliminations of both benzene-d and benzene can occur from the ion of m/z 226, 2.1c, Scheme: 2.3. The CI-CAD mass spectrum, Fig.2.10, reveals that eliminations of both benzene-d and benzene take place from the ion of m/z 225 to yield ions of m/z 147 and 148 respectively.
Scheme: 2.3. Fragmentation processes of the [M+D]$^+$ of 2-acetoxybenzal acetophenone.

2.2.2. Mass spectra of 2-benzyloxybenzal acetophenone

For further understanding of the mechanism of the carboxylic acid elimination of protonated acyloxybenzal acetophenones, the FAB, CI and ESI mass spectra of 2-benzyloxybenzal acetophenone, compound 2.2, an analogue of 2-acetoxybenzal acetophenone, was investigated.
The FAB mass spectrum, Fig. 2.11, of 2-benzoyloxybenzal acetophenone (compound 2.2) shows the molecular ion, [M+H]⁺ of m/z 329 and abundant fragment ion of m/z 207. The formation of the ions of m/z 207 involves the elimination of the elements of benzoic acid from the protonated molecule, analogous to the fragmentation of compound 2.1. The FAB – MI and CAD spectra of [M+H]⁺ of compound 2.2 (Fig. 2.12) exhibit peak corresponding to m/z 207 indicating that this fragment ion is formed by the direct dissociation of the molecular ion.

Fig. 2.11 Partial FAB spectrum of compound 2.2

Fig 2.12. FAB-MI (a) and (b) CAD mass spectra of compound 2.2
The ion of m/z 105 observed in both spectra is due to benzoyl cation. Moreover, the complementary ion \([\text{M+H-105}]^+\) (m/z 224) and an ion of m/z 147 are present in the CAD mass spectrum indicating that the formation of these fragment ions involve high-energy processes. This also suggests that the ion of m/z 147 is formed from the \([\text{M+H}]^+\) ion in two steps via the intermediacy of the ion of m/z 224.

Further, FAB – CAD spectrum of the fragment ion of m/z 207, Fig.2.13a, from compound 2.2 was compared with that of flavylium cation, Fig.2.13b, from its chloride (compound 2.5) taken as reference standard. The two spectra are closely similar indicating that the \([\text{M+H- C}_6\text{H}_5\text{COOH}]^+\) ion has indeed flavylium cation structure, 2.1a. This supports the cyclisation of the molecular ion during the elimination of benzoic acid from \([\text{M+H}]^+\) of compound 2.2.

Fig: 2.13. FAB-CAD spectra of fragment ion of m/z 207 from (a) compound 2.2 and (b) flavylium chloride (2.5)
For further understanding of the mechanism of fragmentation of the protonated 2-benzoyloxybenzal acetophenone the chemical ionization (CI) mass spectrum, Fig. 2.14, was also examined. The CI mass spectrum of compound 2.2 showed an abundant molecular ion, [M+H], of m/z 329 and fragment ions of m/z 207 and 105.

