100 as the only active principle in major quantity. The investigation of *Zanthoxylum simulans* was taken up because other species of this plant had yielded novel alkaloids and coumarins. In the present investigation efforts were made towards separation of a number of phenolic compounds which on TLC plates of the extracts appeared as fluorescent spots. Chromatographic work up of the chloroform extract of *Zanthoxylum simulans*, afforded limonin 101 and flavone 106. This is the first report on isolation of these two compounds from *Z. simulans*. The third chapter is devoted to the antimicrobial screening of the compounds synthesized in the laboratory and isolated from the plant. All compounds showed very good antimicrobial activity against bacteria and fungi including *Candida albicans*. 
CHAPTER 1

Synthesis and Characterization of Heterocycles
1. Introduction

Chromone and its derivatives have been reported to perform important biological activities such as oestrogenic, antibacterial, anti-inflammatory, anti-spasmodytic, antitumor and anti-hepatotoxic activities\(^1\)\(^-\)\(^5\). Thus, it has always attracted the attention of chemists for synthesis of new heterocycles from it.

A number of methods have been reported in the literature for the preparation of 3-formylchromone. Eiden et al have synthesized it for the first time by formylating 2-formyl-2'-hydroxyacetophenone derived from o-hydroxyacetophenone using ethyl orthoformate and acetic anhydride\(^6\).

![Chemical structure of 3-formylchromone](image)

This method however is not good enough as it affords 1 with poor yield. The compound 1 also has been synthesized from 2, 2-difluoro-4-methylnaphtho-1,3,2-dioxaborin by the Vilsmeier Haack reaction\(^7\).

Though various methods are known for carrying out C-formylation Vilsmeier Haack reaction seems best as this reagent is very mild and can be applied to even polyfunctional molecules. In addition to this, Vilsmeier Haack reaction affords 1 in good yield and less time
period. Thus, Nohara et al\textsuperscript{8} reported for the first time synthesis of I by Vilsmeier Haack reaction of o-hydroxyacetophenone using DMF and POCl\textsubscript{3} (Scheme 1).
Recently Linghua and co-workers\(^9\) studied several molar ratios of substituted o-hydroxyacetophenone-POCl\(_3\)-DMF such as 1:1:1, 1:3:3, 1:3:6 and 1:6:12 for synthesis of 6-alkyl and halo substituted 3-formylchromone. They found that in the ratio 1:6:12, the yield of the product was best, even higher than that reported earlier. Some authors have also reported synthesis of hydroxy substituted 3-formylchromone in connection of synthesis of 2-oxopyranochromone-3-carboxaldehyde, by choosing appropriately hydroxy substituted o-hydroxy acetophenone and DMF/POCl\(_3\)^10.

In the IR spectrum of 1 a band at 1692-1700 cm\(^{-1}\) is due to aldehydic group. The chromone carbonyl group usually appears as a strong band at 1645-1665 cm\(^{-1}\).
The $^1$H NMR spectrum of 1 gives singlets at $\delta$ 10-11, 8.4-8.5 for aldehydic and C-2 protons respectively. The double doublet of C-5 proton is usually found at $\delta$ 8.2-8.3. The remaining three aromatic protons are in the form of multiplet in the aromatic region $\delta$ 7.4-7.8. In the mass spectrum the molecular ion peak at m/z 174 loses CO to give the base peak at m/z 146 which again loses CO to give the peak at m/z 118. Another mode of breaking down of the m/z 146 ion occurs through loss of C$_2$H$_2$O and CO groupings to give peaks at m/z 76. The ion at m/z 146 further suffers RDA cleavage to give fragment ions at m/z 120 which eliminates CO to form fragment ions at m/z 92 and 64 (Scheme-2). The m/z 146 ion produced from 1 seems to be same as the molecular ion of chromones$^8$ but the degradations giving peaks at m/e 104 and m/z 76 are not important pathways in the latter. This mode of break down which differs from those of flavones and chromones seems to be characteristic feature of 1.
Scheme-2
2. Theoretical

3-Formylchromones are useful synthons for the synthesis of a large number of heterocycles as it contains several electron deficient centres namely C-2, C-4 and carbon of formyl group which can be modified into various types of derivatives by the attack of nucleophile. In the context of further work on 3-formylchromone, a survey of literature of about 30 years was done and some relevant examples are discussed below.

2.1 Reaction of 3-formylchromone (1) with active methylene compounds:

One of the earliest example in this category is synthesis of o-hydroxyacetophenone 2 from 3-formylchromone 1 and ethylacet-oacetate in the presence of a base \(^{11}\).

![Chemical reaction image]

The mechanism involves initial condensation of 1 with ethyl acetoacetate followed by Michael addition, opening of pyrone ring and subsequent cyclization (Scheme-3).
\[
\text{CH}_3-C-\text{CH}_2-C-\text{O}_2\text{H}_5 + \text{NaOAc} \rightarrow \text{CH}_3-C-\text{CH}-C-\text{O}_2\text{H}_5
\]

Scheme-3
An extension to such type of reaction is the treatment of 1 with methyl acetoacetate in presence of ammonia to give dihydropyridine derivatives 3\textsuperscript{12}.

