I) STUDIES ON 5-CHLOROMETHYL-2-HYDROXY ACETOPHENONE AND
ω-BROMO ACETOPHENONE
An appreciable number of compounds in use today as chemotherapeutic and pharmacotherapeutic agents have been laboratory synthesized through the miracles of chemistry. How true the words of Marcelin Berthelot sound when he remarked "Chemistry resembles the arts; the potential of its creativity is terrifying". Medicinal chemistry remains a challenging science and with hundreds of thousands of new organic chemicals prepared annually throughout the world (of which a few enter into pharmacological screens to determine if they have useful biological activity), medicinal chemists have a chance to participate in the fundamentals of prevention, therapy and understanding of diseases and thus contribute to a healthier and happy life.

Various useful synthetic analogs with improved therapeutic properties can be obtained from a single lead compound by structural modifications. The same applies to the group of flavonoids, one of the most diverse and widespread system of natural products with a broad spectrum of biological activity that include, to name a few, action on central vascular system, anti-inflammatory activity, diuretic action, anti-microbial activity etc.

The amino group containing compounds too occupy a prominent position in medicinal chemistry due to a number of physiological actions. So important is this group in case of adrenergic agents, antibiotics and the sulfonamides etc. that its removal from the structure leads to a virtual loss of activity.

And hence, the following studies were undertaken:
ω-Hydroxy acetophenone was subjected to chloromethylation to get 2-hydroxy-5-chloromethyl acetophenone.

The chlorine of the chloromethyl group can be replaced by amino group containing compounds. Thus the presence of a nitrogen atom and an unsubstituted phenolic hydroxyl (of the ω-hydroxy acetophenone moiety) in the same compound was expected to impart anti-inflammatory, analgesic and anti-microbial properties of the N-(3-acetyl-4-hydroxy benzyl) anilines and miscellaneous derivatives synthesized.

ω-Bromoacetophenone was synthesized and the bromine was sought to be replaced by aromatic primary amines and then test them for anti-microbial activity.

The acetophenone moiety of the chloromethyl product was proposed to be converted into chalcones and flavones. And so was the conversion of some N-(3-acetyl-4-hydroxy benzyl) derivatives into flavonoids. The presence of nitrogen atom was expected to increase the anti-microbial activity already associated with chalcones and flavones, and therefore, the synthesis of compounds with potent anti-microbial and anti-inflammatory activity had been anticipated.

The information, relevant to the work carried out has been incorporated in the text of this thesis under various headings.
B) THEORETICAL
The term chloromethylation refers to the replacement of a hydrogen atom by a chloromethyl group in a single operation. The earliest example of such a reaction may be illustrated by the synthesis of benzyl chloride carried out by Grassi and Maselli\textsuperscript{1} who used benzene, hydrogen chloride, paraformaldehyde and zinc chloride.

\[
\text{C}_6\text{H}_6 + \text{CH}_2\text{O} + \text{HCl} \rightarrow \text{C}_6\text{H}_5\text{CH}_2\text{Cl} + \text{H}_2\text{O}
\]

Chloromethylation has been carried out in aliphatic and aromatic compounds and is a valuable synthetic tool, in as much as the \text{CH}_2\text{Cl} group can be converted to other groups such as \text{CH}_3, \text{CH}_2\text{CN}, \text{CHO}, \text{CH}_2\text{NH}_2 and \text{CH}_2\text{OH}. The original classical reaction consists essentially of the interaction of formaldehyde and hydrogen chloride in the presence of a catalyst such as zinc chloride and is frequently referred to as the "Blanc reaction"\textsuperscript{2,3} or Blanc Chloromethylation reaction. The method may be illustrated by the preparation of benzyl chloride from benzene\textsuperscript{4}.

Chloromethylation is generally applicable to aromatic hydrocarbons. Benzene, naphthalene, anthracene, phenanthrene, biphenyl, aromatic ketones and many of their derivatives form a part of the numerous compounds that have converted to chloromethyl derivatives. There have been many compounds reported wherein a second chloromethyl group has been introduced, these include metaxylene and mesitylene\textsuperscript{5,6} as well as 2-hydroxyacetophenone\textsuperscript{7} and naphthalene\textsuperscript{8,10}.
Substituents which are electron donating promote substitution whereas those which are electron withdrawing retard substitution. The presence of a halogen atom on the ring causes the reaction to be more difficult to effect and more highly halogenated derivatives generally tend to resist chloromethylation. However halogen derivatives of polymethylbenzenes sometimes react readily to give high yields of chloromethyl compounds, bromomesitylene is an example. Nitrogroups tend to inhibit the reaction but satisfactory yields from nitrophenols have been reported. Phenols react so readily that the reaction generally goes too far, yielding polymeric materials. It is also reported that in case of phenols there is a tendency of the reaction to proceed with the formation of diphenylmethane derivative. Ketones are generally unreactive though acetophenones have been reported to undergo chloromethylation. The reaction has also been successful with other ketones such as acetomesitylene, acetoisodurene and 2, 4, 6-triethylacetophenone.

Flavones and flavanones have also been chloromethylated successfully. Aromatic amines react very readily, but it has not been possible to isolate their simple chloromethyl derivative. Chloromethylated aromatic amines could be unstable because of the ability of the chloromethyl group to condense with any amino group that might be present in the molecule.

The most important side reaction is the formation of diarylmethanes. Highly reactive compounds like naphthalene, anisole, phenols, polymethyl benzenes etc. yield this type of product and it is often difficult or impossible to isolate the intermediate chloromethyl derivative. Chloromethylation in the presence of arsenous chloride or arsenous oxide is claimed to inhibit the
formation of diarylmethanes and other byproducts\textsuperscript{20}. To avoid the formation of diarylmethane, chloromethylation is carried out using chloromethylmethylether in the presence of $\text{AlCl}_3$ as in the case of 2-hydroxy-5-nitrobenzaldehyde\textsuperscript{21}.

\[
\begin{array}{c}
\text{OH} \\
\text{CHO} \\
\text{NO}_2
\end{array} + \text{CH}_3\text{OCH}_2\text{Cl} \xrightarrow{\text{AlCl}_3} \begin{array}{c}
\text{OH} \\
\text{CHO} \\
\text{Cl}_2\text{H}_2\text{C} \\
\text{NO}_2
\end{array}
\]

The reagents and methods used for chloromethylation have been modified in a number of ways. The most common reagents have been formaldehyde and hydrochloric acid. The formaldehyde may be added as formalin or it may be generated in the mixture by depolymerization of formaldehyde. When chloromethyl ether or dichloromethyl ether are employed, hydrochloric acid is not needed. However, a byproduct/intermediate formed by the mixture of formaldehyde and hydrochloric acid is bis(chloromethyl)ether, a potent carcinogen. Van Duuren and associates found this ether carcinogenic to rats when injected subcutaneously or repeatedly applied to their skin\textsuperscript{22}. The ether is also an acute lung irritant. Methylchloromethyl ether, which is occasionally used as a reagent for chloromethylation, is also quite toxic although less so than bis(chloromethyl)ether\textsuperscript{23}. Chloromethylation with above reagents must be done with caution in a well-ventilated hood and wearing neoprene gloves.

Alternative methods of chloromethylation using reagents other than formaldehyde and hydrochloric acid have been reported. These include using methoxyacetyl chloride\textsuperscript{24} in the presence of anhydrous aluminium chloride. There has been a recent report\textsuperscript{25} on the improvements achieved with either chloromethyl methyl ether or methoxyacetyl chloride using SnCl\textsubscript{4} as catalyst instead of AlCl\textsubscript{3}.

A two step procedure for chloromethylation has also been described\textsuperscript{26}. The first step involves amidomethylation of the aromatic nucleus by reaction with paraformaldehyde and acetamide in presence of sulfuric acid (conc.) the isolated intermediate is treated with phosphorus oxychloride in dimethyl formamide and xylene to get the chloromethylated product.
A method for the chloromethylation of cross-linked polystyrenes has been reported, which involves reaction with trioxane and chlorotrimethylsilane in the presence of stannic chloride in chloroform. An activated formaldehyde generated from trioxane in the presence of stannic chloride as Lewis acid may react with chlorotrimethylsilane to form a trimethylsilyl ether of the chlorohydrin having a structure similar to chloromethylmethylether to act as chloromethylating agent.

Chloromethylation of thioanisole, under the usual conditions, using HCHO and HCl gives rise to both para and ortho products, but if the reaction is done with methylal and AlCl₃ in CH₂Cl₂, the para isomer is almost the exclusive product. 
Regioselective chloromethylation can also be done indirectly from phenol using benzene boronic acid\textsuperscript{29, 30}.

Chloromethylation has been carried out at temperatures between 0° to 60° and above. Higher temperatures and zinc chloride favors the formation of diphenylmethane derivatives and also dichloromethylation products.

Catalysts may or may not be used. In a study of the effect of substituents on the ease of chloromethylation of benzene by chloromethylether in the absence of a catalyst, Vavon, Bolle and Calin\textsuperscript{31} have found that the rate is increased by \(-\text{CH}_3\), \(-\text{C}_2\text{H}_5\), \(-\text{C}_3\text{H}_7\), \(-\text{OCH}_3\) and \(-\text{OC}_3\text{H}_7\) and diminished by \(-\text{Cl}\), \(-\text{Br}\), \(-\text{I}\), \(-\text{CH}_2\text{Cl}\), \(-\text{COOH}\) and \(-\text{NO}_2\).

Phenols and their ethers react much more readily than the hydrocarbons. For anisole and methyl cresyl ethers, monochloromethylation with 35-40% formalin and hydrochloric acid is most successful if conducted at 0-15° and without a catalyst\textsuperscript{4}. Phenyl esters, hydroxy aldehydes, ethers of hydroxy aldehydes, nitrophenols, nitrophenyl ethers and highly alkylated ketones undergo chloromethylation under mild conditions. The chloromethylation of highly alkylated benzenes generally can be carried out without a catalyst. It is sufficient to treat the aromatic compound with a mixture
of formaldehyde and concentrated hydrochloric acid\textsuperscript{32,33}. Chloromethylation of \( p \)-xylene is carried out in this manner.

Ketones having mesityl, duryl, isoduryl or other highly alkylated aryl radicals undergo chloromethylation in yields of 25-88\%. The procedure involves the use of formaldehyde or paraformaldehyde and concentrated hydrochloric acid without any catalyst. The chloromethylation of acetomesitylene gives yields of about 75-80\% by shaking a mixture of acetomesitylene, paraformaldehyde and hydrochloric acid on a mechanical shaker at room temperature.

The synthesis of 5-chloromethyl-2-hydroxyacetophenone\textsuperscript{7} by chloromethylation of 2-hydroxyacetophenone requires no catalyst and a temperature below 25 - 30\°.

\[
\text{HCl gas} \rightarrow \text{HCHO} + \text{HCl} \xrightarrow{<25-30\°} \]

The catalysts which are used include zinc chloride, sulfuric acid, acetic acid and perchloric acid mixture\textsuperscript{34}, aluminium chloride, phosphoric acid\textsuperscript{30}, stannic chloride and phase transfer catalysts\textsuperscript{35}. Ambiphilic surfactants like \( \text{C}_{16}\text{H}_{35}\text{N}^+\text{Me}_3\text{X}^- \) are good micellar catalysts forming an emulsion in a two-phase system, providing a high surface area for reactivity\textsuperscript{36}.

The preparation of benzylchloride\textsuperscript{4} usually is carried out using zinc chloride, as a catalyst. Sulfuric acid and aluminium chlorides, among others have also been used. However, sometimes, the above catalysts favor the formation of diphenylmethane derivatives. Stannic chloride\textsuperscript{19} have sometimes been found to be a superior catalyst for compounds which do not undergo chloromethylation easily. The preparation of 2, 4, 6-triisopropyl benzyl chloride is interesting because chloromethyl ether is used in place of formaldehyde and the catalyst used is stannic chloride.

The chloromethylation of naphthalene has been done in various ways including the use of petroleum ether in the Blanc method which gives yield\textsuperscript{5} of 30\%. Use of glacial acetic acid in large volumes as a solvent\textsuperscript{37-42} has been found to give better yields. Cole and Dodds proposed to carry out the reaction in an aqueous mixture\textsuperscript{41} with sulfuric acid as a catalyst. For deactivatedarenes, conversion is good if fuming (60\%) sulfuric acid is added dropwise to substrate dissolved in excess of chloromethyl ether\textsuperscript{43}.

Chloromethylation is similar in some respect to that of Friedel and Crafts. The kinetic studies of Agata and Okano have shown that the
mechanism of chloromethylation is based upon an electrophilic attack on an aromatic nucleus by the hydroxymethyl cation\textsuperscript{44}. This electrophile reacts with the aromatic ring to give the benzylic alcohol which is then converted into the chloromethyl derivative by hydrogen chloride.

\[
\begin{align*}
H_2C &= \text{O} + \text{HCl} \rightarrow \text{Cl}^- + [\text{H}_2\text{C} = \text{OH} \leftrightarrow \text{H}_2\text{C}^+ - \text{OH}] \\
\text{ArH} + \text{CH}_2\text{OH}^+ &\rightarrow \text{Ar.CH}_2\text{OH} + \text{H}^+ \\
\text{Ar.CH}_2\text{OH} + \text{HCl} &\rightarrow \text{Ar.CH}_2\text{Cl} + \text{H}_2\text{O}
\end{align*}
\]

Reactions where methoxyacetyl chloride is used the cation (1) formed substitutes in the aromatic nucleus to give the benzylmethyl ether (2) which is subsequently converted into the chloromethylated product (3)\textsuperscript{24}.

\[
\begin{align*}
\text{MeO.CH}_2\text{COCl} + \text{AlCl}_3 &\rightarrow \text{MeO} \xrightarrow{\text{AlCl}_4} \text{CH-C=O} \xrightarrow{-\text{CO}} \text{MeO=CH}_2 \xrightarrow{\text{AlCl}_4} (1) \\
\text{ArH} + \text{MeO}=\text{CH}_2 &\rightarrow \text{Ar.CH}_2\text{O.Me} \rightarrow \text{Ar.CH}_2\text{Cl} (2) (3)
\end{align*}
\]

The chloro atom of the chloromethyl group can be converted to -OH, -COOH, -CN, -NHR, etc. Chloromethylation of phenanthrene to give 9-chloromethyl phenanthrene and conversions of this compound into other 9-phenanthrylmethyl derivatives in useful yields have been described by Fernandez et al.\textsuperscript{35} 8-quinolinol has also been chloromethylated and converted to a number of derivatives\textsuperscript{46} of pharmacological interest.

Some reactions analogous to chloromethylation have been tried with aldehyde other than formaldehyde and halogen acids other than HCl with successful results. These include:

\textbf{Bromomethylation}

Bromomethyl derivatives\textsuperscript{46} have been prepared by using hydrogen bromide in place of hydrochloric acid. Bromonaphthalene\textsuperscript{33}, benzyl bromide\textsuperscript{47}, p-chlorobenzyl bromide\textsuperscript{47} and dibromo-p-xylene\textsuperscript{47} have been prepared in this way. It has been stated\textsuperscript{48} that the method is general but yields are
comparatively lower than chloromethylation reactions. Bromomethylation has also been reported in excellent yields using phase transfer catalysis.\(^{35}\)

**Iodomethylation**

Iodomethylation has been reported by Sandin and Fieser\(^ {49} \) who converted 9-methyl-1, 2-benzanthracene through the intermediate iodomethyl derivative. The iodomethylation was carried out by treating the hydrocarbon with chloromethyl ether or paraformaldehyde in glacial acetic acid solution and adding hydroiodic acid. The bright yellow iodomethyl compound is obtained in yields of 90%.

\[
\begin{array}{c}
\text{CH}_3 \\
\end{array}
\begin{array}{c}
\text{CH}_3 \\
\end{array}
\rightarrow
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_2
\end{array}
\]

**Chloroethylation**

Chloroethylation has been effected by using paraaldehyde in the place of formaldehyde. Anisole and its homologs when treated with paraaldehyde and hydrochloric acid, give the corresponding chloroethyl derivatives in yields of 40-60\(^ {50-54} \). The synthesis of 4-methoxychloroethyl benzene is an example.

\[
\begin{array}{c}
\text{CH}_3 \text{CHO} + \text{HCl} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{CH}_{3}\text{CHCl}_3 \\
\text{OCH}_3
\end{array}
\]

Xylene has also been chloroethylated\(^ {52} \). The chloroethyl derivatives readily lose hydrogen chloride yielding the corresponding vinyl derivatives. In place of paraaldehyde, chloroacetaldehyde has also been used and with anisole it gives \(\alpha, \beta\)-dichloroethyl anisole\(^ {55} \).
Chloropropylation

The synthesis of anethole\textsuperscript{53} has been carried out by chloropropylation of anisole followed by dechlorination.

\[
\begin{align*}
\text{OCH}_3 & \quad \rightarrow \quad \text{CHClCH}_3 \quad \rightarrow \quad \text{CH=CHCH}_3
\end{align*}
\]

Anethole

Chlorobutylation

Chlorobutylation of anisole has also been reported\textsuperscript{52, 53, 56}. By using butyraldehyde, Ducasse\textsuperscript{55} obtained 2-methoxy-5-methyl-chlorobutyl benzene in 30% yield. Chloroisobutylation of anisole has likewise been effected\textsuperscript{51}. 
REFERENCES


11. Fuson; Kneisley; Lindsey; Rabjoh; Sperati, unpublished work.


32. Sommelet, M., Compt. Rend., 157, 1443 (1913); C. A., 8, 1088 (1914).


52. Sommelet, M.; Marszak, I., Fr. Pat., 787,655; *C. A.*, 30, 1185 (1936).


Flavonoids comprises of a large group of naturally occurring compounds and basically are benzo-pyrone derivatives which resemble coumarin and are ubiquitous in photosynthesizing cells. Geissman et al. (1952) have applied the term flavonoid to embrace all compounds whose structure is based on C₆-C₃-C₆ skeleton. Flavonoids have been known to possess marked physiological activity and high specificity of function. Flavonoids are widely distributed in plants as water soluble glycosides.