The ion of m/z 207 corresponds to the loss of benzoic acid from the molecular ion. The CI-MI and CAD spectra (Fig: 2.15) of compound 2.2 exhibit the peaks corresponding to the ions of m/z 224, 207, 147 and 105 similar to the FAB-MI and CAD mass spectra, Fig. 2.12. The CI – MI and CAD mass spectra of [M+H]⁺ of compound 2.2 (Fig. 2.15) exhibit peak corresponding to the ion of m/z 207 indicating that this fragment ion is formed by the direct dissociation of the molecular ion. The ion of m/z 105 observed in both spectra is due to benzoyl cation. Moreover, the complementary ion [M+H-105]⁺ (m/z 224) and an ion of m/z 147 are present in the MI and CAD mass spectra indicating that the ion of m/z 147 is formed from the [M+H]⁺ ion in two steps via the intermediacy of the ion of m/z 224.
Further, the ESI mass spectrum, Fig. 2.16a and the ESI-CAD (low energy in comparison with the FAB and CI-CAD) mass spectrum of compound 2.2, Fig. 2.16b, do not show peaks corresponding to the ions of m/z 224, 147 and 105. The only fragment ion observed is the ion of m/z 207, indicating that the elimination of benzoic acid from 2.2 is the low energy process. Therefore, the formation of ion of m/z 147 is a high-energy process involving the intermediacy of fragment ion of m/z 224.
A mechanism is proposed for the formation of the ion of m/z 224, 207 and 147 from protonated 2-benzoyloxybenzal acetophenone, **Scheme: 2.4**, similar to the mechanism for the fragmentations of compound **2.1 (Scheme: 2.2)**. The initial protonation occurs on the carbonyl oxygen atom of the keto group leading to a cyclic structure, **2.2d**, for the protonated molecule. The fragment ion of m/z 224 is formed by the elimination of benzoyl group from **2.2d**. There are three pathways for the formation of flavylium cation **2.2a**. The first pathway envisages protonation of the carbonyl oxygen atom of the benzoyloxy group followed by the nucleophilic displacement of a molecule of benzoic acid by the oxygen atom of keto group. The second is 1,2-elimination of the elements of benzoic acid from **2.2d** and third pathway involves the elimination of an OH\(^-\) from **2.2c**, [M+H-benzoyl]**+** fragment. Subsequent elimination of a phenyl group from the ion of m/z 224, **2.2c**, produces the fragment ion of m/z 147 having protonated coumarin, **2.2f**.

**Scheme: 2.4.** Proposed mechanism of fragmentation of [M+H]**+** of **2.2**
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The proposed mechanism is further supported by the confirmations of the structures of the fragment ions of m/z 207 and 147 by comparison of their CAD mass spectra with that of suitable reference compounds. The CI – CAD spectrum of the fragment ion of m/z 207 from compound 2.2 and that of flavylium cation from its chloride (compound 2.5) taken as reference standard are given in **Fig.2.17a** and **Fig.2.17b** respectively. The two spectra are closely similar indicating that the \([\text{M} + \text{H} - \text{C}_6\text{H}_5\text{COOH}]^+\) ion has indeed flavylium cation structure, 2.2a. This observation is in agreement with the FAB- CAD mass spectral studies of fragment ion of m/z 207 obtained from compound 2.1, and thus it confirms the structure of ion of m/z 207 as flavylium cation.

![Fig: 2.17. CI-CAD spectra of ion of m/z 207 produced from (a) compound 2.2 and (b) flavylium chloride (2.5)](image-url)
To confirm the proposed structure, 2.1f, for the fragment ion of m/z 147, its CI-CAD mass spectrum, Fig. 2.19c, was compared with that of protonated coumarin, Fig. 2.18b and with that of the fragment ions of m/z 147 from compound 2.1, Fig. 2.18a. The three spectra are similar but there are additional fragment ions of m/z 131, 119, 117, 115 and 105 present in Fig. 2.18c, the CI-CAD mass spectrum of the ion of m/z 147 from compound 2.2 indicating that ion of m/z 147 has a mixture of two structures.

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**Fig. 2.18.** CI-CAD spectra of ion of m/z 147 from (a) compound 2.1 (b) protonated coumarin (c) compound 2.2
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The relative abundances of the characteristic fragment ions of m/z 91, 103 and 118 are nearly identical in all the three CAD mass spectra indicating that the major fraction of the ions of m/z 147 may have protonated coumarin structure but there is minor contribution from another structure, probably an open chain structure which will be discussed later. Furthermore, the proposed structure 2.1f can easily explain the expulsions of CO\(_2\) (44 amu) and CHO (29 amu) to produce the characteristic fragment ions of m/z 103 and 118 respectively.