Another interesting example in this category is the condensation of 1 with amino esters such as glycine ester in the presence of a catalytic amount of p-toluenesulfonic acid. The reaction mixture gave a mixture of pyridine 4 and pyrrole 5\textsuperscript{13}.
2.2 Synthesis of Styryl chromones:

Styrylchromones belong to a group of oxygen heterocycles and are reported to exhibit biological properties. Heck reaction used for the synthesis of styrylchromone from 3-bromochromone with styrene in the presence of Pd (OAc)_2, tri-o-tolylphosphine and (Et)_3N in DMF has some disadvantages as the method has been used for the formation of only unsubstituted 3-styryl chromones and taking polyhalogenated chromone derivatives, the coupling of several alkenes to the chromone systems at the halogenated sites takes place to give undesired product.

In this connection Portugees authors have reported synthesis of 3-styryl chromones from 1 and benzylic ylides (Witting reaction). The reaction involves treatment of benzylidene triphenyl phosphorane with 1 to give a mixture of cis and trans 3-styryl chromones, 7 and 8 (Scheme 4).

![Chemical structure diagram]
The reaction mechanism involves following steps:

(i) Formation of ylide 6

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{CH}_2\text{Cl} \quad + \quad \text{Ph}_3\text{P} \quad \rightarrow \quad \text{O}_2\text{N} \quad \text{CH}_2\text{P}^+\text{Ph}_3 \\
\text{NaH}/\text{THF} & \quad \downarrow \\
\text{O}_2\text{N} & \quad \text{CH}=\text{PPh}_3 \\
\end{align*}
\]

(ii) Reaction of ylide 6 with 1

\[
\begin{align*}
\text{C} & \quad \text{O} \quad \text{H} \\
\text{1} & \quad + \quad \text{Ph}_3\text{P}=\text{CH}\rightarrow \quad \text{Ph}_3\text{PO} \\
\text{O} & \quad \text{O} \quad \text{Ph} \\
\text{6} & \quad \rightarrow \\
\text{8} \\
\end{align*}
\]

Scheme 4

Another simple transformation of 1 to styrylchromone 9 involves treatment of 1 with 2,4-dinitro toluene in the presence of base such as pyridine.

\[
\begin{align*}
\text{1} & \quad + \quad \text{CH}_3\text{NO}_2 \quad \text{Ph}_3\text{NO}_2 \\
\text{Pyridine/} & \quad \text{reflux} \\
\text{9} \\
\end{align*}
\]
2.3 Synthesis of chalcones:

The chalcones are important precursors of flavonoids and isoflavonoids. A large number of chalcones and their derivatives have been prepared from Claisen-Schmidt condensation of aldehydes with methyl ketones under basic conditions. These compounds have shown in vitro anti-malarial activity against chloroquine sensitive and chloroquine resistant strains of Plasmodium falciparum. A variety of chalcone derivatives also have been reported for acting as potent tyrosinase inhibitors, antioxidants and are thus used as new depigmentation agents. Recently Tshkaev et al have reported conversion of 1 to cholcone 10 with acetophenones under acidic conditions using acetic acid and perchloric acid and have shown a variety of biological activities.

\[
\begin{align*}
\text{CHO} & \quad \text{H}_3\text{C} \quad \text{AcOH} \\
\text{1} & \quad \text{HClO}_4 \quad \text{10}
\end{align*}
\]

2.4 \((4\pi + 2\pi)\) Cyclo addition reactions of 1

Due to presence of carbonyl group at position 3, which may conjugate with double bond, 1 also functions as diene in \((4\pi+2\pi)\) cycloaddition reaction with dienophiles. The intermediate adduct as obtained can be transformed to other useful heterocycles either by rearrangement or through ring opening reactions. An interesting...
example in this context is the reaction of 1 with vinyl ethers to give 11 which has the heterocyclic unit similar to that present in fungal metabolite, fulvic acid.\textsuperscript{25}

\[
\begin{align*}
\text{CH}_2\equiv\text{C}(\text{OR}) + \text{H} & \rightarrow \text{R'} \quad \text{R'} = \text{H}, \text{Me}, \text{OEt}, \text{nBu}\n\end{align*}
\]

The adduct 11 has been converted to aldehyde 12 with methoxide ion through ring opening followed by rearrangement and cyclization\textsuperscript{26} (Scheme 5).

\[
\begin{align*}
\text{OR} \quad \text{MeOH} & \rightarrow \text{OMe} \\
\text{CHO} & \rightarrow \text{OH}
\end{align*}
\]

Scheme 5

The aldehyde 12 has also been synthesized by Ghosh et al\textsuperscript{27} indirectly from 3-substituted chromone.

Sandhu et al\textsuperscript{28} synthesized Schiff's base 13 of 1 and then used it as a diene in the reaction with dienophile 14 to form the adduct 15 in the presence of triflate as a catalyst (Scheme 6).
The compound was identified on the basis of $^1$H NMR which showed disappearance of diagnostic peak of H-2 proton at δ 8.65 in 1 and presence of upfield signal at δ 5.34. This confirmed that cycloaddition had occurred at C-2 position of the chromone unit.

Diels Alder reaction of 3-formylchromone with o-benzoquinone dimethane 16 forms bezoxanthones 18 through oxidation of cycloadduct 17.
Xanthones, which are naturally occurring compounds and are present as minor constituents in plants, can be synthesized, thus, in one step reaction as shown in Scheme 7.

2.5 Ring Opening Reactions

Chromone and its derivatives react with nitrogen bases and form the products by opening of pyrone ring followed by cyclisation. Schonberg et al. refluexed a mixture of chromone 19 and hydrazine hydrate in ethanol and believed that the product was hydrazone (absence of chromone carbonyl group in IR spectrum) but Baker et al. showed that the product was pyrazole 20 (absence of diagnostic signal of H-2 proton of chromone moiety).