The flavonoids are C₁₅ phenolic compounds wherein two benzene rings (A and B) are linked by a propane bridge in A-C₃-C₂-C₁-B (I) fashion, exceptions being isoflavonoids and neoflavonoids. In the case of isoflavonoids the linkage is in the form of A-C₃-C₂ -(B) -C₁ (II) e.g. Diadzem, genistein and orobal while a pattern of A-C₃-(B) -C₂-C₁ (III) is present in neoflavonoids of which dalbergin is an example.

The basic skeleton of flavonoids can be derived from variable nature of heterocycle which is derived from pyran (IV) or pyrylium (V) or pyrone (VI). Variations in the structure of the flavonoids depends upon the various levels of saturation and oxidation of 1-benzopyran which occurs when the cyclization takes place between the third carbon (C₁) of the chain and OH group of the ring-A-ortho to this chain leading to the formation of chroman (VII), 2H-chromene (VIII), 4H- chromene (IX), 4-chromanone (X) and chromone (XI).
Identification of flavonoids

Colour reactions:

The colour reactions of the flavonoids gives a broad indication of the structural features depending upon the pattern of hydroxylation and substitution.

The reagents used for colour reactions include magnesium/hydrochloric acid\(^2\), sodium amalgam/hydrochloric acid, Wilson boric acid\(^3\) and zinc/hydrochloric acid\(^4\).
Biflavonoids are found to react in more or less the same manner as the monomers. With zinc/hydrochloric acid, biflavonoids give a pink colour.

Synthesis of flavonoids

Synthesis of Chalcones:

Chalcones characterized by a C₆ (A)-CO-CH=CH-C₆ (B) structure are important intermediates in the synthesis of flavones.

Various methods are available for the preparation of chalcones and can be obtained by the acid or base catalysed aldol condensation of o-hydroxy acetophenones with substituted benzaldehydes. Although possessing certain limitations, condensation by means of alkali give high yields specially polymethoxy derivatives condense best with 50%, 60% and 70% potassium hydroxide in aqueous alcohol and as a rule give the chalcone. Similarly, non methylated polyhydroxy acetophenones afford good yields if condensed at 0°C in presence of a base. The use of sodium hydride as a base catalyst has also been reported and has been employed for the preparation of the chalcone precursors in the synthesis of xanthomicrol and the flavanone moiety of silymarin. Synthesis of 4-hydroxy 2,4-dimethoxy chalcone from m-methoxy phenol and p-methoxyacetophenone has been reported to have been carried out in four steps.

Acids favor the cyclization of chalcones to flavanones and so acid catalyzed reactions favor the formation of chalcone-flavanone isomers or even the flavanone alone. However, the formation of flavanone is inhibited by the presence of a free-OH group at position 4 of the chalcone. Methoxylated chalcones can be obtained from isomeric flavanones by ring opening in alcoholic potassium hydroxide and precipitation in cold with dilute acid.

Trans isomers are always produced in the synthesis of chalcones. These can be converted to cis isomers by UV irradiation.

Flavanones:

Flavanones, isomeric with the chalcones are obtained from the latter by acid or alkali catalyzed ring closure. Ring closure is favored by an OH group at 6 position of the chalcone.

The 6-, 8- methyl flavanones, strobopinin (1) and cryptostrobin (2) are synthesized in a single step by the condensation of of 2-hydroxy-4, 6-dibenzylxoxy-3- methyl acetophenone or 2,4-dihydroxy-6-methoxy-5-methyl acetophenone with benzaldehyde in ethanolic potassium hydroxide, followed by cleavage of the protecting groups.
Polyhydroxy flavanones were synthesized in a one step process by reacting the appropriate hydroxyacetophenones and hydroxybenzaldehydes in the presence of boric acid, in a mixed solvent system. Aromatic aldehydes when condensed with malononitrile gave \( RCH:C(CN)\text{H}_2 \), which when treated with phloroglucinol give corresponding flavanones. Flavonoids have also been synthesized from corresponding benzopyrans using a free radical process involving the Bu₃SnH/AlBN system in refluxing benzene. Synthesis of 6-prenyl flavanones has also been reported and is based on Claisen rearrangement and cyclization reaction.

Flavones:

Flavones can be synthesized from chalcones or flavanones or from simple precursors by condensation.

Dehydrogenation of chalcones and flavanones:

\( \text{o-Hydroxy chalcones are converted to flavones through acetylation and addition of bromine, the dibromides with ethanolic potassium or sodium hydroxide lose hydrogen bromide to give flavones.} \)

Partially acetylated flavanones such as naringenin (3), hesperetin (4) and homoeriodyctiol (5) get converted to the respective flavones, apigenin (6), diosmetin (7) and chrysoeriol (8) by the benzoyl peroxide catalyzed bromination with 1 mol of N-bromo succinimide in carbon tetrachloride, followed by acid hydrolysis.

Good yields of the desired flavones have been obtained by oxidising \( \text{o-hydroxy chalcones and flavanones in presence of selenium dioxide.} \)

Dehydrogenation with selenium dioxide has been utilized for the synthesis of partial methyl ethers of luteolin (9) and 6-hydroxy luteolin (10) and of polyhydroxy flavones with completely disubstituted A ring. Bose et al. (1970) have reported a cyclization and simultaneous dehydrogenation of 2',4'-dihydroxychalcones to the corresponding flavones by heating with palladium on charcoal.
A novel flavonoidal alkoloid, Ficine\textsuperscript{23}, isolated from \textit{Ficus pantoniana} was synthesized\textsuperscript{24} by reaction of 1,3,5-trimethoxybenzene with $\gamma$-amino butyric acid followed by basification, and reduction to give 1-methyl-2-(2',4',6'-trimethoxy phenyl)-pyrrolidine. The pyrrolidine was subjected to Friedel Crafts acylation to form a hydroxy acetophenone which was converted to chalcone, then to a flavanone and finally dehydrogenated to give the flavone, ficine.

A one step conversion of flavanones into isoflavones was achieved by the use of Tl(NO$_2$)$_3$\textsuperscript{25}.

Similarly, flavanones, when treated with Tl(OAc)$_3$ in acetic acid or methyl cyanide, undergo facile dehydrogenation to afford flavones whereas, upon treatment with Tl(O$_3$SC$_6$H$_4$Me-4)$_3$ or Tl(NO$_3$)$_3$ in ethylcyanide or methylcyanide respectively; they undergo oxidative 2,3-aryl migration to give isoflavones II in high yield\textsuperscript{26}.

3-Benzyl flavones were synthesized either by the reaction of flavanone with aromatic aldehydes or by the conversion of 3-arylidene flavanones, both catalyzed by piperidine\textsuperscript{27}.
Allan Robinson Condensation:

In this method, flavones are obtained in one step by the condensation of o-hydroxy acetophenones with the anhydride of an aromatic acid in the presence of the salt of the same acid or in the presence of trimethylamine or pyridine as catalyst at oil bath temperature. Side products such as o-aroyloxy acetophenones and o-hydroxy dibenzoyl methanes are also formed in this synthesis and are not isolated.

To avoid side reactions the temperature of the oil bath should not be much higher than that of the melt. If a partial demethylation occurs during the Allan Robinson acylation it is accompanied by a simultaneous ring isomerization. In a variant of these two methods, flavones that are partially or completely alkylated in the side phenyl can be prepared by melting together phloroglucinol and benzyloxy or methoxy benzyl acetic acid ethyl esters.

Kostanecki Synthesis:

This is a general method for synthesizing flavones and consists in condensing the ester of an alkylated salicylic acid with an acetophenone in the presence of sodium metal. For flavone this reaction is carried out with methyl o-methoxy benzoate and acetophenone.

By cyclization of β-diketones:

o-Methoxy dibenzoyl methanes with hydrogen iodide undergo simultaneous demethylation and ring closure to give flavones.

a) Claisen Condensation:

o-Hydroxy acetophenone and ethyl propionate gives the diketone cyclised by acetic acid and hydrochloric acid to 2-ethyl chromene. This condensation can be effected in pyridine or xylene or by means of sodium hydride.

b) Baker-Venkataraman Rearrangement:

In this method o-hydroxy acetophenones are acylated at oil bath temperatures with aromatic acid chlorides in acetone/potassium carbonate or pyridine and the resulting esters are converted into diketone with potassium hydroxide in pyridine or with sodium hydride. Ring closure is finally performed in ethanol-sulfuric acid, in glacial acetic acid and anhydrous sodium acetate or simply by heating the diketone in a vaccum. An o-aroyloxy acetophenone, formed by base catalysis, is first cyclized to compound of the 2-hydroxy flavanone, type, which then rearranges to o-hydroxy dibenzoyl
methane derivative. Under acid catalysis, cyclization again results in a 2-
hydroxy flavanone which is finally dehydrated to the flavone.

The aldol condensation of α-chloro-2-hydroxyacetophenone with
aromatic aldehydes in aqueous alcohol containing 5-10% sodium hydroxide at
room temperature yields the corresponding flavones\(^1\).

**Modern Analytical Techniques in Flavonoids**

**Ultraviolet and Visible absorption spectroscopy:**

Among the instrumental methods of analysis of flavonoids, UV
spectroscopy has become an important technique not only because a very
small quantity of the compound is required but also because the quantum of
structural information gained from a UV spectra is enhanced by the use of
specific reagents which react with one or more functional groups of the
flavonoid nucleus.

The UV spectra of flavonoids consists of two major absorption maxima,
one of which occurs in the range of 240-285 nm (band II) and the other in the
range of 300-400 nm (band I).

Flavones absorb between 304-350 nm, while flavonols absorb in the
range 352-385 nm. Absorption at longer wavelength occurs for highly
oxygenated flavones and flavonols as compared to those with fewer oxygen
substituents.

Band II is less affected by changes in the ring B oxygenation although
3’, 4’ dihydroxylated flavones generally show two peaks in this region, while
4’-hydroxylated flavone show only one\(^3\). A change in the ring A oxygenation
pattern however significantly affects band II and increases from 250 nm in
flavone itself to 252 nm in 7-hydroxyflavone, 268 nm in 5-hydroxyflavone and
5,7-dihydroxyflavone, 274 nm in 5,6,7-trihydroxyflavone and 281 nm in 5,7,8-
trihydroxyflavone. The absence of hydroxyl group in either ring is evidenced
by relatively weak intensity of the relevant band. Hypsochromic shifts,
particularly of band I results upon methylation or glycosylation of hydroxyl
groups on the flavonoid nucleus. The extent of these shifts range from a 3-10
nm shift (band I) on substitution of a 4’-hydroxyl group to a 5-15 nm shift
(bands I and II) on substitution of a 5-hydroxyl group. A 12-17 nm shift for 3-
hydroxy substitution becomes 22-25 nm with 5-deoxy flavonols.

Similar substitution, other than 3,5,4’-hydroxy groups has little effect of
UV spectra. Acetylation can be useful for locating alkoxy groups in a flavonoid
because acetylation tends to nullify the effect of phenolic hydroxy groups on
the spectra.
### Ultraviolet Absorption spectra of Flavones

<table>
<thead>
<tr>
<th>Flavone</th>
<th>MeOH (λ&lt;sub&gt;max&lt;/sub&gt;, nm)</th>
<th>NaOMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavone</td>
<td>250, 294, 307 sh</td>
<td>250, 294, 309 sh</td>
</tr>
<tr>
<td>5-hydroxy flavone</td>
<td>268, 296 sh, 333</td>
<td>272, 380</td>
</tr>
<tr>
<td>7-hydroxy flavone</td>
<td>252, 268, 307</td>
<td>266, 307, 359</td>
</tr>
<tr>
<td>4-methoxy flavone</td>
<td>253, 317</td>
<td>254,316</td>
</tr>
<tr>
<td>3',4'-dihydroxy flavone</td>
<td>242, 314 sh, 333</td>
<td>249 sh, 278 sh, 302, 404</td>
</tr>
<tr>
<td>3',4'-dimethoxy flavone</td>
<td>242, 314 sh, 333</td>
<td>241,314 sh, 334</td>
</tr>
</tbody>
</table>

Isoflavones, flavanones and dihydroflavonols lack conjugation between the A and B ring. These exhibit a low intensity band I absorption which often appears as a shoulder to the band II peak and thus can be distinguished from flavone.

The spectra of these compounds are largely unaffected by changes in the oxygenation and substitution patterns in the B ring. However increased oxygenation in A ring leads to a bathochromic shift in band II absorption.

In isoflavones band II appears in the 245-270 nm region and the band I shoulder in the range of 300-340 nm.

Flavanones and dihydroflavonols on the other hand have E<sub>max</sub> (band II) in 270-295 nm region and as with the isoflavones the lack of a free 5-hydroxyl group causes a 10-15 nm shift of these maxima to shorter wavelengths.

**Chalcones and Aurones:**

The UV spectra of chalcones and aurones are characterized by the presence of a dominant band I absorption and a relatively minor band II. The band I absorption occurs in the range 340-390 nm whereas band II appears in 220-270 nm. Increase in oxygenation in chalcones leads to bathochromic shifts, particularly in band I whereas methylation or glycosylation has negligible effect on the spectrum unless it is at the 2'- position wherein a 15-20 nm hypsochromic shift is observed.

In aurones, band I is generally found in the 370-430 nm region although with simple oxygenation pattern it appears at shorter wavelength. In naturally occurring aurones, band I ranges from 388 nm to 413 nm. Methylation or glycosylation of hydroxyl groups on the aurone nucleus does not affect the spectrum greatly, unless the substitution is at 7-position in 6,7-dihydroxyaurones.
Proton Magnetic Resonance Spectroscopy (\( ^1 \text{HNMR} \)):

The application of \( ^1 \text{HNMR} \) spectroscopy has proved to be an important tool in the structure determination of flavonoids\(^{34,36,37} \).

DMSO\(_6\) is by far the most commonly used solvent for underivatized flavonoids\(^{38-42} \), though CCl\(_4\), C\(_6\)D\(_6\), CDCl\(_3\), pyridine, D\(_2\)O or a mixture of these have also been used\(^{34,43-46} \).

DMSO\(_6\) however, has its disadvantages like its high boiling point (which makes recovery of flavonoid difficult), the possibility of flavonoid decomposition in its presence\(^{40,49} \) and of an additional signal due to possible water absorption.

In order to avoid these difficulties, the TMS ether derivative of flavonoids with CCl\(_4\) as solvent has been preferred\(^{34,50-52} \).

The chemical shifts of protons of A ring and B ring prove to be independent of each other but are affected by C ring. All chemical shift values quoted in this section are in parts per million (ppm) on the \( \delta \) scale.

A-ring protons:

\[ \text{C}_6 \text{ and C}_8 \text{ protons in 5,7-dihydroxy flavonoids:} \]

The C\(_6\) and C\(_8\) protons in 5,7-dihydroxy flavonoids appear as doublets (\( J=2.5 \) Hz) in the range of 5.7-6.9 ppm. The H\(_8\) doublet occurs consistently at higher fields than the signal for H\(_6\). A downfield shift is observed for both H\(_6\) and H\(_8\) upon glycosylation of the 7-hydroxyl group. The H\(_6\) and H\(_8\) signals can also be distinguished by their widely different paramagnetic induced shifts.

7-hydroxyflavonoids (5-deoxyflavonoids).

The C-5 proton in such compounds is strongly deshielded by the 4-keto group and thus appears near 8.0 ppm as a doublet (\( d_1, J = 9 \) Hz) due to ortho coupling with H\(_6\). Signals for H\(_6\) (\( q, J = 9 \) and 2.5 Hz) appear downfield than 5,7-dihydroxyflavonoids and may even reverse their positions relative to one another.
Chemical shift data for \( \text{C}_6 \) and \( \text{C}_8 \) protons in 5,7-dihydroxyflavonoids\(^{34}\).