Further, the CI-CAD mass spectrum, Fig.2.19, of [M+D]\(^+\), (m/z 330), show fragment ions of m/z 225, 207, 148 and 105. The presence of the ion of m/z 225 shows that the deuterium is retained in the product ion during the elimination of benzoyl group from the molecular ion as shown in Scheme: 2.5. However, the formation of flavylium cation involves loss of D to afford fragment ion of m/z 207. The ion of m/z 225 (2.2c) dissociates via the elimination of a phenyl group to from, the fragment ion of m/z 148, 2.2f, Scheme: 2.5, with the retention of D.
**Scheme: 2.5.** Mechanism showing the fragmentation processes of [M+D]$^+$ of 2-benzoyloxybenzal acetophenone (2.2)

2.2.3. Mass spectra of 4-acetoxybenzal acetophenone (2.3)

![FAB mass spectrum of compound 2.3](image)

**Fig.2.20.** FAB mass spectrum of compound 2.3
For further understanding of the mass spectral behavior of protonated acetoxy and benzoyloxybenzal acetophenones the FAB, CI and ESI mass spectra of 4-benzoyloxybenzal acetophenone, compound 2.3, was examined.

The FAB mass spectrum, Fig. 2.20, of 4-acetoxybenzal acetophenone (compound 2.3) shows molecular ions, [M+H]^+ of m/z 267 and abundant fragment ion of m/z 225. The formation of the ions of m/z 225 involves the elimination of a ketene molecule from the protonated molecule, analogous to the fragmentation of compound 2.1. But this molecule does not show fragment ion of m/z 207 which was observed in the FAB mass spectrum of compound 2.1, the ortho isomer, indicating that the elimination of acetic acid from the protonated molecule,[M+H]^+ of 2.3, is not feasible for the para isomer. This suggests that the elimination of acetic acid from 2.1 is indeed due to the intramolecular
cyclisation of the protonated molecule. In addition the FAB – MI and CAD spectra of [M+H]$^+$ of 2.3 (Fig.2.21) does not show a fragment ion of m/z 207 but exhibit peaks corresponding to m/z 225 and 147. The ion of m/z 147 is formed from the [M+H]$^+$ ion in two steps via the intermediacy of the ion of m/z 225. If the mechanism proposed for the formation of the ion of m/z 147 from compound 2.1 and 2.2 involving cyclisation are true, the fragment ion of m/z 147 formed from 2.3 must have a structure different from that proposed in Schemes: 2.2 and 2.4 (protonated coumarin).

Further, the CI mass spectrum, Fig.2.22, of 2.3 showed an abundant molecular ion, [M+H]$^+$, of m/z 267 and fragment ions of m/z 225 and 147.

![Fig: 2.22. CI-mass spectrum of 4-Acetoxybenzal acetophenone (2.3)](image)

The CI-MI and CAD mass spectra, Fig. 2.23a and b, of 2.3 exhibit the peaks corresponding to the ions of m/z 225, 207, 147 and 105. The ion of m/z 105 observed in both spectra is due to benzoyl cation. Moreover, the intensity of peak corresponding to m/z 207 is low indicating that the formation of this ion is a high-energy process i.e. it may be formed due to the dissociation of the ion of m/z 225.
To determine the structure of the ion of m/z 147, its CAD spectrum, Fig. 2.24d, was compared with that of protonated coumarin, Fig. 2.24b, 2.1, Fig. 2.24a, and 2.2, Fig. 2.24c. The CAD spectrum of the ion of m/z 147 from 2.3 is not similar to that of either protonated coumarin or that of the ion of m/z 147 from 2.1, indicating that ion of m/z 147 from 2.3 has a structure different from protonated coumarin. The elimination of CO$_2$ is not prominent in the CAD spectrum of ion of m/z 147 from 2.3, a characteristic elimination of protonated coumarin. Whereas the CAD mass spectrum, Fig. 2.24d, shows a characteristic fragment ion of m/z 119 due to the elimination of CO, indicating an open chain structure that can eliminate CO as shown in Scheme: 2.6. Therefore, the presence of the ion of m/z 119 in the CAD mass spectrum, Fig. 2.24c, indicates that a minor fraction of the ions of m/z 147 from 2.2 may have an open chain structure.
Fig: 2.24. CI-CAD spectra of ion of m/z 147 from (a) 2.1 (b) protonated coumarin (c) 2.2 and (d) 2.3
The ESI mass spectrum, Fig.2.25a and CAD mass spectrum, Fig.2.25b, of 2.3, show the molecular ion of m/z 267 and m/z 225. The formation of fragment ion of m/z 225 is due to the elimination of a ketene molecule from the [M+H]^+ of 2.3. The ESI-mass spectra do not show fragment ions of m/z 207 and 147 indicating that the formation of these ions involves high energy process. The measured accurate mass 225.0916 corresponds to C_{13}H_{13}O_{2} (calc. 225.0915), loss of ketene from [M+H]^+ ion.