Phenylhydrazine also causes ring cleavage of chromones but under some conditions the phenylhydrazone 22 is also formed

Scheme 8
Treatment of 1 with guanidine in the presence of ethoxide affords pyrimidine 23\textsuperscript{34}.

The authors explained the formation of 23 by 1,2 addition of guanidine, generated from its salt by alkali or alkoxide, to the aldehydic group of 1 followed by ring opening and subsequent cyclization. This, however, effects the yield of the product as extensive degradation takes place in the presence of alkali which also changes the course of the reaction.
Ghosh et al.\textsuperscript{35} obtained 23 along with 24 by the reaction of 1 with guanidine without using alkali or alkoxide. This method afforded pyrimidine derivatives in good yield.

The formation of 23 and 24 take place by initial 1,4 addition of nucleophile to pyrone ring followed by subsequent elimination and cyclization (Scheme 9)

\begin{align*}
\text{formation of 23 and 24} & \text{take place by initial 1,4 addition of nucleophile to pyrone ring followed by subsequent elimination and cyclization (Scheme 9)}
\end{align*}
2.6 Synthesis of tetrahydrofuran derivative

In another useful transformation 1 was treated with halohydrin to give tetrahydro furan derivative\textsuperscript{25}. This reaction was investigated when an effort was made to carry out hydroxyethylation of 1 under basic conditions.

\[
\begin{array}{c}
\text{CHO} \\
\text{1}
\end{array} + \text{HO-CH}_2\text{-CH}_2\text{-I} \rightarrow \begin{array}{c}
\text{CHO} \\
\text{25}
\end{array}
\]

The reaction involves attack of halohydrin on C-2 carbon followed by cyclization and deformylation.

2.7 Synthesis of 5-nitro-2,3-benzofurandione-(z)-2-oxime

In an attempt of synthesize 6-nitro-3-formylchromone\textsuperscript{8} using fuming nitric acid, Curran et al\textsuperscript{38} reported formation of oxime 26 as shown in Scheme 10. The mechanism involves oxidation of 3-formylchromone to carboxylic acid\textsuperscript{37} 27 which undergoes electrophilic addition by nitronium ion followed by retroaldol reaction, hydrolysis and decarboxylation.
Scheme 10
3. Discussion

3.1 The reaction of 3-formylchromone with 3-alkyl-4-amino-5-mercapto-1,2,4-triazole.

Chromones usually undergo opening of pyrone ring via nucleophilic attack at C-2. Thus, chromone 19 reacts with nitrogen nucleophiles such as hydroxyl amine to give 28.

Aniline also behaves in a similar manner. When diethylamine is allowed to react with 3-bromochromanone, 29 the product is 30 and not 31. The formation of 30 involves chromone, 19 as intermediate which undergoes ring opening via nucleophilic attack of diethylamine (Scheme 11).
The reactivity of chromone system towards nucleophiles is however, changed when certain substituent are present at position 3. Thus, the reaction of 1 with aniline proceeds without opening of the chromone ring to give 32.

\[
\begin{align*}
\text{1} & \quad \text{NH}_2\text{Ph} \\
\text{32}
\end{align*}
\]

It appeared, therefore, interesting against this background to study the reaction of 1 with alkyl substituted 4-amino-5-mercapto-1,2,4-triazole. Thus, the reaction with different types of triazoles viz. 4-amino-3-methyl-5-mercapto-1,2,4-triazole 33, 4-amino-3-ethyl-5-mercapto-1,2,4-triazole 34, and 4-amino-3-propyl-5-mercapto-1,2,4-triazole 35, afforded T3Fc(B), 3FcTP, 3FcTB and their structures are discussed below.

3.1.1 The reaction of 1 with 4-amino-3-methyl-5-mercapto-1,2,4-triazole:

The compound shows M^+1 peak at m/z 287 in its mass spectrum (Fig. 1). The IR spectrum (Fig. 2) exhibits a broad band at 3424 cm^{-1} which is assigned to NH group. The IR spectrum also displays a strong
absorption band at 1649 cm\(^{-1}\) and a moderate band at 1284 cm\(^{-1}\). These bands are due to chromone carbonyl and C=S functional groups. Since the compound does not give the characteristic colour test with FeCl\(_3\), the opening of the chromone ring is ruled out. Therefore, a structure like 36 cannot be assigned to the compound.

\[ \text{36} \]

The other structure 37 compatible with the value of M\(^+\) is again not possible because of absence of aldehydic band at 1700 cm\(^{-1}\) in the IR spectrum and negative test with ferric chloride.

\[ \text{37} \]

The nmr spectrum (Fig. 3) of compound shows presence of methyl group as a sharp singlet at \(\delta\) 2.37. The doublet of H-5 proton of
chromone nucleus is clearly seen at $\delta$ 8.17. The characteristic singlet of H-2 proton of chromone nucleus at $\delta$ 9.13 is at rather high value and this may be due to presence of electron withdrawing triazole nucleus at C-3. The most ambiguous feature of the nmr spectrum is down field singlet at $\delta$ i0.37 for imine proton, as in literature the resonance of this proton usually appears at $\delta$7.5 to 8.7.41

On the basis of these spectral features one arrives at structure 38 for compound which prefers to be present in the form 38a involving thiol-thione tautomerism.