<table>
<thead>
<tr>
<th></th>
<th>( \text{H}_6 ) (ppm)</th>
<th>( \text{H}_8 ) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones, flavonals, Isoflavones</td>
<td>6.0-6.2d</td>
<td>6.3-6.5d</td>
</tr>
<tr>
<td>Flavanones, dihydroflavonols</td>
<td>5.75-5.95d</td>
<td>5.9-6.1d</td>
</tr>
</tbody>
</table>

B-ring protons:

B-ring protons are located downfield from the region where A-ring protons absorb and appear in the range 6.5-7.1 ppm.

4'-oxygenated flavonoids:

Due to the free rotation of the B-ring, protons at \( \text{C}_2',\text{C}_3',\text{C}_5' \) and \( \text{C}_6' \) appear as two pairs of ortho coupled doublets (\( d \ J = 8.5 \text{ Hz} \)) in the range 6.5-7.9 ppm. The \( \text{H}_3',\text{H}_5' \) doublet always occurs upfield from \( \text{H}_2',\text{H}_6' \) doublet due to the shielding effect of oxygen substituents as also because of the deshielding influence of the C-ring functions on \( \text{H}_2',\text{H}_6' \). The position of the \( \text{H}_2',\text{H}_6' \) doublet is dependent upon the level of oxidation of the C-ring.

Chemical shift data for \( \text{C}_2',\text{C}_3',\text{C}_5',\text{C}_6' \) protons in 4'-oxygenated flavonoids\(^{34}\).

<table>
<thead>
<tr>
<th></th>
<th>( \text{H}_2',\text{H}_6' ) (ppm)</th>
<th>( \text{H}_3',\text{H}_5' ) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanones</td>
<td>7.1-7.3 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Dihydroflavonols</td>
<td>7.2-7.4 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>7.2-7.5 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Chalcones (( \text{H}<em>{26} ) and ( \text{H}</em>{35} ))</td>
<td>7.4-7.6 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Aurones</td>
<td>7.6-7.8 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Flavones</td>
<td>7.7-7.9 d</td>
<td>6.5-7.1 d</td>
</tr>
<tr>
<td>Flavonols</td>
<td>7.9-8.1 d</td>
<td>6.5-7.1 d</td>
</tr>
</tbody>
</table>

3',4'-dioxygenated flavonoids:

The \( \text{C}_6 \) proton of 3',4'-dioxygenated flavone and flavonols appear as a doublet centred between 6.7 and 7.1 ppm (\( d \ J = 8.5 \text{ cps} \)) whereas the \( \text{C}_2' \) and \( \text{C}_6' \) protons signals which often overlap arise between 7.2 and 7.9 ppm.

The \( \text{C}_2 \) proton signal is usually centred at slightly higher field than the \( \text{C}_6' \) proton signal in flavonoids containing the 4'-methoxy group. Their
positions are however reversed when a 3'-methoxy group is present in 3',4' oxygenated flavonol.

In 3',4'-dioxygenated isoflavones, flavanones and dihydroxy flavonols the C2',s' and C6' protons appear as a complex multiplet in the range 6.7-7.1 ppm.

Chemical shift data for C2' and C6' protons in 3',4'-dioxygenated flavonoids\textsuperscript{34}

<table>
<thead>
<tr>
<th></th>
<th>H\textsubscript{2}' (ppm)</th>
<th>H\textsubscript{6}' (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones (3',4'—OH and 3'-OH, 4'-OMe)</td>
<td>7.2-7.3 d</td>
<td>7.3-7.5 q</td>
</tr>
<tr>
<td>Flavonols (3,4'-OH and 3'-OH, 4'-OMe)</td>
<td>7.5-7.7 d</td>
<td>7.6-7.9 q</td>
</tr>
<tr>
<td>Flavonols (3'-OMe, 4'-OH)</td>
<td>7.6-7.8 d</td>
<td>7.4-7.6 q</td>
</tr>
</tbody>
</table>

3',4',5' - trioxygenated flavonoids:

The C2' and C6' proton signals usually overlap in the region 6.5-7.5 ppm whereas methylation or glycosylation of the 3' or 5' hydroxyl may lead to nonequivalence of C2' and C6' protons with the consequence that they appear as distinct doublets (J = Ca 2 Hz).

C-ring protons:

![C-ring structure](image)

The oxidation level of the C-ring leads to variations in the chemical shifts among different classes of flavonoids.

The C\textsubscript{3} proton in flavones appears as sharp singlet near 6.3 ppm which usually overlaps the signals produced by A ring protons.

In 8-methoxy flavones, long range coupling of H\textsubscript{6} with the 8-methoxyl protons causes the H\textsubscript{6} signal to be slightly broadened and hence of lower intensity. It has been found\textsuperscript{53,54} that selective detrimethylsilation of the 5-hydroxyl group affected the chemical shifts of C6, C8 and C3 proton signals in different ways. The H\textsubscript{3} signal shifts downfield by at least 0.15 ppm while H\textsubscript{6} shifts upfield by about 0.15 ppm whereas H\textsubscript{6} remains virtually unaffected.
Chalcones and Aurones:

In case of chalcones $\alpha$H and $\beta$H appear as doublets ($J = Ca 17$ Hz) in the range 6.7-7.4 and 7.3-7.7 ppm respectively. The benzylic proton in aurones appears as singlet in the range 6.5-6.7 ppm. Increased hydroxylation in both A and B rings causes the signal to move upfield, relative to that of the unsubstituted aurone especially hydroxylation at C$_4$ (0.19 ppm) and C$_6$ (0.16 ppm) positions.

Mass Spectrometry:

Mass spectrometry serves as a valuable tool in determining the structures of flavonoid aglycones and glycosides. Much data on electron impact Mass spectrometry of flavonoids and their fragmentation has been reported.$^{55}$

For flavonid aglycones an intense molecular ion ($M^+$) peak often represents the base peak whereas the underivatized flavonoid glycosides rarely give the molecular ion peak and even permethylated or peracetylated derivatives give a peak of low intensity. In addition to molecular ion, flavonoid aglycones usually afford major peaks for $[M-H]^+$ and when methoxylated $[M-CH_3]^+$. The rings A and B, in flavonoids, are the first to undergo fragmentation under the electron impact. The most important fragmentation in terms of flavonoid identification are those which involve cleavage of intact A and B ring fragments. Such ions are designated here as A$_1$, A$_2$,... and B$_1$, B$_2$,... etc. Some of these ions are derived by Retro-Diels-Alder (RDA) processes.

Pathway I process, as designated in figure-1 corresponds to an RDA cleavage and usually produces two different ions A$^+$ and B$^+$, the ratio of one to the other indicates the charge distribution with the parent ion. Pathway II, in contrast, yields predominantly a single charged species B$_2$.

These two fragmentation processes are noted to be competitive$^{56}$ and the combined intensities of the B$_2^+$ and [B2-CO]$^+$ ions approximately inversely proportional to those of A$^+$ and B$^+$.

Flavones were the first in the group of flavonoids to be analyzed by mass spectrometry.$^{56-60}$ Simple flavones give a strong molecular ion peak since they are not fully conjugated and thus do not possess sites for facile bond rupture.
Although base peak for most flavone aglycones is the molecular ion $M^+$; peaks are prominent in the spectra for $[M-CO]^+$; and for the pathway-I fragments $A_1^+$ and $B_1^+$.

Flavone itself gives the molecular ion as the base peak with other major peaks corresponding to $[M-H]^+$, $[M-CO]^+$, $A_1^+$, $[A_1-CO]^+$ and $B_1^+$.

Substitution in the A-ring can be detected by examining the m/e value for the $A_1^+$ fragment, for example, 5,7 -dihydroxy flavone gives the same $B_1^+$ fragments as does flavone (at m/e 102) but produces an $A_1^+$ ion 32 mu (mass
units) higher, that is m/e 152 instead of 120 thus indicating two additional oxygen atoms in the A-ring.

It has been reported,\textsuperscript{56,61} that flavones with four or more hydroxyl or methoxyl groups give only weak fragments derived via the primary Retro-Diels Alder reaction (pathway 1) while luteolin and its derivatives give moderately intense A\textsubscript{1}\textsuperscript{*} and B\textsubscript{1}\textsuperscript{*} fragments.

Fragmentation via RDA reactions is comparatively favored in an highly oxygenated flavone than an unsubstituted flavone due to the stabilization of the initially produced ion radical by mesomerism over a number of oxygen atoms. These minor breakdowns may still prove to be of diagnostic value as they frequently represent only, the even numbered peaks in their particular region and hence, are readily distinguished.

Flavanones:

Flavanones fragment by the RDA reaction (pathway 1) and yield ions as those observed for flavones (A\textsubscript{1}\textsuperscript{*} and [A\textsubscript{1}+H]\textsuperscript{*}) however the important B ring ion (designated as B\textsubscript{1}) from pathway-1 contains an ethylenegroup\textsuperscript{56,60} which is present together with other B ring fragments even when the B-ring is in the quinonoid form\textsuperscript{62}.

The substitution patterns of A and B ring, determines the intensity of the two ring fragments from flavanones.

Mass Spectrometry of Chalcones:

Chalcones give strong ions for M\textsuperscript{*}, [M-H]\textsuperscript{*} and [M-CH\textsubscript{3}]\textsuperscript{*} (for methoxy chalcones)and structurally informative fragments derived by fission on either side of the carbonyl group. The relative intensities of the latter ions, designated as A\textsubscript{2}\textsuperscript{*} and B\textsubscript{5}\textsuperscript{*} and the other ions derived from them depend upon the substitution pattern of the parent compound\textsuperscript{56,59,63,64} as is outlined in figure-2.
Chalcones with 2-hydroxyl groups give MS ions which are derived by fragmentation of both the chalcone and its corresponding flavanone\textsuperscript{56,64,65}. The later investigators further established that an intramolecular equilibrium exists between chalcone and flavanone type molecular ion though complete isomerization to one or the other molecular ions does not occur. Vande Sande et al.\textsuperscript{64} were able to establish the pathways involved in the formation of some fragments (figure-2) by using a series of deuterated compounds. For example, they noted that in the formation of flavanone ion, the hydrogen of the hydroxyl group in a 2-hydroxy chalcone shifts to C\textsubscript{3} position however, in some instances, cleavage of the chalcone adjacent to the carbonyl group is much
faster than isomerization to the flavanone and thus the spectrum of the chalcone predominates.

It is also emphasized that in most cases, it is difficult to determine with certainty from mass spectral data, which of the two tautomers the chalcone was originally present.

**Biological Activity of Flavonoids**

Flavonoids play an important role in the biological system of human beings and animals with pharmacological activities influencing the cardiovascular, cerebrovascular and neuromuscular systems. In addition, flavonoids or chalcones have also been reported to be antibiotic in nature, whereas some of them possess biological properties, which are detrimental to growth of microbes. Flavonoids structurally resemble nucleosides-isoalloxazine and folic acid, and this forms the basis of many hypothesis of their physiological action. Some chalcones have been found to be toxic to animals and insects, and exhibit inhibitory action on several enzymes, ring etc.

The structure and features of flavonoids considered to be of importance in biological functions include:

i) The presence of an extended conjugated resonating system with a carbonyl chromophore.

ii) The presence of aromatic hydroxyl groups and

iii) Their molecular shape.

The first feature is responsible for the presence of important pigments in the plants. The aromatic hydroxyl groups lead to the ability of flavonoids to interact with certain enzyme systems whereas the molecular shape, particularly in case of isoflavonoids is responsible for physiological activities due to their similarity in the structure to the animal hormones.

**Cardiovascular System:**

Studies show an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease which is explained in part by the inhibition of low density lipoprotein oxidation and reduced platelet aggregability. Hesperidin and eriodictiol were introduced as therapeutic agents to increase capillary resistance. Similarly, quercetin and rutin have been used as effective constituents for treatment of capillary fragility and phlebosclerosis. The unsubstituted parent compound, flavone, exerts coronary dilatory activity. Its combination with rutin and isoquercetin is useful in the treatment of arteriosclerosis.
ω-amino alkoxychalcones and their acid addition salts are associated with coronary vasodilatory properties. Various chalcones are reported to affect venous circulation, restore capillary resistance and decrease capillary fragility. Indole analogues of chalcones possess weak hypotensive activity. 2’, 4’, 6’-tri-hydroxy chalcone has been reported to exhibit hypotensive as well as anticoagulant properties.

(3-phenyl-7-flavanoxy) propanolamines have been shown to exhibit antihypertensive activity through depletion of myocardial norepinephrine rather than β adrenoceptor inhibition although these compounds are structurally similar to classical β-adrenergic blocking agents. Removal of the 3-phenyl group decreased the CNS side effects. Flavodilol was found to be most effective of all compounds in the series of 7-flavonoxy propanolamines.

Antimicrobial Activity:

The presence of enone function in the molecule confers antibiotic property to the chalcone. This property is enhanced by substitutions at the α-(nitro and bromo) and β-(bromo and hydroxyl) positions. Antibiotic activity is also associated with the C=C bond of the chalcone molecule. Addition of cystein to chalcones hampers activity owing to reduction with the SH group. Chalcones show a significant bacteriostatic action against a number of test organisms like E. coli, S. aureus, B. subtilis, and S. lutea. This bacteriostatic effect could not be reversed by cystein, in contrast to the effect of this compound on other antibiotics. This antibacterial activity is associated with α, β-unsaturated ketone group of the molecule. Chalcones having 4’-hydroxyl and halogen substitution (2 and 4 positions) possess marked antibacterial activity. Chalcone with fluoro substituent have better antibacterial activity compared to chalcones with bromo and chloro substitution when tested against S. albus and S. aureus. 3’-nitro-4’-hydroxy -2- methoxy chalcone has the highest antibacterial activity. The other active chalcones include 3’-nitro -4’-hydroxy -2, 3-dimethoxy chalcones and 3’-nitro -4’-hydroxy -2,5-dimethoxy chalcone. 4 (and 4’) amino chalcones possess bacteriostatic activity against S. aureus and S. hemolyticus.

Among the other chalcones possessing antibacterial activity include the alkylthio chalcones, hydroxycarboxy chalcones and their dihydro derivatives and the methylene dithioacetic acid derivative of chalcone.

Quercetin, among the naturally occurring flavonoids was found to completely inhibit the growth of S. aureus at a concentration of 100μg/ml. Fistenedin chloride at low concentration, inhibited the growth of S. albus, S. aureus, B. subtilis and C. albicans.
Flavones containing bromine at 3-position are reported to be antibacterial in nature.\textsuperscript{65}

The compound 2'-hydroxy chalcone sulfonylic acid possesses weak antifungal activity\textsuperscript{66} whereas carboxy and sulfonic acid derivatives of chalcone show fungicidal action\textsuperscript{65}. Some heterocyclic analogs of chalcone like furan and 8-hydroxy quinoline type compounds have also been reported to exhibit antifungal activity.\textsuperscript{76, 87} Substituted isoflavones, carrying a double bond in the oxygenated ring, possess antiviral activity higher than that of the corresponding isoflavones.\textsuperscript{68}

**Anti-inflammatory activity:**

Several studies on this activity in flavonoids have been conducted in the last two decades. A pharmaceutical composition consisting of neomycin and a topical anti-inflammatory flavonoid in a suitable carrier has been patented for the treatment of acne.\textsuperscript{69} Another patent was taken on xanthorhamnin as an anti-inflammatory agent. The flavonoids from Astragalus had a significant effect when injected (i.p.) into rats with experimental inflammation induced by formalin.\textsuperscript{90}

Ferrandiz et al.\textsuperscript{91} have studied the mechanism of anti-inflammatory action and have data which supports the inhibition of arachidonic acid metabolism as one of the mechanism by which flavonoids exert their anti-inflammatory effects. 5,7 -dihydroxy flavonols having hydroxyl group(s) in B ring and a isoflavone were found to show broad inhibitory activities (14-52%) against croton oil or arachidonic acid induced ear edema by oral or topical administration.\textsuperscript{92}

Flavone and its 2',4'-,3- and 6-hydroxy derivatives have also been reported to produce dose related anti-inflammatory effects in both acute and chronic models of inflammation in rats and the study indicates that hydroxylation favors the anti-inflammatory activity of the flavone nucleus more than does methoxylation. Panthong et al. have also studied the anti-inflammatory activity of flavonoids and have reported the structural features necessary for activity to be the presence of methoxy groups at C\textsubscript{5} and C\textsubscript{7} of the flavonoid molecule.\textsuperscript{64, 93} 2' -and 3' -hydroxy chalcones and 2,5' -dihydroxy chalcones have shown inhibitory effects on the activation of mast cells and neutrophils which play a important role in inflammatory disorders.