![Graphical representation of fragmentation processes](image)

**Scheme: 2.6.** Fragmentation processes of protonated 4-acetoxybenzal acetophenone
The fragmentation processes of protonated 4-acetoxybenzal acetophenone are shown in Scheme: 2.6. The [M+H]$^+$ of 2.3, eliminates a molecule of ketene to form product ion of m/z 225 which can exist in two isomeric forms, 2.3a and 2.3b. The fragment ion of m/z 225 then eliminates a benzene molecule to form the ion of m/z 147.

2.2.4. Mass spectra of 4-benzoyloxybenzal acetophenone

Further the FAB, CI and ESI mass spectra of 4-benzoyloxybenzal acetophenone, 2.3, para isomer of 2-benzoyloxybenzal acetophenone, was investigated. The FAB mass spectrum, Fig.2.26, of 2.4 shows the [M+H]$^+$ ion of m/z 329. It does not show peaks corresponding to fragment ions of m/z 207 and 147. The FAB – MI and CAD spectra of [M+H]$^+$ of compound 2.4 (Fig.2.27) exhibit peaks corresponding to m/z 224 and 105 indicating that these fragment ions are formed by the direct dissociations.

Fig. 2.26. FAB spectrum of 4-benzoyloxybenzal acetophenone

The CI mass spectrum (Fig: 2.28) of 2.4 showed an abundant molecular ion of m/z 329 and fragment ions of m/z 251 and 105. The fragment ion of m/z 105 is due to the formation of benzoyl cation and the fragment ion corresponding to m/z 251 shows benzene loss from the molecular ion, m/z 329.
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Fig: 2.27. FAB-MI (a) and (b) CAD mass spectra of [M+H]^+ of 2.4

Fig: 2.28. CI mass spectrum of 4-benzyloxybenzal acetophenone, 2.4
The ESI mass spectrum of \textbf{2.4}, \textbf{Fig.2.29a}, showed the molecular ion of m/z 329. The CAD mass spectrum, \textbf{Fig.2.29b}, showed very small peaks due to fragment ions of m/z 251 and 105. This shows that the fragmentation of protonated 4-benzoyloxybenzalacetophenone is a high energy process.

\textbf{Scheme: 2.7.} Fragmentations of protonated 4-benzoyloxybenzalacetophenone (2.4)
2.3. Conclusion

The gas-phase rearrangements and consequent fragmentations of acetoxy and benzoyloxy benzalacetophenones, upon protonation in the FAB, CI and ESI modes of ionizations, were investigated. The \([\text{M+H}]^+\) ion of 2-acetoxybenzal acetophenone cyclises and then eliminates either acetic acid to yield flavylium cation or ketene to generate protonated 2-hydroxybenzal acetophenone. The elimination of acetic acid may be considered as nucleophilic displacement of the acetic acid by the oxygen atom of the keto group. The \([\text{M+H-Ketene}]^+\) ion fragments by eliminating a molecule of benzene to yield protonated coumarin (m/z 147). The \(\text{para}\) isomers also yield the ions of m/z 147 but have an open chain structure. The mechanism of elimination of acetic acid, ketene and benzene from the protonated molecule was proposed based on tandem mass spectrometric experiments and D-labeling. Upon protonation 2-benzoyloxybenzalacetophenone cyclises and then eliminates either benzoic acid or benzoyl radical. The structure of the fragment ion, \([\text{M-benzoic acid}]^+\), was established to be flavylium cation. The \([\text{M-C}_6\text{H}_5\text{CO}]^+\) fragment by eliminating a phenyl group to yield protonated coumarin (m/z 147).
2.4. Experimental