The mass spectrum (Fig. 1) is also consistent with structure 38 showing fragment ions at m/z 107, 120, 172, 165 as shown in Scheme 12.
Scheme 12
3.1.2 The reaction of 1 with 4-amino-3-ethyl-5-mercapto-1,2,4-triazole:

The compound 39 shows (M$^+$+1) at m/z 301 in its mass spectrum (Fig. 4).

![Chemical Structure of 39]

IR spectrum (Fig. 5) exhibit a broad band at 3451 cm$^{-1}$ which is assigned to NH group. The $^1$H NMR spectrum (Fig. 6) shows clear triplet and quartet for CH$_3$ and CH$_2$ protons at $\delta$ 1.33 and 2.80. Three aromatic protons H-6, H-7 and H-8 of chromone nucleus appear as two multiplets centred at $\delta$ 7.48-7.56 and 7.73-7.78. The H-5 proton is discernible as doublet at $\delta$ 8.33 (J=9Hz) whereas H-2 proton appears as sharp singlet at $\delta$ 8.70 which is quite a normal value. The proton of imine group again resonates at higher value $\delta$ 10.37 and NH protons appears as a broad singlet at $\delta$ 10.46 which is D$_2$O exchangeable.

3.1.3 The reaction of 1 with 4-amino-3-propyl-5-mercapto 1,2,4-triazole:

The mass (Fig.7) and IR spectra (Fig. 8) agree with the structure 40.

![Chemical Structure of 40]
The \(^1\)H NMR spectrum (Fig. 9) displays signals for CH\(_3\), CH\(_2\), CH\(_2\) protons as triplet at \(\delta\) 1.04, multiplet at \(\delta\) 1.81 and triplet at \(\delta\) 2.77 respectively. Besides it, H-5 proton appears as double doublet at \(\delta\) 8.32 and H-2 proton as a sharp singlet at \(\delta\) 8.71. Another sharp singlet at \(\delta\) 10.35 and a broad singlet at \(\delta\) 11.22 which is exchangeable with D\(_2\)O, has been assigned to imine and NH protons respectively.

3.1.4 Cyclization of Schiff's bases, 38-40:

Cyclization of Schiff's bases take place by refluxing in a high boiling solvent\(^{41}\). In order to get cyclized product Schiff's, bases 38-40, were refluxed in nitrobenzene for sometime. After removal of solvent under reduced pressure, the reaction mixture was chromatographed over silica gel. Elution of the column with benzene-petrol with varying ratio afforded two compounds, 3FCTNB as a major product and 3FCTPNB as a minor one.

3FCTNB:

The IR spectrum (Fig. 10) of the compound shows a slightly broad and strong absorption band at 1654 cm\(^{-1}\) showing the presence of more than one chromone carbonyl group. The \(^1\)H NMR (Fig. 11) of the compound does not show any methyl signal in the region \(\delta\) 2.2 to 2.4. This rules out the possibility of a structure \(^{41}\) for the compound which is also not compatible with the mass spectrum (Fig. 12).
Fig. 12
The \(^1\)HNMR spectrum clearly shows the characteristic C-5 signals of chromone units as a doublet integrating for two protons at \(\delta 8.24\). This indicates the presence of two chromone nuclei in the compound. In addition to this, the spectrum also shows two sharp singlets, each integrating for one proton at \(\delta 9.14\) and 9.58. The former can be assigned to C-2 proton (\(H_a\)) of the chromone nucleus whereas the later at higher value can be assigned to a proton which is under the influence of some electron withdrawing group. On the basis of these structural features structure 42 may be assigned to the compound.

On the basis of structure 42 the singlet at \(\delta 9.58\) is now assigned to the proton which is \textit{peri} to oxygen (\(H_b\)) and experiences deshielding effect of electron withdrawing \(>\text{C}=\text{O}\) group of chromone unit. Since structure 42 does not have any alkyl group, it seems that the compound...
42 on refluxing in nitrobenzene, is obtained as a result of dimerisation of the Schiff's bases 38-40 involving (4+2) cycloaddition reaction with the expulsion of triazole moiety as shown in Scheme 13.

![Scheme 13](image)

The structure 42 is also in conformity with its mass spectrum (Fig. 12) which shows M$^+$ at m/z 343. (M+1 peak). Elimination of HCN or CN groups is a common feature in the mass spectrum of pyrimidines/pyridazines$^{42}$. Molecular ion peak, therefore, gives peak at m/z 315 by removal HCN. The other relevant peaks are obtained as a result of break down of the compound as shown in Scheme 14.
RDA

m/z 120

m/z 222

m/z 144 - CO

m/z 116

m/z 342

RDA

m/z 171

41
Scheme 14

3FCTPNB:

The compound shows $M^+$ at $m/z$ 343 (M+1 peak) in its mass spectrum (Fig. 13) Since it has the same molecular ion peak as that of 42, it was thought that it is the same compound but repeated TLC, Co TLC and melting point confirmed that two compounds are different. The IR spectrum (Fig. 14) of the compound shows a broad and strong absorption band at
1672 cm⁻¹ indicating the presence of chromone carbonyl group in the compound. The nmr spectrum (Fig. 15) exhibits a sharp singlet integrating for one proton at δ 10.30 and a doublet of C-5 proton (Hc) of chromone nucleus at δ 8.24 (J=7.8 Hz). Another doublet is situated at δ 7.63 which may be assigned to C-5 proton of other chromone nucleus. Since the molecular weight of the compound is the same, it appears that the compound is a functional/positional isomer of 42.