**Action on enzymes:**

Flavonoids are active over a wide range of enzymes. The inhibitory activities of flavonoids on hyaluronidase, histidine decarboxylase, xanthine oxidase and choline acetylase have been known since long.
Among the hydroxy chalcones, 3,3', 4,4' - tetrahydroxy chalcone is an efficient inhibitor of liver xanthine oxidase activity whereas the 2,4,4' trihydroxy chalcone is a potent stimulator of indole acetic acid oxidase. Recent studies have found that C₄ substituted flavonoids stimulated the action of indole acetic acid oxidase. The stimulating effect increased considerably on addition of C₇ hydroxyl group. On the contrary, an inhibition of enzyme activity occurred with flavonoids containing hydroxyl groups at C₃ and C₄. Flavonoids were found to inhibit liver COMT in-vitro to various degrees. The glycosides being weaker inhibitors than the corresponding genins. cAMP phosphodiesterase inhibition has also been reported in flavonoids. Flavonoids demonstrate moderate to good Angiotensin I converting enzyme (ACE) inhibitory activity in in-vitro tests. Chalcones and furan analogs of chalcone inhibit the activity of Papain, cholineserase in horse serum. The furan analog of chalcone has a marked ability to inhibit activity of the enzyme dihydroxyphenylalanine decarboxylase. A few salicylic chalcones inhibit aromatic L-amino acid carboxylases.

Diuretic activity:
The diuretic effect of myrcetin and kaempferol was observed in rabbits (s.c. 25 mg/kg) as early as 1931. The potency increased with an increase in number of hydroxyl groups. Flavone glycosides e.g. quercetin, rutin, kaempferol -3- rhamno glucoside and luteolin were also found to inhibit diuretic activity.

Anti-tumor activity/cytoxic activity:
2,4,4'-tri hydroxy chalcone and 2', 4', 6'-tetra hydroxy chalcone has been found to have antineoplastic action on Ehrlich's ascitic sarcoma in mice. Nitro chalcones having nitro group in 2 (and 2') and 4 (and 4') positions show cytotoxic activity and have been tested against normal and Roux virus transformed hamster fibroblasts. 3' and 4' methyl-3-hydroxy chalcones showed highest potency in inhibiting tumorigenesis in a group of 40 chalcones. They also showed a marked inhibitory effect on the proliferation of HGC-27 cells derived from human gastric cancer. Various Mannich bases of chalcones and related compounds display significant cytotoxicity towards murine P388 and L1210 leukemia cells as well as a number of tumor cell lines. Cis-3-hydroxy-3'-methyl chalcone is reported to inhibit TPA-activated tumor promotion at a concentration of 5μg/ml.

Naturally occurring flavonoids inhibit HL-60 cell growth with a nontoxic mechanism, possibly via cessation of DNA, RNA and/or protein synthesis of the leukemic cells.
Antiparasitic activity / Antimalarial activity

Thiophene analogs of chalcones possess some antiparasitic activity\(^{120}\). The compounds with following general formula possess antimalarial activity\(^{121}\).

\[
\text{CR=CH—C} \quad \text{NCH(=NH)NH}_2 \text{HCl}
\]

Anti-allergic activity:

Flavonoids are found to have anti-allergic activity. Activity is associated with possible conversion of flavones to 2'-hydroxy chalcones\(^{122}\). In a related report flavonoid aglycones show a stronger activity for histamine release inhibition than glycosides on antigen induced histamine release from IgE-sensitized RBL-2H3 cells\(^{123}\).

Anti-HIV activity:

A known flavone, chrysin was found to be the most promising compound among a group of natural and synthetic flavonoids when evaluated as inhibitors of HIV replication in H9 cell\(^{124}\). Flavonoids with hydroxy groups at C\(_5\) and C\(_7\) and with a C\(_2\)-C\(_3\) double bond were more potent inhibitors of HIV growth and the presence of substituents (hydroxyl and halogen) in the B ring increased toxicity and/or decreased activity.

Anti-oxidant activity:

Flavonoids were found to have the best relative antioxidant efficiency (RAE) when tested along with coumarins and cinnamic acids when compared to that of \(\alpha\)-tocopherol\(^{125}\).

Hypoglycemic activity:

Isorhamnetin 3-rhamnosyl galactoside isolated from *Dodonea viscosa* showed 15% blood lowering effect at 200 mg/kg. Mishra et al.\(^{126}\) in their review have pointed out the correlation of structure of some flavonoids to their hypoglycemic activity.
Anthelmintic activity:

The 2,2'-dihydroxy chalcone has anthelmintic activity against *Amoeba*. Chalcone derivative 3,5-diphenyl isoalloxazine has shown anthelmintic activity against *Pinworms*. The hydroxy chalcones and derivatives show lesser toxicity and local irritation than hexyl resorcinol against the test organism *Ascaris*.

Other activities:

Chalcones and some furan analogs exhibit acaricidal activity. Some chalcones are reported to possess anesthetic, anti-convulsant and anti-ulcer activity. Thiényl pyrazolines derived from appropriate chalcones are described as potential schistosomicidal agents. Chalcone derivatives have also been claimed to have insect repellant properties while N-substituted o-carbamoyloxime of chalcone is reported to exhibit a weak insecticidal activity and herbicidal activity.

Toxicity to animals:

The furan analog of chalcone has been found to be markedly toxic against rats while for polyhydroxy chalcones, the toxicity increases if all the hydroxyl groups are methylated.
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The Reagents/ Chemicals/ Solvents used during the course of these studies were obtained from Merck (India), Qualigens, BDH, SD fine and CDH Laboratories and were of the Laboratory grade. The solvents were purified by distillation before their use.

Silica Gel used for thin layer chromatography was of Central Drug House brand.

Iodine chamber and UV lamp were used for visualization of TLC Spots. Whatman filter papers (No. 1, England) were used for filtration (Vacuum or ordinary).

The solvent systems used for thin layer chromatography were
1) Irrigant 'a'  Petrol: Toluene: Ethyl Acetate (5:4:3)
2) Irrigant 'b'  Toluene: Ethyl acetate: Formic Acid (5:4:1)
3) Irrigant 'c'  Benzene
4) Irrigant 'd'  Benzene: Ethyl Acetate (7:3)

These have been accordingly mentioned at appropriate places.

The U.V. Spectras were recorded on Perkin Elmer, UVMIS Spectrophotometer Lamda Bio 20

The IR Spectras were recorded on Hitachi IR Spectrometer model 270-30. Potassium Bromide was used for making pellets.
The NMR Spectras were recorded on 60 MHz, 90 MHz or 300 MHz instruments.

The Mass Spectras were recorded on a GCI MS instrument.

Melting points of all the compounds were recorded on liquid paraffin bath in open capillary tubes and are uncorrected.

Detection of elements present (Nitrogen, Sulphur and Halogens)

The detection of nitrogen, sulphur and halogens was carried out by sodium fusion method (Lassaigne’s test) as follows:

In a fusion test tube, a small cube of freshly cut sodium metal was introduced. The tube was heated slowly until sodium vapour rises in the test tube. The test compound (about 0.05g) was added to the molten sodium. The tube was then heated to redness for about two minutes and then allowed to cool. The tube was crushed into a crucible containing 10 ml of distilled water. Three such tubes were added to the crucible. The mixture was slightly concentrated and filtered. The filtrate was used for the various tests detailed below:

a) Test for nitrogen: To 2-3ml of the filtered fusion solution in a test tube was added 0.1-0.2g of powdered ferrous sulphate crystals. The mixture was heated gently with shaking till it boiled, then dilute sulfuric acid was added. A prussian blue color or precipitate indicated the presence of nitrogen. When sulphur was also present the mixture was boiled after addition of ferrous sulphate and then acidified with dilute sulfuric acid.

b) Test for sulphur: The fusion solution (2ml) was acidified with dilute acetic acid and a few drops of lead acetate solution was added. A black precipitate indicated the presence of sulphur.

c) Test for halogens:

1) A portion of the fusion solution was acidified with dilute nitric acid and an excess of silver nitrate solution was added. A precipitate indicated the presence of halogen, which was treated with dilute aqueous ammonia solution. A white precipitate soluble in ammonia indicated chlorine while a yellow precipitate difficultly soluble indicated bromine.

2) When nitrogen and/or sulphur was present in the compound the fusion solution (5ml) was acidified with dilute nitric acid and the solution evaporated to half its volume, diluted with an equal volume of water and then tested for halogens as above.
(a) Exploitation of 5-Chloromethyl-2-hydroxy acetophenone nucleus to synthesize pharmaceutically important compounds.

i) Synthesis of benzyl anilines and miscellaneous compounds

Synthesis of 2-hydroxy-5-chloromethyl acetophenone (I)

Hydrochloric acid gas was generated by pouring conc. sulfuric acid over sodium chloride and passed into a mixture of o-hydroxy acetophenone, formalin and conc. hydrochloric acid, with constant stirring, at a temperature below 15° for a period of ten hours. The contents after diluting with water were filtered and crystallized from petroleum ether to give a white crystalline compound (I) m. p. 93-95°. It gave a positive test with alcoholic ferric chloride and was TLC pure. It tested positive for chlorine by Lassaigne's test.

\[
\begin{array}{ccc}
\text{HCHO, HCl} & \text{aq.} & \text{HCl} \\
\text{< 15°} & \text{ClH}_2\text{C} & \text{OH} \\
\text{O} & \text{O} & \text{O} \\
\text{OH} & \text{ClH}_2\text{C} & \text{O} \\
\end{array}
\]

In the IR spectrum it showed bands at 3250 cm\(^{-1}\)(b, OH), 1640 cm\(^{-1}\) (CO), 1580 cm\(^{-1}\) (aromatic C=C str.), 810 cm\(^{-1}\) (C-Cl str.)

The NMR spectrum of the compound showed two singlets at \(\delta\) 2.7 and \(\delta\) 4.6 in the ratio of 3:2. In the aromatic region, a one proton doublet at \(\delta\) 7.05 (J=8Hz), one proton double doublet at \(\delta\) 7.6 and a one proton doublet at \(\delta\) 7.9 (J=2Hz) suggested 1:2:4 trisubstitution. Hence the compound formed is 2 acetyl-4-chloromethyl phenol (2-hydroxy-5-chloromethyl acetophenone).

The mass spectrum of the compound showed the molecular ion peak located at m/z 185 analysing for the molecular formula \(\text{C}_9\text{H}_9\text{O}_2\text{Cl}\). Other important peaks could be located at m/z 169, 149 and 131. The fragmentation pattern follows.
Synthesis of N-(3-acetyl-4-hydroxy benzyl) aniline (II) from (I)

To a mixture of aniline, and sodium bicarbonate in hot water was added (I) gradually in small portions and with constant stirring while maintaining the temperature between 90°-100°. Stirring was continued for two hours. A product which separated was filtered, dried and crystallized from methanol to give crystals of (II) m.p.186-90°. The product was TLC pure and gave a positive test for nitrogen by sodium fusion test.

In the I.R. spectrum it showed bands at 2950 cm⁻¹ (chelated OH), 1660 cm⁻¹ (CO), 1620 cm⁻¹ (aromatic C=C str.) 780, 720 cm⁻¹ (Ph. ring C-H bend). The NMR spectrum of the compound showed two singlets integrating for three protons and two protons each located at δ 2.52 and δ 4.53 which could arise from methyl and methylene groupings. The singlet for chelated hydroxyl group could be picked up at δ 12.16. In the aromatic region there was a multiplet centered at δ 6.79 integrating for three protons and another multiplet centered at δ 7.21 integrating for two protons which could arise from the protons H-D, F, H and protons H-E, G respectively of the aniline moiety. The protons H-A, H-B and H-C could be picked up as an ortho coupled doublet (J=9Hz), ortho-meta coupled doublet (J=9Hz, 2Hz) and meta coupled doublet (J=2Hz)
located at δ 6.93, δ 7.35 and δ 7.52 respectively. These data are satisfactory for the above structure assigned to the compound.

The mass spectrum of the compound showed a molecular ion peak located at m/z 241, which could be analyzed for the molecular formula C₁₅H₁₅O₂N. Other important peaks could be located at m/z 149, 131, 93 and 77. The fragmentation pattern is as follows.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III) from (I)

A hot mixture of o-toluidine, sodium bicarbonate and water was treated with (I) as above. Stirring was continued for two hours. A solid mass, which separated out was filtered, dried and purified from methanol to give (III) m.p. 104-06°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

The IR spectrum showed the following bands at 3450 cm⁻¹ (b,OH + NH), 1640 cm⁻¹ (CO), 1610 cm⁻¹, 1580, 1520 (aromatic C=C str.), 780, 740 cm⁻¹ (toluidine ring C-H bend.).

The NMR spectrum of the compound showed three singlets at δ 2.1, δ 2.6 and δ 4.3 in the 3:3:2 ratio corresponding to two methyls and one methylene group. A broad absorption at δ 3.3 could be due to -NH. The aromatic region showed a multiplet between δ 6.5 and δ 7.2 for five hydrogens which might arise from the protons of the o-toluidine moiety and a hydrogen ortho to the hydroxyl group on the other ring. A double doublet of
one proton \((J=8\ \text{Hz} \text{ and } 2\text{Hz})\) at \(\delta 7.5\) and a doublet \((J=2\text{Hz})\) at \(\delta 7.7\) accounted for the two protons deshielded by the carbonyl group respectively. These results showed successful formation of the compound.

It was further supported by the mass spectral data, which showed the molecular ion peak located at \(m/z\) 255, which analyzed for the molecular formula \(\text{C}_{16}\text{H}_{17}\text{O}_{2}\text{N}\). Other, important peaks could be picked up at \(m/z\) 149, 120, 107 and 77. The fragmentation pattern has been given below.

\[
\begin{align*}
\text{m/z 255} & \\
\text{m/z 149} & \\
\text{m/z 120} & \\
\text{m/z 107} & \\
\text{m/z 77} & \\
\end{align*}
\]

**Synthesis of N-(3-acetyl-4-hydroxy benzyl)-\(p\)-toluidine (IV) from (I)**

A mixture of \(p\)-toluidine, sodium bicarbonate and water was heated over a hot plate and to the mixture was added (I) gradually and with stirring. Stirring was continued for another three hours. The mixture was extracted with ether and the residue crystallized from a mixture of petroleum ether and diethyl ether to give (IV) m. p. 188-90°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

In the IR spectrum it showed the following bands at 2920 cm\(^{-1}\) (chelated OH), 1650 cm\(^{-1}\) (CO), 1620, 1560 cm\(^{-1}\) (aromatic C=C str.), 840 cm\(^{-1}\) (p-disubstituted phenyl ring C-H bend.).

Its structure was proved on the basis of NMR data. The NMR spectrum of the compound showed two singlets at \(\delta 2.3\) and \(\delta 2.7\) for the methyl group.
and protons of the acetyl group. A singlet at δ 4.5 accounted for methylene group attached to nitrogen. A one proton doublet (J=8 Hz) at δ 6.7 is probably due to proton ortho to the phenolic hydroxyl while the doublet at δ 8.2 (J=2 Hz) arises from the proton ortho to the acetyl function. A five proton multiplet at δ 7.2 accounted for the remaining protons.

It was further supported by mass spectral data, which showed a molecular ion peak located at m/z 255 analysing for the molecular formula C_{16}H_{17}O_{2}N. Other, important peaks could be located at m/z 149, 131 and 107. The fragmentation pattern is as follows.

**Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-anisidine (V) from (I)**

To a hot mixture of o-anisidine, sodium bicarbonate and water was added (I) gradually as stated above. Stirring was continued for another two hours. The product obtained was filtered, dried and crystallized from methanol to give buff colored crystals (V) m. p. 86-88°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.
The IR spectrum showed the following absorption peaks at 3500 cm\(^{-1}\) (b,NH+OH), 1640 cm\(^{-1}\) (CO), 1610 cm\(^{-1}\), 1520 cm\(^{-1}\) (aromatic C=C str.), 800, 725 cm\(^{-1}\) (o-anisidine ring CH bend).

The NMR spectrum of the compound showed a singlet arising from the acetyl group located at \(\delta 2.6\). There was another singlet located at \(\delta 3.8\) which accounted for the methoxyl function. A broad singlet at \(\delta 4.2\) could be due to the NH group. There was another singlet located at \(\delta 4.4\) which could arise from the methylene function. There was a multiplet centered at \(\delta 6.6\) which accounted for the protons A and D. The protons B and C were located at \(\delta 6.72\) as a multiplet. The protons G and F were located at \(\delta 6.83\), the proton E could be located at \(\delta 7.95\) while the chelated hydroxyl group was located at \(\delta 12.17\). The above data satisfactorily accounted for the structure assigned to the compound.

The mass spectrum of the compound showed the molecular ion peak located at m/z 271, analysing for the molecular formula C\(_{16}\)H\(_{17}\)O\(_3\)N. Other important peaks could be located at m/z 149, 131, 123 and 108. The fragmentation pattern follows.
Attempted synthesis of N-(3-acetyl-4-hydroxy benzyl)-p-anisidine (VI) from (I) which resulted in the formation of the ketimine (Vla).

A mixture of p-anisidine, sodium bicarbonate and water was heated as described in earlier cases. To this hot mixture was gradually added (I) in portions with stirring. Stirring was continued for another two hours. The mixture was extracted with ether, crystallized from a mixture of petroleum ether and ether to give greenish needles of (Vla) m. p. 180-82°, which were found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

\[
\text{(I) } \xrightarrow{\text{NaHCO}_3, \text{H}_2\text{O}} \text{H}_3\text{CO}-\text{HNNH}_2\text{C} \xrightarrow{\text{N}} \text{CH}_3 \\
\text{(VIa)}
\]

The IR spectra showed the following bands at 3370 cm\(^{-1}\) (b, NH+OH), 1600 cm\(^{-1}\) (CO), 1580 cm\(^{-1}\), 1510 cm\(^{-1}\) (aromatic C=C str.), 860, 840, 820 cm\(^{-1}\) (p-anisidine ring CH bend).