2.4.1. Preparation of 2-Hydroxybenzal acetophenone [31, 32]

Salicylaldehyde - 5.5 ml
Acetophenone - 6.0 ml
Sodium hydroxide - 2.5 gm in 25 ml distilled water

2.5 gm of sodium hydroxide was dissolved in 25 ml of distilled water. 6.0 ml of acetophenone was slowly added followed by the dropwise addition of 5.5 ml of salicylaldehyde with stirring. The mixture was kept in a water bath for acquiring the room temperature and stirred for four hours, acidified with dilute hydrochloric acid. The mixture was then added to ice water. The precipitated 2-hydroxybenzalacetophenone was filtered, washed with water and dried. It was purified by recrystallisation from ethanol.

Yield 8.5 gm. Melting point 156° C (Reported [32, 33] 154-155° C).

IR (KBr) cm\(^{-1}\): 3210(aromatic O-H stretch), 3084 (aromatic CH stretch), 1640(\(\alpha,\beta\)-unsaturated C=O stretch), 1562-1600 (C=C stretch), 1231(C-O stretch).

\(^1\)H NMR (in CDCl\(_3\)): \(\delta\) 6.3 (s, 1 H), 6.9 (d, 1 H), 7.0-8.0 (m, 9H), 8.2 (d, 1H).

2.4.2. Preparation of 2-Acetoxybenzalacetophenone (2.1) [32, 34, 35]

2-hydroxybenzalacetophenone - 2 gm
Acetic anhydride - 3 ml
Sodium bi carbonate - 1 gm

Two gram of powdered 2-hydroxybenzalacetophenone is taken in a 100 ml R.B.flask; 3ml of acetic anhydride is added. The R.B. flask is kept immersed in water. The content is stirred for four hours. After 2 hours 1 gm of sodium bi carbonate is added. The content is added to the ice cold water and extracted with ether. Ether layer is washed with dilute solution of NaHCO\(_3\) and then with distilled water. Ether is evaporated off. Ether layer is dried by adding anh. Na\(_2\)SO\(_4\). A dense liquid is obtained which is solidified
on standing for 48 hours. Yield 1.8 gm, purified by recrystallisation from ethanol. Melting point 64 °C (Reported [32] 65-66 °C)

**IR (KBr) cm\(^{-1}\):** 3037-3060 (aromatic CH stretch), 2940(methyl CH stretch), 1759(ester C=O stretch), 1660(α,β-unsaturated C=O stretch), 1573-1602 (C=C stretch ), 1177-1201(C-O stretch).

**\(^1\)H NMR (CDCl\(_3\)):** δ 2.4 (s, 3H), 7.1 (d, 1H), 8.0 (d, 1H), 7.3-7.9 (m, 9H)

### 2.4.3. Preparation of 2-Benzoyloxybenzal acetophenone (2.2)[32 ]

2-hydroxybenzal acetophenone - 2 gm

Benzoyl chloride - 2 ml

Sodium hydroxide (20%) - 15 ml

To 2.0 g of powdered 2-hydroxybenzal acetophenone is taken in an R.B. flask, 15 ml of 20% NaOH is added with shaking. The R.B. flask is kept immersed in water and 2 ml of benzoyl chloride is added drop wise with stirring. The content is stirred for 4 Hrs. The mixture is added to the ice and shaken well. The precipitated compound is filtered at the pump, washed with water and dried. Yield 2.4 gm. The product is further purified by recrystallisation from ethanol. Melting point 99°C (Reported [32]101-102° C).