In the light of these spectral features structure 43 can be assigned to the compound the formation of which has been shown in Scheme 15.
The only ambiguous part of the nmr spectrum is the presence of a slightly broad singlet integrating for one proton at $\delta$ 10.0 which is assigned to $H_a$ proton of chromone unit. The broadening of singlet may be due to restricted rotation of chromone unit in compound 43 which is not noticed for the same proton due to more freedom to rotation of chromone unit in compound 42 as shown in structures 42a and 43a

![Structures 42a and 43a](image)

3.2 The reaction of 3-formylchromone with dimedone:

3-Formylchromone undergoes ring opening reaction with active methylene compounds such ethylacetoacetate to give o-hydroxyacetophenone derivatives\(^\text{11}\). It seemed, therefore interesting what course would be followed if the reaction is carried out with active methylene compound, dimedone. Thus, one would expect 44 from the reaction of 1 and dimedone. The mechanism leading to 44 involves the sequence of reactions shows below.
The mass spectrum (Fig. 16) of the product agrees with the assigned structure showing $M^+$ at 295. Since the compound does not have any nitrogen in its structure, therefore, $m/z$ 296 was considered as molecular ion peak. The IR spectrum (Fig. 17) shows slightly broad and strong absorption band at 1630 cm$^{-1}$ indicating the presence of more than one carbonyl groups. Thus, if 44 is the structure for the compound, then IR spectrum should display the band at 1660-1670 cm$^{-1}$ rather than at 1630 cm$^{-1}$, which is the absorption region for chromone carbonyl group. The NMR, spectrum (Fig. 18) shows a sharp singlet integrating for six protons at $\delta$ 0.98 indicating the presence of two methyl groups in the compound. However, two methylene groups which usually appear as singlet or sometimes multiplet at $\delta$ 2.2-2.5 are not visible in the spectrum because of presence of water and DMSO peaks in the same region. In the NMR spectrum, the ortho coupled
doublet of C-5 proton of chromone nucleus is clearly discernible at δ 8.12 (J=7.8 Hz) whereas its counterpart is seen at δ 7.65. The sharp singlet at 8.321 which is usually the diagnostic peak of C-2 proton of chromone moiety appear at the same value as reported in literature\textsuperscript{44}. These spectral features, thus, fit better structure 45 which is formed by simple condensation reaction.

![Structure 45](image)

The structure 44 is less likely for the compound as the chemical shift for vinylic and under oxygen proton is usually at δ 7.0\textsuperscript{45} as against δ 8.32 here. Though the NMR spectrum shows a singlet at δ 5.91 which may be assigned to allylic proton, the integration for this singlet does not match with the integration for other protons. Retro-Diels-Alder cleavage giving fragment ions at m/z 120 and 176 further confirm structure 45 for the compound.
The work on the synthesis of new heterocyclic compounds of biological interest has made extensive use of 4-hydroxycomarin, triacetic acid lactone and their derivatives. This is due to readiness with which these compounds react at position 3 with electrophiles and propensity of 4-position to nucleophilic attack. Another possibility is opening of pyrone ring followed by loss of carbon dioxide or further cyclization. We have discussed here the reaction of enol lactones viz. 4-hydroxycomarin and triacetic acid lactone with starting materials such as 2-amino-3-formylchromone and 2-ureidomethylenecyclohexane-1,3-dione in order to obtain novel heterocycles.

3.3 The reaction of 4-hydroxycoumarin with 2-ureidomethylene-cyclohexane-1,3-dione:

This reaction was investigated in the context of earlier studies on the reaction of active methylene compounds and 2-ureidomethylene-cyclohexane-1,3-dione which had formed tetrahydrocoumarins. The reaction is, thus, a modified form of Knovenagel reaction for the synthesis of coumarin from salicylaldehyde and malonic ester/malononitrile. The authors have chosen here 2-ureidomethylenecyclohexane-1,3-dione in place of salicylaldehyde because due to presence of NHCXNH₂ (X=O, S) group, 3 position of 2-ureidomethylenecyclohexane-1,3-dione group acts as an electrophilic centre for nucleophilic attack in the synthesis of 5-oxo-5,6,7,8-tetrahydrocoumarins (Scheme 17).
It was of interest, therefore, to see what course the reaction would take with 2-ureidomethylene-cyclohexane-1,3-dione and 4-hydroxycoumarin. Thus, the compound 47 is expected from the reaction of 2-ureidomethylene-cyclohexane-1,3-dione and 4-hydroxycoumarin. The mechanism leading to 47 involves the sequence of reaction shown below.

Scheme 17

Scheme 18
It is pertinent to mention here that the reaction of 4-hydroxycoumarin with aldehydes and ketones yields dicoumarols\textsuperscript{49}.

The reaction however, did not proceed as thought and afforded 48 instead of 47.