The NMR spectrum of the compound showed three singlets for a methyl group at \(\delta\) 2.3 and for two methoxyls at \(\delta\) 3.7 and \(\delta\) 3.8. There was a methylene signal located at \(\delta\) 4.22. In the aromatic region there were four two proton doublets forming two A2/B2 systems around \(\delta\) 6.65, \(\delta\) 6.77, \(\delta\) 6.84, \(\delta\) 6.91 indicating incorporation of two p-anisidine rings. The first two doublets can be assigned to hydrogens of the p-anisidine condensed at the CH2Cl while the other two may arise from the anisidine ring condensed with the carbonyl group to give a ketimine. The three protons of the other ring gave an ortho coupled doublet at \(\delta\) 6.98, a double doublet centred at \(\delta\) 7.35, and an meta coupled doublet at \(\delta\) 7.6. These data suggest that the compound has the structure (Vla) as shown above.

**Synthesis of N-(3-acetyl-4-hydroxy benzyl)-morpholine (VII) from (I)**

(I) was gradually added in portions and with stirring to a hot mixture of morpholine, sodium bicarbonate and water stirring was continued for another
five hours. The mixture was extracted with ether and the ether evaporated off. The residue was crystallized from a mixture of methanol and acetone to give a cream colored compound (VII) m. p.28-30°, which was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

The NMR spectrum of the compound showed the presence of two methylene groups attached to the nitrogen atom located at δ 2.43, there were another signals centred at δ 3.70 which could arise from the two methylene groups attached to oxygen. These data are satisfactory for the morpholine moiety. The two singlets located at δ 2.63 and δ 3.44 integrating for three and two protons respectively could arise from the methyl function of the acetyl group and the methylene function of the chloromethyl group of the parent compound. In the aromatic region, there were two multiplets located at δ6.92 and δ 7.44 which could arise from the protons ortho and meta to the hydroxyl group. There was another doublet located at δ 7.6, which could arise from the proton ortho to the acetyl function. The proton of the chelated hydroxyl group appeared at δ 12.2. These data are satisfactory for the structure assigned to the compound.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-nitroaniline (VIII) from (I)

To a hot mixture of o-nitroaniline, sodium bicarbonate and water was added (I) in small portions with continuous stirring maintaining the reaction conditions as described earlier. Stirring was continued for another four hours. The mixture was extracted with ether. The residue after evaporation of ether, was crystallized from methanol to give orange colored crystalline compound (VIII) m.p.112-16°. It gave a positive test for nitrogen by sodium fusion test and was found to be TLC pure.
The IR spectrum showed the following bands at 3400 cm\(^{-1}\) (b,NH+OH), 1620 cm\(^{-1}\) (CO), 1580, 1490 cm\(^{-1}\) (aromatic C=C str.), 780, 740 cm\(^{-1}\) (o-nitroaniline ring CH bend.).

The NMR spectrum of the compound showed signals for acetyl group at \(\delta\) 2.6 and methylene group at \(\delta\) 4.5. In the aromatic region it showed three one proton doublets at \(\delta\) 6.8, \(\delta\) 7.0 and \(\delta\) 8.2 showing ortho coupling, besides it showed a one proton triplet at \(\delta\) 6.67 and a one proton doublet at \(\delta\) 8.34. The doublets obviously arise from proton ortho to hydroxyl group, proton ortho to amino function and proton ortho to nitro group respectively, while the lowest field singlet can be assigned to proton ortho to carbonyl group. There was a signal for chelated hydroxyl group at \(\delta\) 12.25. These data suggested that the expected compound has been formed.

The mass spectrum of the compound showed the molecular ion peak located at m/z 286 analysing for the molecular formula C\(_{15}\)H\(_{14}\)O\(_4\)N\(_2\). Other important peaks could be located at m/z 268, 149, 138 and 131. The fragmentation pattern is given below.

![Fragmentation pattern diagram]

**Synthesis of N-(3-acetyl-4-hydroxy benzyl)-m-nitroaniline (IX) from (I)**

To a mixture of m-nitroaniline, sodium bicarbonate and water was added (I) in small portions and with stirring while maintaining reaction conditions as above. Stirring was continued for another two hours. The product was extracted with ether. After evaporating off the ether, the residue was crystallized from methanol to give yellow colored crystals of (IX) m. p. 106-08°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.
The IR spectrum showed the following bands at 3470 cm\(^{-1}\) (\(\text{b}, \text{NH}+\text{OH}\)), 1620 cm\(^{-1}\) (CO), 1520 cm\(^{-1}\) (aromatic C=C str.), 840, 680 cm\(^{-1}\) (\(m\)-nitroaniline ring CH bend).

The NMR spectrum showed two singlets for an acetyl function at \(\delta\ 2.6\) and the methylene group at \(\delta\ 4.3\). The ketone ring hydrogens gave a doublet at \(\delta\ 6.98\) (H-A), a double doublet at \(\delta\ 7.49\) (H-B) and a meta coupled doublet at \(\delta\ 7.73\) (H-C). The nitroaniline ring hydrogens gave a double doublet at \(\delta\ 6.9\) (H-G), a triplet at \(\delta\ 7.28\) (H-F), a triplet at \(\delta\ 7.44\) (H-D) and a double doublet at \(\delta\ 7.55\) (H-E). These data suggested that the compound is the expected condensation product.

The above structure was further supported by the mass spectral data, which showed the molecular ion peak located at m/z 286, which analyzed for the molecular formula \(\text{C}_{15}\text{H}_{14}\text{O}_{4}\text{N}_{2}\). The other important peaks were located at m/z 268, 149, 138, 131 and 77. The fragmentation pattern is given below.

\[\text{NO}_2^+ \xrightarrow{\text{m/z 286}} [\text{HN} - \text{CH}_2]^+ \xrightarrow{\text{m/z 268}} \]

\[\text{m/z 77} \quad \text{m/z 138} \quad \text{m/z 149} \quad \text{m/z 131} \]

**Synthesis of N-(3-acetyl-4-hydroxy-benzyl)-\(m\)-tolidine (X) from (I)**

(I) was added in portions and with continuous stirring to a hot mixture of \(m\)-tolidine, sodium bicarbonate and water. Stirring was continued for another five hours. A solid mass, so obtained was filtered, dried and crystallized from methanol to give a buff colored compound (X) m. p. 120-22\(^\circ\).
It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

\[
\text{(I)} \xrightarrow{\text{NaHCO}_3; \text{H}_2\text{O} / \sigma - \text{tolidine}} \text{(X)}
\]

The IR spectrum showed the following absorption peaks at 3445 cm\(^{-1}\) (b, NH+OH), 1630 cm\(^{-1}\) (CO), 1600 cm\(^{-1}\), 1500 cm\(^{-1}\) (aromatic C=C str.).

The NMR spectrum of the compound showed a singlet for the two methyl groups at \(\delta 2.2\) while the two acetyl functions gave a singlet at \(\delta 2.6\). The methylene hydrogens gave a singlet at \(\delta 4.35\). The ketone ring hydrogens gave an ortho coupled doublet at \(\delta 7.0\) (H-C), a double doublet at \(\delta 7.5\) (H-A) and a meta coupled doublet at \(\delta 7.75\) (H-B). The toidine ring hydrogen gave an ortho coupled doublet at \(\delta 6.64\) (H-D) and a multiplet around \(\delta 7.27\) (H-E, F). These data are consistent for the symmetrical structure expected.

**Synthesis of N-(3-acetyl-4-hydroxy-benzyl)-p-bromo aniline (XI) from (I)**

To a mixture of \(p\)-bromo aniline, sodium bicarbonate and water was added (I) gradually in small portions with continuous stirring maintaining reaction conditions as stated above. Stirring was continued for another three hours. A solid mass so obtained was filtered, dried and crystallized from methanol and dichloromethane mixture to give colorless crystals of (XI) m. p. 100-02°. It was found to be TLC pure and gave a positive test for nitrogen and bromine on sodium fusion test.

\[
\text{ClH}_2\text{C} \xrightarrow{\text{NaHCO}_3; \text{H}_2\text{O} / \rho\text{-bromoaniline}} \text{BrE} \quad \text{F}
\]

In the IR spectrum it showed following peaks at 3420 cm\(^{-1}\) (NH, OH) 1640 cm\(^{-1}\) (aromatic-C=C str.), 1620, 1600 cm\(^{-1}\) (aromatic), 810 cm\(^{-1}\) (\(p\)-disubstituted ring C-H bend.).

In the aliphatic region of the NMR spectrum there could be located two peaks at \(\delta 2.61\) and \(\delta 4.24\), which could be due to the protons of acetyl and methylene functions. In the aromatic region there was an ortho coupled doublet (J=9Hz) at \(\delta 6.5\) due to proton H-A, two doublet forming an \(A_2B_2\)
pattern located at $\delta$ 6.95 and $\delta$ 7.24 due to the protons H-D, G and H-E, F respectively. Besides, there was a double doublet (J=2Hz, 9Hz) located at $\delta$ 7.45 due to the proton H-B and one metacoupled doublet (J=2Hz) located at $\delta$ 7.69 arising from H-C. These data satisfactorily favour the structure assigned to the compound.

In the mass spectrum molecular ion peaks were located at m/z 319/321 which could analyze for the molecular formula $C_{15}H_{14}O_2NBr$. Other important peaks could be located at m/z 171/173, 149 and 131. The fragmentation pattern is as given below.

![Fragmentation pattern diagram]

**Synthesis of N-(3-acetyl-4-hydroxy benzyl)-norfloxacin (XII) from (I)**

A mixture of norfloxacin, sodium bicarbonate and water was heated as stated above. To this was added (I) in portions with continuous stirring. Stirring was continued for another four hours. The product obtained was filtered, dried and crystallized from a mixture of benzene and methanol to give buff colored compound (XII) m. p. > 350. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.
The IR spectrum showed the following peaks at 3490 cm\(^{-1}\) (b, COOH+OH), 1630 cm\(^{-1}\) (C=O/C00H), 1580 cm\(^{-1}\), 1490 cm\(^{-1}\) (aromatic C=C str.)

The NMR spectrum of the compound showed a triplet at \(\delta\) 1.5 and a quartet at \(\delta\) 4.55 for the N-C\(_2\)H\(_5\) moiety. The singlet at \(\delta\) 2.7 can be assigned to the acetyl function while the singlet at \(\delta\) 4.46 can be assigned to N-CH\(_2\) aryl group. The piperazine ring hydrogens gave a multiplet from \(\delta\) 3.4 to \(\delta\) 4.0. In the aromatic region, aromatic protons of norfloxacin moiety could be located at \(\delta\) 7.0 (H-A) and \(\delta\) 7.70 (H-B) while the pyridone ring hydrogen could be located as a singlet at \(\delta\) 8.1. The three hydrogens of the aromatic ring gave a signal at \(\delta\) 7.15 (proton ortho to hydroxyl) and a two proton multiplet at \(\delta\) 7.9 (protons ortho and para to carbonyl). These data suggest successful condensation.

**Synthesis of N-(3-acetyl-4-hydroxy-benzyl)sulfadoxine (XIII) from (I)**

To a hot mixture of sulfadoxine, sodium bicarbonate and water was added (I) in portions maintaining reaction conditions as stated above. Stirring was continued for three hours. The product obtained was filtered, dried and crystallized from methanol and dichloromethane mixture to give brown colored crystals of (XIII) m. p. 176-78\(^{\circ}\). It was found to be TLC pure and gave a positive test for nitrogen and sulphur on sodium fusion test.
The IR spectrum showed the following bands at 3475 cm\(^{-1}\) (NH), 3275 cm\(^{-1}\) (OH), 1640 cm\(^{-1}\) (C=O), 1580, 1530 cm\(^{-1}\) (aromatic C=C str.), 1325, 1150 cm\(^{-1}\) (S=O), 830 cm\(^{-1}\) (p-disubstituted aromatic ring CH bend).

The NMR spectrum showed an acetyl function by a singlet at \(\delta 2.6\) and two methoxyl groups by two singlets at \(\delta 3.7\) and \(\delta 3.9\). The methylene group appeared as a doublet at \(\delta 4.3\) due to coupling with NH. In the aromatic region the two doublets located at \(\delta 6.58\) and \(\delta 7.7\) could be assigned to the protons of p-disubstituted ring, while the proton attached to nitrogen in NHCH\(_2\) appeared as a triplet at \(\delta 6.68\) and the sulphonamide proton appeared as a singlet at \(\delta 7.9\). The pyrimidine ring proton appeared as a singlet at \(\delta 8.05\). The remaining three protons of the acetophenone moiety appeared as ortho coupled doublet at \(\delta 6.9\) (H-A), a double doublet at \(\delta 7.5\) (H-B) and a meta coupled doublet at \(\delta 7.8\) (H-C). These data suggested that the compound is the expected condensation product XIII.

Synthesis of \(N\)-(3-acetyl-4-hydroxy-benzyl)-2-aminobenzothiazole (XIV) from (I)

Chloromethyl acetonaphone (I) was gradually added in portions to a hot mixture of 2-aminobenzothiazole, sodium bicarbonate and water with continuous stirring. Stirring was continued for another two hours. The mixture was extracted with ether, the ethereal layer evaporated and the residue was crystallized from methanol to give a compound m. p. 144-46\(^{\circ}\), which was found to be a mixture of two components on TLC examination. It gave a positive test for nitrogen and sulphur on sodium fusion test.

\[
\begin{align*}
(I) & \xrightarrow{\text{NaHCO}_3;\text{H}_2\text{O}/\Delta} \text{2-aminobenzothiazole} \\
& \xrightarrow{\text{2-aminobenzothiazole}} \text{(XIV)}
\end{align*}
\]

The IR spectrum showed the following bands at 3010 cm\(^{-1}\) (b,OH), 1640 cm\(^{-1}\) (CO), 1614, 1480 cm\(^{-1}\) (aromatic C=C str.), 800 cm\(^{-1}\) (benzimidazole ring CH bend.)

The NMR spectrum of the compound showed two singlets located at \(\delta 2.48\) and \(\delta 2.57\) arising from the methyl function of the acetyl group and two singlets integrating for two protons each located at \(\delta 4.40\) and \(\delta 5.18\) which suggested that complete reactions has not taken place and the compound is a mixture of (XIV) and the starting ketone.
Synthesis of N-(3-acetyl-4-hydroxy-benzyl)-o-aminothiophenol (XV) from (I) and o-aminothiophenol

Chloromethyl acetophenone (I) was added gradually in portions with continuous stirring to a hot mixture of o-aminothiophenol, sodium bicarbonate and water. Stirring was continued for another two hours. The mixture was extracted with ether and the ethereal layer evaporated off. The residue was crystallized from methanol to give (XV) m.p 148-50° which was found to be pure on TLC examination and gave a positive test for nitrogen on sodium fusion test.

![Chemical Structure](image)

The IR spectrum showed the following bands at 3450 cm⁻¹ (NH+OH), 1640 cm⁻¹ (CO), 1580 – 1500 cm⁻¹ (aromatic C=C str.), 740 cm⁻¹ (o-disubstituted ring CH bend.).

The NMR spectrum of the compound showed a singlet located at δ 2.59 which could arise from the acetyl function. The signals due to methylene group and NH group could be located at δ 4.45 and δ 5.35. In the aromatic region the two protons from the aminothiol moiety appeared as a multiplet centered at δ 6.53 while the other two protons also appeared as a multiplet centered at δ 7.16. The protons H-B, H-C and H-A from the o-hydroxy acetophenone moiety appeared as an ortho coupled doublet at (J=9 Hz) (from the proton H-B), an ortho-meta coupled doublet centered at δ 7.37 (J=9Hz, 2Hz) arising from H- C, the proton H-A appeared as a meta coupled doublet centered at δ 7.69 (J=2Hz). The chelated hydroxyl appeared as a singlet located at δ 12.2. The above data are satisfactory for the structure assigned to the compound.