**IR (KBr) cm\(^{-1}\):** 3035-3063 (aromatic CH stretch), 1740(ester C=O stretch), 1664(α,β-unsaturated C=O stretch), 1602(C=C stretch ), 1178-1217(C-O stretch)

**\(^1\)H NMR (in CDCl\(_3\)):** δ 7.1-7.9(m, 15 H), 8.2 (d, 1H).

### 2.4.4. Preparation of 4-Acetoxybenzal acetophenone (2.3) [32]

4-hydroxybenzal acetophenone - 2 gm

Acetic anhydride - 3 ml

Sodium bicarbonate - 1 gm
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The title compound is prepared by employing the procedure used for the preparation of 2-acetoxybenzal acetophenone (2.1). Yield 2.2 gm, purified by recrystallisation from ethanol. Melting point 128° C (Reported [32, 36] 129° C).

\[ \text{IR (KBr) cm}^{-1}: 3063 (\text{aromatic CH stretch}), 2950(\text{methyl CH stretch}), 1754(\text{ester C=O stretch}), 1659(\alpha,\beta-\text{unsaturated C=O stretch}), 1590-1600 (\text{C=C stretch }), 1161-1213(\text{C-O stretch}). \]

\[ \text{H NMR (in CDCl}_3\text{: } \delta 2.3 (s, 3 H), 7.2 (d, 1 H), 7.3-7.9 (m, 9H), 8.0 (d, 1H) \]

The 4-hydroxybenzal acetophenone used for the preparation was prepared by making use of the procedure used for the preparation of 2-hydroxybenzal acetophenone.

- 4-hydroxybenzaldehyde - 5.5 ml
- Acetophenone - 6.0 ml
- Sodium hydroxide - 2.5 gm in 25 ml distilled water

The product is purified by recrystallisation from ethanol. Yield 8 gm. Melting point 182° C (Reported [32, 36] 182.5-184° C)

\[ \text{IR (KBr) cm}^{-1}: 3235 (\text{O-H stretch}), 3060 (\text{aromatic CH stretch}), 1650(\alpha,\beta-\text{unsaturated C=O stretch}), 1580-1600 (\text{C=C stretch }), 1161-1213(\text{C-O stretch}) \]

\[ \text{H NMR (CDCl}_3\text{: } \delta 6.9 (d, 1 H), 7.1 (s, 1H), 7.4-7.9 (m, 9H), 8.0 (d, 1H) \]

2.4.5. Preparation of 4-Benzoyloxybenzal acetophenone (2.4) [32]

- 4-hydroxybenzal acetophenone - 2 gm
- Benzoyl chloride - 2 ml
- Sodium hydroxide (20%) - 15 ml

The title compound is prepared by employing the procedure used for the preparation of 2-benzoyloxybenzal acetophenone (2.2). Yield 2.4 gm, purified by recrystallisation from ethanol. The 4-hydroxybenzal acetophenone used for the
preparation was prepared by making use of the procedure used for the preparation of 2-hydroxybenzal acetophenone.

**IR (KBr) cm⁻¹**: 3062 (aromatic CH stretch), 1735 (ester C=O stretch), 1658 (α,β-unsaturated C=O stretch), 1576-1598 (C=C stretch), 1166-1211 (C-O stretch)

**¹H NMR (in CDCl₃)**: δ 7.2 (d, 1H), 7.3-8.0 (m, 14 H), 8.2 (d, 1H).

### 2.4.6. Preparation of Flavylium chloride (2.5) [37]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxybenzal acetophenone</td>
<td>1 gm</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>3 ml</td>
</tr>
<tr>
<td>Con. HCl</td>
<td>3 ml</td>
</tr>
</tbody>
</table>

One gram of 2-hydroxybenzal acetophenone is taken in a 100 ml R.B. flask; it is dissolved in 3 ml of ethyl acetate and 1 ml of con. HCl is added. The mixture is stirred for 3 hours. The content is added with 15 ml of water. The precipitated compound is filtered at the pump, washed with water and dried. The product is purified by recrystallisation from hexane. Yield 1.2 gm. Melting point 85°C (Reported [37] 85-6°C)

**IR (KBr) cm⁻¹**: 3060 (aromatic CH stretch), 1576-1598 (alkene C=C stretch), 1450-1600 (aromatic C=C stretch), 1222-1263 (C-O stretch).

**¹H NMR (in CDCl₃)**: 6.7-8.1 (m, 11 H).

### 2.4.7. Coumarin (2.6)

Coumarin was purchased from Aldrich Chemical Co. and used with out further purification.
2.5. References

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