The structure 47 was ruled out because it did not give any colour with ferric chloride. Further the mass spectrum (Fig. 19) of the compound which shows $M^+$ at m/z 456 was also not compatible with structure 47. The IR spectrum (Fig. 20) for the compound 48 shows a broad band at 3432 cm$^{-1}$ and strong band at 1722 cm$^{-1}$ due to OH and coumarin carbonyl groups. The band at 1616 cm$^{-1}$ accompanied by a shoulder at 1630 cm$^{-1}$, is due to carbon-carbon double bond and $\alpha,\beta$ unsaturated carbonyl group. The $^1$HNMR (Fig. 21) clearly shows two sharp singlets at $\delta$ 1.11 and 1.18 for methyl groups. Two methylene groups appear as four doublets at $\delta$ 2.16, 2.39 ($H_a$, $H_b$, $J$=15.9 Hz, 15.0 Hz) and 2.64, 2.79 ($H_c$, $H_d$, $J$=17.7 Hz, 17.7 Hz) as a result of gem coupling which takes place among the methylene protons. A sharp
Fig. 19
singlet integrating for one proton at $\delta$ 5.26 has been assigned to methine (H-7) proton. The aromatic region clearly evidences the presence of two coumarin moieties in the form of two ortho coupled doublets of H-5' and H-1 protons at $\delta$ 8.0 and 7.9. The remaining six protons of coumarin nuclei appear as multiplet situated at $\delta$ 7.73-7.27. This suggests that in the formation of product 48 two 4-hydroxycoumarin molecules are involved as shown below:

Scheme 19
The compound 48 is further confirmed by its mass spectrum (Fig 19) showing $M^+$ at 456. The base peak at m/z 295 is obtained by loss of 4-hydroxycoumarin moiety from molecular ion peak through rout A or B involving RDA cleavage (Scheme 20).

![Scheme 20](image-url)
It has to be noted here that these spectral features can fit also structure 49, which can be rationalized on the basis of the mechanism shown in Scheme 21.

This structure is, however, less probable as the methene proton usually appears as a singlet in the region δ 8.2-8.4^{50} as against δ 5.26 here.
3.4 The reaction of 2-amino-3-formylchromone with triacetic acid lactone:

2-Amino-3-formylchromone 50 is obtained easily by treatment of 3-formylchromone with hydroxylammonium sulfate followed by ring opening and cyclization under basic conditions\(^5\). Schurreit Thomas has synthesised pentacycles 51 and 52 from 50 and 4-hydroxycoumarin under strong acidic conditions\(^6\) (Scheme 22, 23). The mechanism leading to product 51 involves formation of intermediate 53 similar to benzyne by elimination of formyl group and ammonia from 50. The intermediate 53 then adds to another molecule of 50 followed by dehydration of the adduct 54 to form 51.

![Scheme 22](image_url)

The product 52, however, is obtained by simple condensation of 50 and 4-hydroxycoumarin.
Since triacetic acid lactone (TAL) shows nucleophilic properties similar to 4-hydroxycoumarin due to presence of double bond at position 4, attempt was made to synthesised novel polyketo compounds employing 50 as starting material.

Enol lactones react with salicyldehyde to give a product whose structure was assigned as 55 by earlier worker\textsuperscript{53}. The reaction was further investigated because such structures were found to be unstable and therefore, structure 55 was revised to 56\textsuperscript{54}.
In a series of papers Spanish workers\textsuperscript{55} have discussed at length that structures similar to 55, generally rearrange to 56 through intramolecular translactonization. Thus, TAL when reacts with salicyldehyde, the intermediate 57 rapidly undergoes intramolecular translactonization to give 58 (Scheme 25).

\begin{center}
\includegraphics{scheme25.png}
\end{center}

Scheme 25

In an attempt to synthesize 3-formyl derivative of TAL which, was needed in the context of another project with triethylorthoformate, the reaction mixture, however, gave the rearranged product 59\textsuperscript{56} (Scheme 26).
These studies reveal that the intramolecular translactonization takes place by opening up of the lactone ring by nucleophilic attack of hydroxyl group of salicylaldehyde. Since the amino group shows better nucleophilic property than hydroxyl group, it was thought that the intramolecular translactonization will occur much easily if OH group is substituted by NH$_2$ group in a compound to afford the rearranged product. 50 was, therefore, selected for carrying out such types of rearrangement reactions as it contained both NH$_2$ and CHO chromophores at positions similar to OH and CHO groups in salicylaldehyde. The reaction afforded 62 as felt by conducting the reaction of 2-amino-3-formylchromone 50 with TAL 61. A
positive ferric chloride test and strong yellow colour of the product due to extended conjugation further supported that this actually has happened (Scheme 27). The ease with which reaction proceeds is indicated by the fact that product 62 is formed at room temperature and in quantitative yield.

The IR spectrum (Fig. 22) showed strong bands at 1647 with shoulders at 1652 and 1642 cm\(^{-1}\) for chromone, lactam and chelated carbonyl groups. In the \(^1\)H nmr spectrum (Fig. 23) methyl singlet was clearly discernible at \(\delta\) 2.28. A sharp singlet at \(\delta\) 7.09 was assigned to H\(_a\) proton. The presence of chromone ring was established by ortho coupled doublet of C\(_6\) proton at \(\delta\) 8.2 (J=9 Hz). The most deshielded olefinic proton in the compound H\(_b\) appeared as a sharp singlet at \(\delta\) 8.86. Two broad singlets which appeared at \(\delta\) 16.5 and 13.5 (D\(_2\)O exchangeable) indicated the presence of two OH groups in the compound. Thus, 62, also existed in
Fig. 24
its tautomeric form 62a. The structure 62 for the compound was supported by its mass spectrum (Fig. 24) which showed M⁺ at m/z 297. The smaller peaks at m/z 282 and 254 indicated losses of CH₃ and CH₃CO groups. A loss of 57 mass units corresponding to elimination of CH₃COCH₂ group gave fragment ion at m/z 240. The mass spectrum of 3-acetoacetylcoumarin studied by Dean et al⁵⁷ is also characterized by loses of CH₃CO and CH₃COCH₂ groups. However, in our case the base peak is obtained as a result of loss of CH=C(CO)₂ grouping from fragment ion m/z 240. The other relevant peaks are obtained as shown in Scheme 28.