The above structure was further supported by mass spectral data, which showed the molecular ion peak at m/z 273 analysing for the molecular formula, C15H15O2NS. Other important peaks could be located at m/z 149, 131 and 77. The fragmentation pattern is as below.
Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-aminophenol (XVI) from (I)

To a hot mixture of o-aminophenol, sodium bicarbonate and water was added (I) gradually in portions with continuous stirring. Stirring was continued for another three hours. The product obtained was filtered, dried and crystallized from petrol and ether mixture to give compound (XVI) m. p. 194-96° (decomp.). It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

The IR spectrum showed the following bands at 3030 cm⁻¹ (NH+OH), 1620 cm⁻¹ (CO), 1600, 1560 cm⁻¹ (aromatic C=C), 820, 780, 740 cm⁻¹ (aromatic CH bend, of o-aminophenol ring)

The NMR spectrum of the compound showed two signals located at δ 2.64 and δ 4.43 integrating for three protons and two protons each, which could arise from the acetyl and methylene groups. The protons from the o-hydroxy acetophenone moiety H-B, H-C and H-A were located at δ 6.99, δ 7.70 and δ 7.73 as ortho coupled doublet (J=9Hz), meta coupled doublet (J=2Hz) and ortho meta coupled doublet (J=9, 2Hz) respectively. The protons from the o-aminophenol ring along with the proton of the hydroxyl group attached to the same ring appeared as a complex multiplet centered at δ 7.42.
The OH and NH protons of the other amino phenol were located at δ 6.23 while the chelated hydroxyl group appeared as a singlet at δ 12.27. These data are satisfactory for the structure assigned to the compound.

Synthesis of N-\(3\)-acetyl-4-hydroxy benzyl)-anthranilic acid (XVII) from (I)

To a hot mixture of anthranilic acid, sodium bicarbonate and water was added (I) gradually in small portions with continuous stirring. Stirring was continued for another three hours. A solid mass obtained was filtered washed with water, dried and crystallized from ethanol to give (XVII) m. p. 198-200°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

The IR spectrum showed the following bands at 3400 cm\(^{-1}\) (NH), 3000 cm\(^{-1}\) (b,\(\text{COOH} + \text{OH}\)), 1660, 1640 cm\(^{-1}\) (CO, \(\text{COOH}\)), 1580, 1510 cm\(^{-1}\) (aromatic C=C str.), 740 cm\(^{-1}\) (anthranilic acid ring CH bend.).

The NMR spectrum of the compound showed a singlet located at δ 2.60 integrating for three protons and another singlet located at δ 4.40 integrating for two protons which could be accounted for by the protons of the acetyl moiety and methylene group attached to the NH group of anthranilic acid system. In the aromatic region the signals due to the proton H-D and H-B could be located as an ortho-meta coupled doublet at δ 6.62 and a multiplet located at δ 6.59 respectively. The proton H-A appeared as an ortho-meta coupled doublet located at δ 7.91 while the proton H-C was observed located at δ 7.29 as a doublet of triplet. The protons arising from the acetophenone ring system were located at δ 6.92 arising from the proton H-E as an ortho coupled doublet (J=9Hz) while the proton H-F was located at δ 7.48 as an ortho-meta coupled doublet (J=9Hz, 2Hz). The proton H-G was located at δ 7.75 as a meta coupled doublet (J=2Hz). The proton NH was located at δ 7.65 and a chelated hydroxyl group appeared as a singlet located at δ 12.15. These data are satisfactory for the above structure assigned to the compound.
It was further supported by the mass spectral data, which showed the molecular ion peak located at m/z 285 analysing for the molecular formula C_{16}H_{15}O_{4}N. Other important peaks could be located at m/z 241, 149, 135, 92 and 77. The fragmentation pattern is as follows.

```
    COOH  OH
   NH - CH2
     m/z 285

    OH
   H2C
     m/z 149

    OH
   H2C
     m/z 135

    NH - CH2
     m/z 241
```

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-sulfanilamide (XVIII) from (I)

To a hot mixture of sulfanilamide, sodium bicarbonate and water was added (I) in small portions with continuous stirring. Stirring was continued for another three hours. The product obtained was filtered, dried and crystallized from a mixture of methanol and acetone to give a colorless compound (XVIII) m.p. 194-96° (decomp.) It was TLC pure and gave a positive test for nitrogen and sulphur on sodium fusion test.

```
    (I) NaHCO3:H2O //
       sulfanilamide
          \     H2N-S
           \   NH-CH2
            \ (XVIII)
```

The IR spectrum showed the following absorption peaks at 3300 cm\(^{-1}\), 3150 cm\(^{-1}\) (b,NH+OH), 1620 cm\(^{-1}\) (CO), 1580, 1560 cm\(^{-1}\) (aromatic C=C str.), 1320 cm\(^{-1}\), 1160 cm\(^{-1}\) (S=O), 840 cm\(^{-1}\) (\(\rho\)-disubstituted ring CH bend).
The NMR spectrum of the compound showed two signals located at δ 2.63 and δ 4.3 in the aliphatic region, which could be due to acetyl and methylene functions. In the aromatic region it showed two doublets forming an A2B2 pattern located at δ 6.61 and δ 7.57 (J=9Hz) which could arise from the p-disubstituted phenyl ring. The protons H-A, H-B and H-C could be located as ortho coupled doublet (J=9Hz), meta-ortho coupled doublet (J=9Hz, 2Hz) and meta coupled doublet (J=2Hz) located at δ 6.89, δ 7.74 and δ 7.75. The proton of the NH moiety appeared as a triplet located at δ 6.5 while the proton from the chelated hydroxyl grouping was located at δ 12.0. These data are satisfactory for the structure assigned to the compound.

Attempted synthesis of N-(3-acetyl-4-hydroxy benzyl)-diclofenac (XIX) from (I)

Chloromethyl acetophenone (I) was added gradually in portions with continuous stirring to a hot mixture of diclofenac, sodium bicarbonate and water. Stirring was continued for another two hours. The mixture was extracted with ether and after usual work up was crystallized from methanol to give a crystalline compound m.p. 93-95°. It was found to match with the Rf value of (I) on TLC examination and gave a positive test for chlorine on sodium fusion test.

\[
\begin{align*}
\text{(I)} & \xrightarrow{\text{NaHCO}_3\cdot\text{H}_2\text{O}} \text{ClH}_2\text{C} \rightarrow \text{CH}_2\text{COOH} \\
& \text{|} \\
& \text{CH}_2\text{C} \rightarrow \text{CH}_2\text{COOH} \\
& \text{ClH}_2\text{C} \rightarrow \text{ClH}_2\text{C} \\
& \text{OH} \rightarrow \text{OH} \\
& \text{Cl} \rightarrow \text{Cl} \\
& \text{(XIX)} \end{align*}
\]

The IR spectrum showed the following bands at 3100 cm\(^{-1}\) (b,OH), 1620 cm\(^{-1}\) (CO), 1610, 1580 cm\(^{-1}\) (aromatic C=C str.), 800 cm\(^{-1}\) (C-Cl str.).

The NMR spectrum of the compound showed two singlets located δ 2.59 and δ 3.91 integrating for three protons and two protons which could be accounted for by the methyl and methylene groupings respectively. In the aromatic region the protons H-A, H-B and H-C could be picked up located at δ 6.93, δ 7.29 and δ 7.50 as ortho coupled doublet, ortho-meta coupled doublet...
doublet and meta coupled doublet respectively. The chelated hydroxyl function could be located at $\delta 12.16$. These data suggested that the reaction did not proceed satisfactorily and it is the starting ketone only.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-nimesulide (XX) from (I)

Chloromethyl acetophenone (I) was added gradually with continuous stirring in portions to a hot mixture of nimesulide, sodium bicarbonate and water. Stirring was continued for another two hours. A solid, which separated was filtered and crystallized from a mixture of methanol and acetone to give (XX) m.p. 116-18°. It gave a single spot on TLC examination and was found to contain nitrogen and sulphur on sodium fusion test.

\[ \begin{align*}
\text{Cl}2\text{C} & \quad \text{NaHCO}_3 / \text{H}_2\text{O} \quad \text{nimesulide} \\
\| & \quad \text{H}_3\text{CO}_2\text{S} - \text{N} - \text{CH}_2 \\
\text{O} & \quad \begin{array}{c}
(\text{I}) \\
(\text{XX})
\end{array}
\end{align*} \]

The IR spectrum showed the following absorption peaks at 3020 cm$^{-1}$ (NH+OH), 1650 cm$^{-1}$ (CO), 1620, 1580, 1530 cm$^{-1}$ (aromatic C=C str.), 1520 cm$^{-1}$ (NO$\text{O}_2$), 1340, 1320 cm$^{-1}$ (S=O/NO$\text{O}_2$), 1160, 1140 cm$^{-1}$ (S=O), 750, 690 cm$^{-1}$ (aromatic CH str.).

The NMR spectrum of the compound showed two singlets at $\delta 2.6$ and $\delta 4.86$ integrating for three protons and two protons respectively which may be accounted for by the methyl group of the acetyl function and methylene group of the benzyl moiety. The proton H-A appeared as an ortho coupled doublet ($J=9$Hz) located at $\delta 6.90$. The protons H-G, H-K and H-H, H-I, H-J appeared as two multiplets located at $\delta 7.06$ and $\delta 7.37$ respectively. The protons H-B and H-C of the acetophenone moiety could be picked up at $\delta 7.38$ and $\delta 7.625$ as double doublet and meta coupled doublet while the protons H-F, H-E and H-D appeared as ortho coupled doublet ($J=9$Hz) located $\delta 7.355$, $\delta 7.68$ as meta coupled doublet ($J=2$Hz) and $\delta 7.83$ ($J=2$Hz, 9Hz) as meta-ortho coupled doublet. These data are satisfactory for the structure assigned to the compound.
Synthesis of N-(3-acetyl-4-hydroxy benzyl)-2-aminopyridine (XXI) from (I)

To a hot mixture of 2-aminopyridine, sodium bicarbonate and water was added (I) gradually in small portions with continuous stirring. Stirring was continued for another two hours. The product obtained was filtered, dried and crystallized from methanol to give a crystalline compound (XXI) m.p. 128-30°. It was found to be TLC pure and gave a positive test for nitrogen on sodium fusion test.

The IR spectrum of the compound showed the following absorption peaks at 2950 cm⁻¹ (NH+OH), 1640 cm⁻¹ (CO), 1600, 1560 cm⁻¹ (aromatic C=C str.), 780, 760 cm⁻¹ (pyridine ring CH bend.).

The NMR spectrum of the compound showed two singlets located at δ 2.51 and δ 4.72 integrating for three protons and two protons, which could arise from the methyl function and methylene moiety present in the molecule. H-D in the aromatic region could be located as an ortho-meta coupled doublet at δ 6.47 while the proton H-F could be seen as a doublet of triplet located at δ 6.65 and the proton H-E also appeared as a doublet of triplet located at δ 7.43. The proton H-A appeared as an ortho coupled doublet (J=9Hz) located at δ 6.92. The proton H-C could be seen as a meta coupled doublet (J=3Hz) located at δ 7.55 and the proton H-B appeared as a double doublet located at δ 8.24. These data are satisfactory for the structure assigned to the compound.

The mass spectrum of the compound showed a molecular ion peak located at m/z 242 analyzing for the molecular formula C₁₄H₁₄O₂N₂. Other important peaks were picked up at m/z 164, 150, 136, 121 and 93. The fragmentation pattern is as follows.
Synthesis of S-(3-acetyl-4-hydroxy benzyl)-2- mercaptobenzothiazole (XXII) from (I)

A mixture of 2-mercaptobenzothiazole, sodium bicarbonate and water was heated as described in earlier cases. To this hot mixture was added (I) in small portions with continuous stirring. Stirring was continued for another four hours. A solid mass so separated was filtered, washed with water, dried and crystallized from a mixture of methanol and dichloromethane to give (XXII) m. p. 214-16°. It was found to be TLC pure and gave a positive test for nitrogen and sulphur on sodium fusion test.

The IR spectrum of the compound showed the following peaks at 3500 cm⁻¹ (b, OH) 1650 cm⁻¹ (CO), 1630, 1600 cm⁻¹ (aromatic ring C=C str.), 760 cm⁻¹ (benzothiazole ring CH bend.).

The NMR spectrum of the compound showed two singlets located at δ 2. 59 and δ 5.64 integrating for three protons and two protons which could arise from the methyl group of the acetyl function and S-CH₂ grouping. In the
aromatic region there was a doublet centered at \( \delta 6.95 \) (J=9Hz) which could arise from H-A. There was a doublet of triplet located at \( \delta 7.17 \) which may arise from H-G. Another doublet of triplet could be located at \( \delta 7.28 \) which could account for the proton H-D. A double doublet at \( \delta 7.36 \) (J=2Hz, 9Hz) could be due to the proton H-B. The proton H-C appeared as a meta coupled doublet located at \( \delta 7.88 \) (J=2Hz) while the protons H-E and H-F were seen as multiplet centered at \( \delta 7.49 \). These data are satisfactory for the above structure assigned to the compound.

Synthesis of N-phenyl-N'(3-acetyl-4-hydroxy benzyl)-thiourea (XXIII) from (I)

Chloromethyl acetophenone (I) was added gradually in small portions with continuous stirring to a hot mixture of phenylthiourea, sodium bicarbonate and water. Stirring was continued for another three hours. The reaction mixture was extracted with ether and after usual workup the residue was crystallized from methanol to give (XXIII) m. p. 192-94°. It was found to be TLC pure and gave a positive test for sulphur and nitrogen by sodium fusion method.

The NMR spectrum of the compound showed two singlets located at \( \delta 2.52 \) and \( \delta 4.53 \) arising from the protons of the acetyl and methylene functions. In the aromatic region the protons H-A, H-B and H-C of the acetophenone moiety showed signals at \( \delta 6.94 \) as a doublet (J=9 Hz) at \( \delta 7.35 \) as double doublet (J=2 Hz, 9 Hz) and \( \delta 7.52 \) as meta coupled doublet (J=2 Hz) respectively. The protons arising from the aniline moiety could be seen as two multiplets located at \( \delta 6.7 \) and \( \delta 7.22 \) which could be assigned to
H-D, F, H and H-E, G. These data are satisfactory in favor of the structure assigned to the compound.
ii) Synthesis of flavonoidal derivatives

Synthesis of 2'-hydroxy-5'-ethoxymethyl-4-methoxy chalcone (XXIV) from 5-chloromethyl-2-hydroxy acetophenone (I)

Chloromethyl acetophenone (I) was stirred in ethanolic potassium hydroxide solution with \( p \)-anisaldehyde for two hours and then refluxed on a water bath for another two hours. The mixture was poured onto crushed ice, acidified with concentrated hydrochloric acid and extracted with ether. The ethereal layer was dried over sodium sulphate and after filtering off the inorganic salt, ether was evaporated off to dryness. An oily mass obtained was dissolved in ether and petrol mixture and a liquid obtained as a residue after evaporation of the solvent was redissolved in ether/petrol mixture which was again evaporated off and the process of redissolution and evaporation repeated several times till a pure red colored liquid was obtained which was TLC pure. It gave a positive test for chalcone and a negative test for chlorine suggesting structure (XXIV). The NMR spectrum of the compound could not be recorded, however the formation of flavone (XXVI) from this chalcone confirmed successful formation of (XXIV).

\[
\begin{align*}
\text{(I)} & \xrightarrow{\rho \text{-anisaldehyde} \ K\text{OH/}Et\text{OH}} \text{(XXIV)} \\
\text{ClH}_2\text{C} & \text{OH} \quad \text{H}_2\text{C}_2\text{OH}_2\text{C} \\
\text{O} & \text{O}\text{CH}_3
\end{align*}
\]

In the U.V. spectrum it showed absorption maxima at 225, 256 and 332 nm.

Synthesis of 2'-hydroxy-5' ethoxymethyl-3, 5-methylenedioxy chalcone (XXV) from (I)

A mixture of (I), piperonal and oxygen free ethanol was stirred in the presence of potassium hydroxide solution followed by refluxing as described in the above cases. The mixture was processed as usual and crystallized from a mixture of petrol and ether to yield a yellow crystalline compound m. p. 65-67°. It was found to be TLC pure and gave a red color with one drop of concentrated sulfuric acid suggesting successful chalcone formation.
In the U.V. spectrum it showed two absorption maxima at 260.38 and 375.30 and an inflexion at 343.96 nm.

The NMR spectrum of the compound showed successful formation of the chalcone, however the CH$_2$Cl group of the acetophenone moiety got converted into the CH$_2$OC$_2$H$_5$ system which was evident by the chemical shifts present in the aliphatic region of the spectrum. There was a triplet located at $\delta$ 1.27 and a quartet located at $\delta$ 3.57 integrating for three protons and two protons respectively accounting for the ethyl function. There were two singlets located at $\delta$ 4.48 and $\delta$ 6.05, which could arise from the protons of CH$_2$ of the benzyl moiety and CH$_2$ of the methylenedioxy function. A singlet in the most downfield region located at $\delta$ 12.93 could arise from the chelated hydroxyl group. In the aromatic region there could be picked up two ortho coupled doublets ($J$=9Hz) located at $\delta$ 6.86 and $\delta$ 7.0 which could arise from the protons H-B and H-D. The proton H-C was located at $\delta$ 7.2 as a meta coupled doublet. The protons H-A and H-E could be seen as ortho-meta coupled doublets centred at $\delta$ 7.16 and $\delta$ 7.48. The proton H-F merged with the $\beta$ proton of the chalcone system and was located at $\delta$ 7.85. The proton H-$\alpha$ and H-$\beta$ of the chalcone system were located at $\delta$ 7.49 and $\delta$ 7.85 ($J$=15 Hz). These data are satisfactory for the above structure assigned to the compound.