Transformation of 62 to other heterocyclic compounds is in progress.
While attempting the synthesis of 3-acetoacetyl pyranopyridone, 62, labelled as 3HNaL-1 under different reaction conditions, a light yellow product was isolated by conducting the reaction of 2-amino-3-formylchromone 50 with triacetic acid lactone 61 under reflux in the presence of potassium acetate. It was labelled as 3HNaL and gave light brownish blue colour with ferric chloride. The intensity of the colour was somewhat less than 3HNaL-1, 62. The compound, analyzed for C_{22}H_{15}O_{5}N, showed odd molecular ion peak at m/z 373 in the mass spectrum (Fig. 25). Since the molecular weight of 2-amino-3-formylchromone and triacetic acid lactone are 189 and 126 mass units, the addition product would have the molecular weight 189+126=315 which is less than the required molecular weight for
Fig. 25
the compound. Since the NMR spectrum (Fig. 26) shows presence only one methyl singlet and 8 aromatic protons, it is obvious that the formation of 3HNaL with molecular weight 373, would require incorporation of two units of 2-amino-3-formylchromone 50 and one unit of triacetic acid lactone 62 (2 \times 189 + 126 = 504). This is followed by loss of fragments having 131 mass units (504 - 373 = 131). The reaction, thus, involves addition-elimination type of reaction in the formation of 3HNaL. One has, therefore, to look for a sequence of reactions which can cleave the starting materials in the formation of 3HNaL as shown in Scheme 29.
Assuming that amino group of 50 attacks on C-6 carbon of triacetic acid lactone 61, the initial product would be 63 which undergoes decarboxylation and elimination reaction removing CHO and NH₂ groups to form the carbanion 64 and intermediate 65. The addition of carbanion 64 to 65 takes place to give 66. This undergoes cyclization and ring opening of pyrone ring to give 67. The double bond of pyridone 67 reacts further with another molecule of 65 to give the final product 68.

The formation of intermediate 65 is similar to that observed in the synthesis of 9-bromotriptycene 70 from anthranilic acid and 9-bromo naphthalene involving the intermediate benzyne 69 through elimination of N₂ and CO₂.

The sequence of reactions (Scheme 29) leading to the formation of product 68 is supported by the work of S. Garrat. Her work is about the reaction of dehydroacetic acid 71 and alkyl amines. According to her results, dehydroacetic acid reacts with methylamine to form the
Schiff's base 72. On further reaction with methylamine, 72 is converted into bis-2,7-methylaminohepta-2, 5-dien-4-one 73. The final product 74 is formed by removal of one molecule of methylamine from 73 (Scheme 30). The reaction, thus, involves attack by methylamine on C-6 carbon of dehydroacetic acid 71 in the formation of lutidone, 74.

Scheme 30

The attack of amino group an C-6 carbon of dehydroacetic acid is further supported by the work of Japanese workers. In their findings, regarding the reaction of dehydroacetic acid with ammonia, the pyridone, 75 is formed by the attack of nucleophile on C-6 carbon. (Scheme 31)
These studies are relevant in the present context to the extent that they show the possibility of nucleophilic attack at C-6 carbon in preference to attack at the lactone carbonyl. Drawing on the analogy of dehydroacetic acid it is, thus, possible that amino group of 2-amino-3-formylchromone, 50 attacks on C-6 carbon of triacetic acid lactone 61 to form the product, 68.

The IR spectrum of 68 (Fig. 27) shows a strong band at 1616 with shoulders at 1650 and 1600 in addition to bands for NH and OH groups at 3249 and 3398 cm\(^{-1}\) respectively. The strong absorption band at 1616 cm\(^{-1}\) may be assigned to pyridone carbonyl group as the value for pyridones have been reported is the range 1500-1700 cm\(^{-1}\) including pyrones and pyrimidones\(^{61}\). The shoulders at 1650 and 1600 cm\(^{-1}\) have been assigned to chromone carbonyl and o-hydroxybenzoyl groups. The NMR spectrum (Fig. 26) clearly shows a sharp singlet for methyl group at $\delta$ 2.13 with a slight notch which may be due to long range coupling of methyl hydrogens with C-5 proton. This value is, however, slightly higher as the chemical shift of C-5 proton in compounds derived from triacetic acid lactone is usually $\delta$ 6.5-6.7\(^{52}\) as against $\delta$ 7.07 here. The singlet at
δ 7.07 thus has been assigned to C-5 proton. The presence of chromone moiety is established by C'-5 proton in the forms of ortho coupled doublet at δ 8.02 (J=9 Hz). The C'-2 proton of chromone unit is clearly shown as a sharp singlet at δ 8.61. A slightly broad singlet at δ 9.58 and a broad singlet at δ 16.68 (both D_{2}O exchangeable) have been assigned to NH and OH protons respectively.

Mass spectrum (Fig. 25) further supports structure 68 for the compound showing M' at m/z 373. The loss of carbonyl group from M' gives peak at m/z 345. The peak at 345 undergoes RDA cleavage followed by loss of methyl group and a water to give base peak at m/z 192. The other peaks are obtained as outlined in Scheme 32.
Scheme 32
3.5 The reaction of 2-amino-3-formylchromone 50 with active methylene Compounds

3.5.1 The reaction of 2-amino-3-formylchromone 50 with 5,5-dimethyl cyclohexene-1,3-dione

Azaxanthones are heterocycles of pharmacological interest. They have been used as bronchodilators and antiallergy agents. It appeared, therefore, interesting to synthesize azaxanthones from the reaction of 2-amino-3-formylchromone 50 with 5,5-dimethyl cyclohexanes 1,3-dione 75 and 3-methyl-1-phenyl-5-pyrazolone 79 under mild conditions.

Dimedone, 75 is known to form crystalline compound 76 with aldehydes and triethyl orthoformat (Scheme 33).

![Scheme 33](image-url)
Since 2-amino-3-formylchromone 50 has CHO group it should give 77 upon treatment with 75. The compound, however, did not give any colour with ferric chloride. Further mass spectrum (Fig. 28) was also not in agreement with expected structure 77. It showed M⁺ at rather low value i.e. at m/z 294 (M+1 peak) which again ruled out the possibility of dimeric structure 77.

The compound shows strong IR bands (Fig. 29) at 1685 and 1651 cm⁻¹ for chromone and α, β-unsaturated ketone carbonyl groups. A strong band which appears at 1595 cm⁻¹ is due to C=N bond. The ¹H NMR spectrum (Fig. 30) shows a sharp singlet integrating for 6 protons at δ 1.17 and has been assigned to two methyl groups. Two more singlets at δ 2.64 and 3.14, each integrating for 2 protons are given to the methylene groups of dimedone unit. These features indicates participation of only one dimedone moiety in the formation of the product. The presence of intact
chromone moiety is evident from the doublet of C-5 proton of chromone moiety at δ 8.33. The remaining three hydrogens of chromone nucleus give multiplets situated between δ 7.44-7.83. The most down field singlet at δ 9.27 is assigned to H₄ proton which is peri to two carbonyl groups. Combining these spectral data one arrives at structure 78 for the compound and its formation can be rationalized as shown in scheme 34.

Further confirmation for the compound is provided by its mass spectrum (Fig. 28) showing M⁺ at 294 (M+1) peak) which is also the base peak. Other peaks are obtained as shown in Scheme 35.

Scheme 34

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The same compound 78 was synthesized by Helmut et al\textsuperscript{68} by refluxing of the starting materials 50 and 75 under strong basic conditions whereas we have synthesized 78 at room temperature with improved yield (90%). Further we have explored different types of biological activities which are discussed in the last chapter.

\textit{3.5.2 The reaction of 2-amino-3-formylchromone with pyrazolone}
Attempts were directed towards the synthesis of another azaxanthone, viz. pyrazoloazaxanthone 80 by conducting the reaction of 2-amino-3-formylchromone with 3-methyl-1-phenyl-5-pyrazolone 79 under different reactions conditions. Under mild acidic conditions employing catalytic amount of p-toluene sulphonic acid or glacial acetic acid the reaction mixture, however, afforded unexpected and dimeric product 81. Under mild basic conditions using minute quantity of pyridine or sodium/potassium acetate again the dimer 81 was obtained. The ease with which the compound 81 is obtained in all acidic and basic conditions seems due to the formation of intermediate, formyl derivative of pyrazolone 82 which undergoes dimerization by attack of another molecule of pyrazolone 79 (Scheme 36).

The compound gave light blue-black colour with ferric colour.

The IR spectrum (Fig. 31) of 81 is devoid of any chromone carbonyl group possibility of chromone nucleus in the compound is, thus, ruled out. It displays a broad band for OH group at 3414 cm⁻¹ and a strong band at 1628 cm⁻¹ for α,β-unsaturated carbonyl group. The value of carbonyl group is somewhat lower than that mentioned in literature. This discrepancy in the frequency may be due to intramolecular H-bonding in the molecule which shifts the IR frequency for carbonyl group towards longer wavelength. The ¹H NMR (Fig. 32) shows a sharp singlet integrating for six proton at δ 2.32 due to the presence of two
[ Mass Spectrum ]
Data : 4EAGUS74 Date : 30-Aug-2004 16:00
Sample: MA-5 DR(I1R) N SIDDQUI, ALI GARH #7397
Note: Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion (MF-Linear)
RT : 0.12 min Scans : (1,3)
BP : m/z 359.0000 Int. : 20.14
Output m/z range : 71.8101 to 504.3769 Cut Level : 0.00 %

Fig. 33
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methyl group in the compound whereas 80 if assigned to the compound, has only one methyl group. Further mass spectrum (Fig. 33) which shows $M^+$ at 359 ($M^++1$) (peak), is also not compatible with structure 80 and requires odd molecular ion peak instead of even here. The NMR spectrum completely eliminates structure 80 as it does not show any doublet for C-5 proton of chromone moiety and shows a sharp singlet of olefinic proton at $\delta$ 7.18. The aromatic region of the spectrum shows ten aromatic protons of two benzen rings in the form of two multiplets, situated at $\delta$ 7.23-7.28 integrating for two protons, $\delta$ 7.40-7.45, integrating for four protons and in the form of two singlets at $\delta$ 7.88 and 7.91 respectively each integrating for two protons. The down field singlets of four protons of two benzen rings may be due to presence of carbonyl group adjacent to N so that withdrawal by carbonyl group makes nitrogen positively charged which shifts adjacents C-2', C-6' and C-2", C-6" protons, in much down field region.
The compound 81 was obtained by Wallace et al. while synthesizing 4-formyl-3-methyl-1-phenyl 5-pyrazole, 82\textsuperscript{70}.\)