**Synthesis of 4'-methoxy-6-ethoxymethyl flavone (XXVI) from (XXIV)**

Chalcone (XXIV) was refluxed with dry isoamyl alcohol in presence of selenium dioxide on a heating mantle for 48 hours under anhydrous conditions. After cooling the contents to room temperature, a black insoluble mass was filtered off and the filtrate evaporated to dryness. The residue was crystallized from a mixture of ether and chloroform to give a crystalline compound m.p.126-30° which was TLC pure and gave a pink color with Mg/HCl and showed a yellowish green fluorescence under U.V. light.
In the U.V. spectrum it showed maxima at 222, 258 and 321 nm.

The NMR spectrum of the compound showed a triplet and a quartet indicating the presence of one methyl function and one methylene group arising from ethyl function which were located at δ 1.26 and δ 3.57. There was another singlet located at δ 3.9, which could arise from the methoxyl function of the side phenyl ring. Another singlet located at δ 4.6 could arise from the methylene function attached to the A ring. A singlet accounting for one proton located at δ 6.7 could arise from H-3 of the flavone moiety. There were two doublets centered at δ 7.0 and δ 7.85 (J=8Hz) which accounted for the A2B2 pattern of the paramethoxy phenyl ring. The other important signal in the form of multiplets could be located at δ 7.55, δ 7.73 and δ 8.15, which satisfactorily accounted for protons H-8, H-7 and H-5. These data are satisfactory for the structure assigned to the compound.

Synthesis of 3', 4'-methylenedioxy-6-ethoxy methyl flavone (XXVII) from chalcone (XXV)

A solution of chalcone (XXV) in dry isoamyl alcohol was refluxed in the presence of selenium dioxide on a heating mantle under anhydrous conditions for forty eight hours. After usual workup the residual mass was crystallized from a mixture of methanol and chloroform to give the flavone (XXVII) m.p. 158-60°. It was TLC pure and gave a yellow fluorescence in UV light and a pink color with Mg/HCl.
In the U.V. spectrum it showed maxima at 210, 252 and 293 nm.

The NMR spectrum of the compound showed a triplet and a quartet located at δ 1.26 and δ 3.57 which could arise from the methylene and methyl groupings of the ethoxy moiety. The other methylene function attached to the A-ring was located as a singlet at δ 4.59. The methylenedioxy group was located as a singlet at δ 6.07. H-3 of the flavone moiety appeared as a singlet at δ 6.7. The protons of the A ring appeared as two doublets located at δ 7.4 (J=8Hz), δ 8.1 (J=2Hz) which could arise from H-8 and H-5. H-7 appeared as a multiplet located at δ 7.7. The protons of the side phenyl ring appeared as a multiplet located at δ 6.9 and δ 7.5, the former arising from H-5' and the latter from H-2',6'. These data confirmed that the compound has the structure as shown above.

Synthesis of 6-(o-toluidino methyl) flavanone (XXVIII) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III)

To a stirred solution of (III) and benzaldehyde in oxygen free ethanol was added aqueous potassium hydroxide. Stirring was continued for two hours followed by refluxing the contents for three hours. The reaction mixture was processed and extracted with ether as usual. A semisolid residue obtained after evaporating off ether was dissolved in a mixture of petrol and ether which was evaporated and this process repeated as described in an earlier case till a red colored mass which separated was found to be TLC pure. It did not solidify even at low temperatures (0-5°).

In the U.V. spectrum it showed a maximum at 324 and an inflexion at 242.45 nm.

The NMR spectrum of the compound showed a singlet integrating for three protons located at δ 2.18 which may be due to the methyl function of o-toluidine moiety. The methylene function of the benzyl ring was located as a singlet at δ 4.3. By the appearance of two multiplets centred at δ 5.3 arising from CH function at C-2 and another multiplet centred at δ 3.48, arising from proton of CH₂ group at C-3, it could be ascertained that the present compound exists in the cyclised form as flavanone. The protons of the o-toluidine ring
In the U.V. spectrum it showed maxima at 210, 252 and 293 nm.

The NMR spectrum of the compound showed a triplet and a quartet located at δ 1.26 and δ 3.57 which could arise from the methylene and methyl groupings of the ethoxyl moiety. The other methylene function attached to the A-ring was located as a singlet at δ 4.59. The methylenedioxy group was located as a singlet at δ 6.07. H-3 of the flavone moiety appeared as a singlet at δ 6.7. The protons of the A ring appeared as two doublets located at δ 7.4 (J=8Hz), δ 8.1 (J=2Hz) which could arise from H-8 and H-5. H-7 appeared as a multiplet located at δ 7.7. The protons of the side phenyl ring appeared as a multiplet located at δ 6.9 and δ 7.5, the former arising from H-5' and the latter from H-2',6'. These data confirmed that the compound has the structure as shown above.

Synthesis of 6-(o-toluidino methyl) flavanone (XXVIII) from N-(3-acetyl-4-hydroxy benzyl)-o-toluiciine (III)

To a stirred solution of (III) and benzaldehyde in oxygen free ethanol was added aqueous potassium hydroxide. Stirring was continued for two hours followed by refluxing the contents for three hours. The reaction mixture was processed and extracted with ether as usual. A semisolid residue obtained after evaporating off ether was dissolved in a mixture of petrol and ether which was evaporated and this process repeated as described in an earlier case till a red colored mass which separated was found to be TLC pure. It did not solidify even at low temperatures (0-5°).

\[
\text{(III)} \xrightarrow{\text{benzaldehyde, KOH/EtOH}} \text{(XXVIII)}
\]

In the U.V. spectrum it showed a maximum at 324 and an inflexion at 242.45 nm.

The NMR spectrum of the compound showed a singlet integrating for three protons located at δ 2.18 which may be due to the methyl function of o-toluidine moiety. The methylene function of the benzyl ring was located as a singlet at δ 4.3. By the appearance of two multiplets centred at δ 5.3 arising from CH function at C-2 and another multiplet centred at δ 3.48, arising from proton of CH\textsubscript{2} group at C-3, it could be ascertained that the present compound exists in the cyclised form as flavanone. The protons of the o-toluidine ring...
along with the protons H-7, H-8 appeared as a multiplet located between $\delta$ 6.6 and $\delta$ 7.26. The protons H-3', H-4', H-5' could be seen at $\delta$ 7.08 in the form of a multiplet while the protons H-2', 6' were seen as a multiplet centred at $\delta$ 7.86. The proton H-5 was located as a meta coupled doublet centred at $\delta$ 7.86. These data are satisfactory for the structure assigned to the compound.

Synthesis of 4'-methoxy-6-(o-toluidino methyl) flavanone (XXIX) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III).

To a stirred solution of (III) and $p$-anisaldehyde in oxygen free ethanol was added a solution of potassium hydroxide in oxygen free water. The reaction conditions and processing method were exactly the same as described above. The residue was purified by using column chromatography to get (XXIX) by eluting the column with a mixture of petrol and benzene (50:50). It was found to be TLC pure.

\[
\text{OCH}_3
\]

In the U.V. spectrum it showed absorbance maximum at 368.04 and an inflexion at 240 nm.

The NMR spectrum of the compound in aliphatic region showed three singlets located at $\delta$ 2.16, $\delta$ 3.28 and $\delta$ 4.33 integrating for three, three and two protons which could arise from methyl function of the o-toluidine moiety, methoxyl function and methylene grouping respectively. A multiplet at $\delta$ 5.3 (H-2) and another multiplet at $\delta$ 3.5 (H-3) were picked up indicating that the compound formed is a flavanone. In the aromatic region there could be seen absorptions in the following regions which are assigned accordingly.
δ 6.59: d (J=9Hz) H-8
δ 6.69: m H4”
δ 7.04: m H-3”, H-5”, H-6”
δ 7.38: d (A2/B2 J =9Hz) H-3’, 5’
δ 7.52: dd (J = 2Hz, 9Hz) H-7
δ 7.91: d (J = 2Hz) H-5

Synthesis of 4’-hydroxy-3’-(3,4-dimethoxy-cinnamoyl)-benzyl o-toluidine (XXX) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III).

A solution of (III) and veratraldehyde was condensed in ethanolic potassium hydroxide by the method as described earlier. The reaction mixture was processed as usual. A solid mass, which separated out was crystallized from methanol to yield orange crystals m. p. 102-04°. It was TLC pure and developed a red color on adding one drop of concentrated sulphuric acid.

\[
\begin{array}{c}
\text{(III)} \\
\xrightarrow{\text{veratraldehyde}} \\
\xrightarrow{\text{KOH/EtOH/Δ}} \\
\xrightarrow{\text{XXX}}
\end{array}
\]

In the U.V. spectrum it showed maxima at 215.11, 378.89 and an inflexion at 244.15 nm.

The NMR spectrum of the compound showed signals located at δ 2.18, as a singlet, integrating for three protons, two closely spaced singlets located at δ 3.95 integrating for six protons and a singlet at δ 4.35 integrating for two protons, which could arise from the methyl group of o-toluidine moiety, methoxyl protons of veratraldehyde moiety and methylene function respectively. In the aromatic region there was a double doublet for the proton ortho to nitrogen located at δ 6.65 (J=2Hz, 9 Hz). There was a doublet of triplet centred at δ 6.7, which could arise from the proton para to nitrogen. There was a doublet centred at δ 6.85 (J=9Hz) which could arise from proton H-D. Another doublet (J=9Hz) located at δ 7.2 could arise from H-C. The proton H-A appeared as a meta coupled doublet (J=2Hz) centred at δ 7.13. The proton ortho to methyl function in the ortho toluidine moiety plus the
proton H-E could be located at δ 7.24. The proton para to methyl function was located as a multiplet centered as δ 7.10. The proton H-B appeared as a double doublet (J=2Hz, 9Hz), and was located at δ 7.52. The protons H-α and H-β of the chalcone system could be picked up located at δ 7.44 and δ 7.6 (J=15 Hz). The signal of the proton H-I partly merged with the signal of H-β and could be seen as a meta coupled doublet (J=2 Hz) located at δ 7.91. The proton of the chelated hydroxyl group appeared as a singlet located at δ 12.89. These data are satisfactory for the structure assigned to the compound.

**Synthesis of 4'-hydroxy-3' (3,4-methylenedioxy-cinnamoyl)-benzyl-α-toluidine (XXXI) from N-(3-acetyl-4-hydroxy benzyl) α-toluidine (III)**

A mixture of (III) and piperonal in oxygen free ethanol was stirred and refluxed as above after addition of an aqueous potassium hydroxide solution. The mixture was poured onto crushed ice and acidified with concentrated hydrochloric acid. A solid mass, which separated out was filtered and purified using column chromatography to get IX (chalcone) m.p. 128-30° (column was run with 70:30 petrol:benzene mixture). The compound isolated was found to be TLC pure and gave a positive test for nitrogen by sodium fusion method.

![Chemical structure](image)

In the U.V. spectrum it showed two maximum at 259.05 and 295.02 nm.

The NMR spectrum of the compound showed a singlet integrating for three protons located at δ 2.18 which could arise from the methyl group, another singlet located at δ 4.36 integrating for two protons may arise from the methylene function adjacent to the NH grouping. There was another singlet located at δ 6.04 integrating for two protons arising from the methylene function of the methylene dioxy group. In the aromatic region there was a double doublet (ortho and meta coupled) centered at δ 6.65 which may be due to proton H-J. The proton H-H appeared as a doublet of triplet centered at δ 6.74 while the protons H-G and H-I appeared as a multiplet along with the protons H-A, H-D and H-E centred at δ 7.12. The proton H-C appeared as an
ortho coupled doublet (J = 9Hz) centred at δ 7.01 and H-B appeared as an ortho-meta coupled doublet (J = 9Hz, 2Hz) located at δ 7.51. H-F could be seen as meta coupled doublet located at δ 7.88 (J=2Hz). α and β protons of the chalcone moiety appeared as doublets (J=10Hz) located at δ 7.39 and δ 7.82. The chelated hydroxyl function was located as a singlet at δ 12.80. These data satisfactorily explained formation of the above chalcone.

The mass spectrum of the compound showed a molecular ion peak located at m/z 387 analysing for the molecular formula C_{24}H_{21}O_{4}N. Other important peaks could be picked up at m/z 281, 149, 133, 107, and 77. The fragmentation pattern is as follows.

![Fragmentation Pattern](image)

Synthesis of 1-[2-hydroxy 5-(o-toluidino-methyl)-phenyl]3-β-thenyl-2-propen-1-one (XXXII) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III).

To a solution of (III) and thiophene-2-aldehyde in oxygen free ethanol was added dropwise and with stirring a solution of potassium hydroxide in oxygen free water. Stirring was continued for two hours followed by refluxing for another five hours on a water bath. The contents were processed and extracted with ether as usual. A reddish oily mass obtained was dissolved in a mixture of petroleum ether and ether, the solvent was evaporated off and the dissolution and evaporation process repeated several times as in earlier cases till the mass was found to be TLC pure and gave a red color with concentrated sulfuric acid.
In the U.V. spectrum it showed two maximum 242.82 and 354.55 nm and an inflexion at 280.0 nm.

The NMR spectrum showed a signal for the methyl group at $\delta$ 2.18 and a signal for a methylene group at $\delta$ 4.36. In the aromatic region signal for the two $\beta$ protons of the thiophene moiety appeared as a doublet (H-A) at $\delta$ 6.65 and a triplet at $\delta$ 6.7 (H-B) while the $\alpha$-proton H-C appeared as a doublet at $\delta$ 7.47. A doublet at $\delta$ 8.05 could be assigned to $\beta$ proton of the chalcone moiety. A doublet at $\delta$ 7.0 and a double doublet at $\delta$ 7.53 could be assigned to proton H-D and H-E while the proton H-F appeared as a doublet at $\delta$ 7.87. The remaining signals in the spectrum at $\delta$ 7.15 and $\delta$ 7.4 could not be analyzed in detail but are consistent for the four protons of the toluidine ring, H-C and H-$\alpha$ of the chalcone moiety. These data prove that the compound is the expected product.

**Attempted synthesis of 4'-hydroxy-3'-cinnamoyl-benzyl-o-anisidine (XXXIII) from N-(3-acetyl-4-hydroxy benzyl) o-anisidine (V).**

A mixture of (V) and benzaldehyde was stirred and refluxed in ethanolic potassium hydroxide solution as described in earlier cases. The contents after cooling to room temperature were poured into ice cold water, acidified and extracted with ether as usual to give a red colored mass which was found to be a mixture on TLC examination. It gave a red color with a drop of concentrated sulfuric acid indicating successful chalcone forming, however, it could not be purified and hence further studies was abandoned.
Attempted synthesis of 4'-hydroxy-3'-(p-methoxy-cinnamoyl) benzyl-o-anisidine (XXXIV) from N-(3-acetyl-4- hydroxy benzyl)-o-anisidine (V)

To a solution of (V) and p-anisaldehyde in oxygen free ethanol was added a solution of potassium hydroxide in oxygen free water, dropwise and with stirring. Stirring was continued for two hours followed by refluxing the contents on a water bath for five hours. A semisolid mass was obtained by usual work up mentioned in similar cases above. It gave a red color on treatment with one drop of concentrated sulfuric acid indicating chacone formation but was found to be a complex mixture on TLC examination and could not be purified, hence further studies to characterize the compound was not carried out.

\[ \text{(V)} \xrightarrow{\text{anisaldehyde, } \text{KOH/EtOH/\Delta}} \text{(XXXIV)} \]

Synthesis of 4'-hydroxy-3'-(3,4-dimethoxy-cinnamoyl)-benzyl-o-anisidine (XXXV) from N-(3-acetyl-4- hydroxy benzyl)-o-anisidine (V)

A solution of (V) and veratraldehyde in oxygen free ethanol was stirred and refluxed in ethanolic potassium hydroxide as described above. The reaction mixture was processed by usual workup as mentioned in earlier cases to give a TLC pure compound, m. p. 110-12°. It gave a red color on treatment with one drop of concentrated sulfuric acid.

\[ \text{(V)} \xrightarrow{\text{veratraldehyde, } \text{KOH/EtOH/\Delta}} \text{(XXXV)} \]
In the U.V. spectrum it showed an absorption maximum at 248.61 and an inflexion at 280 nm.

The NMR spectrum of the compound showed three separate singlets located at $\delta$ 3.85 integrating for nine protons arising from methoxyl functions and one singlet integrating for two protons located at $\delta$ 4.29 arising from the methylene function. The proton H-A appeared as a metacoupled doublet located at $\delta$ 7.74. The protons H-2, H-6 of the side phenyl ring appeared as a doublet (J=9 Hz) located at $\delta$ 7.49 while H-$\beta$ of the chalcone moiety appeared as a doublet (J=15 Hz) and located at $\delta$ 7.88. The rest of the protons i.e. H-B, H-C, H-D, H-E, H-F, H-G, H-$\alpha$ and H-5 appeared as a complex multiplet located between $\delta$ 6.57 and $\delta$ 7.00 integrating for eight protons. These data are satisfactory for the above structure assigned to the compound.

Synthesis of 4'-hydroxy-3' (3,4-methylenedioxy-cinnamoyl)-benzyl-o-anisidine (XXXVI) from N-(3-acetyl-4-hydroxy benzyl)-o-anisidine (V)

A solution of potassium hydroxide in water was added dropwise to a stirred solution of (V) and piperonal in oxygen free ethanol. The mixture was stirred for two hours followed by refluxing for another four hours. The contents were processed as usual. A solid mass obtained was crystallized from methanol to give TLC pure crystals of (XXXVI) m. p. 88-90°. It gave a red color on treatment with a drop of concentrated sulfuric acid indicating successful chalcone formation.

In the U.V. spectrum it showed the following maximum at 245.24 and 436.96 nm.

The NMR spectrum of the compound showed two singlets in the high field region located at $\delta$ 3.8 and $\delta$ 4.3 arising from methoxyl and methylene groupings. There was another singlet located at $\delta$ 6.03 which could arise from the protons of the methylene dioxy group. In the low field region there was a double doublet located at $\delta$ 6.64 which could arise from the proton H-G. H-I appeared as a triplet located at $\delta$ 6.7 while the protons H-C, H-H and H-J could be seen as a multiplet centered at $\delta$ 6.82. The proton H-F could be seen...
as an ortho coupled doublet located at $\delta$ 6.9 (J=8 Hz), while the protons H-E and H-D could be seen as a multiplet located at $\delta$ 7.12. The proton H-B could be picked up as a double doublet (J=3Hz, 8Hz) located at $\delta$ 7.49 while the proton H-A was picked up in the extreme downfield region located at $\delta$ 7.87 as a meta coupled doublet (J = 2Hz). The olefinic protons H-α and H-β of the chalcone system were located as doublets at $\delta$ 7.38 (J=15 Hz) and $\delta$ 7.79 (J=15 Hz). These data are satisfactory for the above structure assigned to the compound.

Attempted synthesis of 1-[2-hydroxy-5-(o-anisidinomethyl) phenyl]-3-β-thienyl-2-propen-1-one (XXXVII) from N-(3-acetyl-4- hydroxy benzyl)-o-anisidine (V)

A solution of (V) and thiophene-2-aldehyde in ethanolic potassium hydroxide solution was stirred for two hours followed by refluxing the contents for another five hours. The reaction mixture was processed as usual and extracted with ether. After evaporating the ether a red colored compound was obtained which was found to be a complex mixture on TLC examination and could not be purified. Hence, further characterization studies were abandoned.

\[
\text{(V)} \xrightarrow{\text{thiophene-2-aldehyde,} \text{KOH/EtOH/Δ}} \text{(XXXVII)}
\]
(b) Exploitation of ω-Bromoacetophenone nucleus

i) Synthesis of phenacylamine derivatives

**Synthesis of ω-Bromoacetophenone (XXXVIII)**

Bromine was added gradually dropwise to a mixture of acetophenone and acetic acid with constant stirring. Sufficient heat was generated upon addition of each lot of bromine. The contents were cooled to room temperature and poured into crushed ice. A solid mass, which separated out was filtered, dried and crystallized from ethanol to give a colorless crystalline compound (XXXVIII) m. p. 50° (lit. m. p. 50°). On TLC examination it showed a single spot and gave a positive test for bromine on sodium fusion test.

*(a) Reid, R., J. Am. Chem. Soc., 41, 77 (1919)*

*(b) Davidson, C., Org. Syn., 19, 24 (1939)*

![Chemical Structure](image)

Attempted synthesis of N-phenacylaniline (XXXIX) from (XXXVIII)

A mixture of aniline, sodium bicarbonate and water was heated on a hot plate at 50-60°. To this hot mixture was added (XXXVIII) in small portions over a period of ten minutes with stirring which was continued for another two hours. The contents were extracted with ether, the ethereal layer was washed with water and dried over sodium sulphate. After filtering the inorganic salts, ether was evaporated to dryness and the residue crystallized from ethanol to give a compound, m. p. 180-82°. It gave two spots on TLC examination and a positive test for nitrogen by sodium fusion method.
In IR spectrum it showed the following peaks 3375 cm⁻¹ (NH), 1623 cm⁻¹ (CO), 1520, 1470 cm⁻¹ (C=C aromatic str.), 765, 705 cm⁻¹ (phenyl ring CH bend).

The NMR spectrum showed two doublets of equal intensity at δ 4.7 and δ 5.6 besides a singlet at δ 5.0. Further the aromatic region showed a number of multiplets which suggested that the compound is the mixture of two entities. Further analysis suggested that the product could be a mixture of A and B in the ratio of 3:2 on the basis of signals at δ 5.0 and δ 5.6.

Synthesis of N-phenacyl-o-toluidine (XL) from (XXXVIII)

To a hot mixture of o-toluidine, sodium bicarbonate and water was added (XXXVIII) gradually with continuous stirring as stated above. The reaction mixture was processed and extracted with ether as usual. The residue was crystallized from methanol to give yellow crystals of (XL) m. p. 166-68°. It was TLC pure and gave a positive test for nitrogen by Lassaigne’s test.

In the IR spectrum the following absorption peaks could be located at 3375 cm⁻¹ (NH), 3150-3050 cm⁻¹ (aromatic C-H str.), 1660 cm⁻¹ (CO), 1520,
1480, 1440 cm\(^{-1}\) (C=C aromatic str.) 740, 720 cm\(^{-1}\) (o-toluidine ring C-H bend)

The NMR spectrum of the compound showed a singlet located at \(\delta\) 2.27 and another singlet located at \(\delta\) 4.61 arising from the methyl group and the methylene moiety of the molecule. In the aromatic region there was a multiplet located at \(\delta\) 6.59 arising from the proton H-3', 6'. The proton H-5' appeared as a doublet of triplet located at \(\delta\) 6.7 while the proton H-4' could be located as a doublet of triplet located at \(\delta\) 7.15. The protons H-3, H-4, H-5 appeared as a complex multiplet located at \(\delta\) 7.5 while the protons H-2, H-6 were located at \(\delta\) 8.02. These data are satisfactory for the structure assigned to the compound.

The above structure was further supported by the mass spectral data which showed the molecular ion peak located at m/z 225 which analyzed for the molecular formula \(\text{C}_{15}\text{H}_{15}\text{ON}\). The other important peaks were located at m/z 120, 91 and 77. The fragmentation pattern is given below.

![Diagram of fragmentation pattern](image)

**Synthesis of N-phenacyl-p-toluidine (XLI) from (XXXVIII)**

To a mixture of \(\rho\)-toluidine, sodium bicarbonate and water was added (XXXVIII) maintaining the same reaction conditions throughout as described above. Upon usual work up and crystallization from methanol, (XLI) m. p. 158-60° was obtained which was TLC pure and gave a positive test for nitrogen on sodium fusion test.
In the IR spectrum, the following peaks could be picked up at 3050 cm⁻¹ (NH), 3000-2925 cm⁻¹ (C-H aromatic str.), 1600 cm⁻¹ (CO), 1510, 1440 cm⁻¹ (aromatic C=C str.), 810, 740 cm⁻¹ (p-di substituted ring C-H bend).

The NMR spectrum showed two singlets located at δ 2.3 and δ 4.7 integrating for three protons and two protons arising from methyl and methylene groups. A broad singlet located at δ 4.3 may arise from NH grouping. In the aromatic region there were two doublets located at δ 6.8 and δ 7.2 forming an A2/B2 pattern, which may arise from the protons of the p-disubstituted ring. The protons of the benzoyl ring showed two multiplets located at δ 7.6 and δ 8.2 integrating for three and two protons, the latter may arise from the protons ortho to the carbonyl ring and the former from the remaining three protons of this ring. These data are satisfactory for the structure assigned to the compound.

The structure was further supported by the mass spectral data, which showed the molecular ion peak located at m/z 225, analyzing for the molecular formula C₁₅H₁₅ON. The other important peaks could be picked up at m/z 120, 105, 91 and 77. The fragmentation pattern has been given below.

Attempted synthesis of N-phenacyl-o-anisidine (XLII) from (XXXVIII)

To a mixture of o-anisidine, sodium bicarbonate and water was added (XXXVIII) maintaining reaction conditions as described in earlier cases. The mixture was processed as usual and residue crystallized from methanol to
give crystals m. p. 136-38°, which were found to be mixture on TLC examination and gave a positive test for nitrogen by sodium fusion method. Since this product could not be purified inspite of several attempts and hence further studies were abandoned.

![Chemical structure of compound](image)

**Synthesis of N-phenacyl-p-anisidine (XLIII) from (XXXVIII)**

$p$-Anisidine was condensed with (XXXVIII) in presence of sodium bicarbonate and water under reaction conditions as stated earlier. On usual processing a residual mass obtained was crystallized from methanol to give a crystalline compound (XLIII) m. p. 170-74°. It was TLC pure and gave a positive test for nitrogen by sodium fusion method.

![Chemical structure of compound](image)

In the IR spectrum the following peaks could be picked up 3100 cm⁻¹ (NH), 3000-2950 cm⁻¹ (aromatic C-H str.), 1620 cm⁻¹ (CO), 1540, 1480 cm⁻¹ (aromatic C=C str.), 840, 780 cm⁻¹ (p-di substituted ring C-H bend).

The NMR spectrum of the compound showed two singlets located at δ 3.7 and δ 4.9, which could arise from the protons of methoxyl and methylene functions. In the low field region there were two doublets forming an A2/B2 system located at δ 6.54 and δ 6.75 arising from the $p$-disubstituted phenyl ring. A multiplet located at δ 8.01 may arise from the two protons ortho to the carbonyl function while the remaining three protons appeared as two multiplets centred at δ 7.5 and δ 7.6. These data are satisfactory for the structure assigned to the compound.
Attempted synthesis of N-phenacyl-o-nitroaniline (XLIV) from (XXXVIII)

Compound (XXXVIII) was added to a mixture of o-nitroaniline, sodium bicarbonate and water maintaining similar reaction conditions of heating etc. as mentioned in earlier cases. On usual processing and crystallization from methanol, a compound m. p. 75-77° was obtained. It showed two spots on TLC examination and gave a positive test for nitrogen by sodium fusion method. Inspite of several attempts it could not be purified and hence further studies were abandoned.

\[
\begin{align*}
\text{(XXXVIII)} & \rightarrow \text{NaHCO}_3/\Delta \\
\text{o-nitroaniline} & \rightarrow \text{(XL IV)}
\end{align*}
\]

Synthesis of N-phenacyl-m-nitroaniline (XLV) from (XXXVIII)

m-Nitroaniline was condensed with (XXXVIII) in presence of sodium bicarbonate and water under reaction conditions as described above. Upon usual work up of the reaction mixture and crystallization from methanol, compound (XLV) m. p. 62-64° was obtained which was TLC pure and gave a positive test for nitrogen by sodium fusion method.

\[
\begin{align*}
\text{(XXXVIII)} & \rightarrow \text{NaHCO}_3/\Delta \\
m\text{-nitroaniline} & \rightarrow \text{(XLV)}
\end{align*}
\]

The IR spectrum showed the presence of following peaks 3375 cm\(^{-1}\) (NH), 1620 cm\(^{-1}\) (CO), 1530, 1340 cm\(^{-1}\) (aromatic C=C str.), 1220, 1000 cm\(^{-1}\) (m-nitroaniline ring C-H bend), 850, 750, 730 cm\(^{-1}\) (m-nitroaniline ring C-H bend).

The NMR spectrum of the compound showed a singlet located at \(\delta 5.2\) which accounted for the methylene group. In the aromatic region there could be seen two closely spaced multiplets located at \(\delta 7.7\) and \(\delta 8.0\) which could arise from the protons H-2', 5', 6' and H-3, H-4 and H-5. The proton H-4', could be located as a multiplet centred at \(\delta 8.5\) while the protons H-2, H-6
appeared as a multiplet centred at $\delta$ 8.6. These data are satisfactory for the structure assigned to the above compound.

The mass spectrum of the compound showed the molecular ion peak located at m/z 256 analysing for the molecular formula $\text{C}_{14}\text{H}_{12}\text{O}_{3}\text{N}_2$. Other important peaks could be located at m/z 151, 105 and 77. The fragmentation pattern has been shown below.
EXPERIMENTAL

(a) Exploitation of 5-Chloromethyl-2-hydroxy acetophenone nucleus to synthesize pharmaceutically important compounds.

i) Synthesis of benzyl anilines and miscellaneous compounds

Synthesis of 2-hydroxy-5-chloromethyl acetophenone (I)

Hydrochloric acid gas was generated by pouring dropwise sulfuric acid (conc.) on sodium chloride (sulfuric acid was taken in a 2 lit. separating funnel which was fitted on the mouth of a 2 lit. filtration flask which contained sodium chloride – details given below) and passed into a suspension of o-hydroxy acetophenone (40.0 g = 35.39ml; 0.29mole), 40% aqueous formaldehyde (26.5g = 26.5ml; 0.88 mole) and 37% hydrochloric acid (230g = 230ml) for ten hours with continuous stirring of the contents. The temperature was maintained below 15° throughout the reaction. A product, which separated out was poured into water (2.0 lit.) and filtered. It was washed with water and air-dried. In order to purify, it was extracted with petroleum ether (80ml). The extract on concentration and cooling gave colorless needles of the desired
product, 2-hydroxy-5-chloromethyl acetophenone (I) (24.0g; 44.23%) m.p. 93-95° (lit. m. p. 94-95°).

It gave a single spot on TLC examination (irrigant 'a'). On Lassaigne's test, the sodium extract after acidification with nitric acid and addition of silver nitrate gave a white precipitate of silver chloride which readily dissolved in ammonia.


Generation of Hydrochloric acid gas:

In a 2.0 lit. filtration flask, a dropping funnel was fitted with the help of a rubber cork at its mouth and the side tube was connected through a catch to the reaction mixture. Sodium chloride 99% was introduced into the filtration flask which was moistened with hydrochloric acid and through the dropping funnel sulfuric acid (conc.) was added dropwise over sodium chloride at the rate of about 20 drops per minute which generated hydrochloric acid gas.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)- aniline (II) from (I)

A mixture of aniline (0.5g = 0.49ml; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated on the hot plate maintaining the temperature between 90-100°. To this hot mixture was added I (1.0 g; 5.4 m mol) in small portions with continuous stirring during a period of fifteen minutes. Stirring was continued for two hours. A solid product obtained in the end was filtered, washed with water to remove sodium bicarbonate and air dried. It was dissolved in methanol and filtered. The filtrate on concentration and leaving at room temperature gave a crystalline compound II (0.9 g; 69.2%), m. p. 186-90°. It was found to be pure on TLC examination (irrigant 'a') and gave a positive test for nitrogen on sodium fusion test.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III) from (I)

A mixture of o-toluidine (0.58g = 0.57 ml, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated on a hot -plate maintaining the temperature conditions as stated above. To this hot mixture was added I (1.0g; 5.4 m mol) in small portions with continuous stirring during a period of fifteen minutes. Stirring was continued for another two hours. A solid mass which separated out was filtered, washed with water to remove sodium bicarbonate and crystallized from methanol to give a cream colored TLC (irrigant 'a') pure crystalline compound III (1.1g; 70.71%) m. p. 104-06°. It gave a positive test for nitrogen on sodium fusion test.
Synthesis of N-(3-acetyl-4-hydroxy benzyl)-p-toluidine (IV) from (I)

A mixture of p-toluidine (0.58g; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as described above. To this hot mixture was added I (1.0g; 5.4 m mol) in small portions with continuous stirring during a period of ten minutes. Stirring was continued for another three hours. After completion of the reaction, the contents were extracted with ether. The ethereal layer was washed with water, dried over anhydrous sodium sulphate and filtered. It was evaporated to dryness and the residue dissolved in a mixture of petroleum ether and diethyl ether and filtered. The filtrate on concentration and leaving at room temperature gave a crystalline compound IV (0.4g; 28.98%) m. p. 188-90° which gave a single spot on TLC examination (irrigant ‘a’). It also gave a positive test for nitrogen by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-anisidine (V) from (I)

A mixture of o-anisidine (0.67g = 0.6 ml; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated maintaining the temperature conditions as stated above. To this hot mixture was added I (1.0g; 5.4 m mol) in small portions during a period of ten minutes with continuous stirring. Stirring was continued for another two hours. A solid mass so obtained was filtered, washed with water to remove sodium bicarbonate and crystallized from methanol to give a buff colored compound V (1.1g, yield 75.3%) m. p. 86-88°. It gave a single spot on TLC examination (irrigant ‘a’). On Lassaigne’s test it gave a positive test for nitrogen.

Attempted synthesis of N-(3-acetyl-4-hydroxy benzyl)-p-anisidine (VI) from (I) which resulted in the formation of ketimine (VI a)

A mixture of p-anisidine (0.67g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated in small portions with continuous stirring during a period of ten minutes. Stirring was continued for another two hours. After completion of the reaction, the contents were extracted with diethyl ether. The ethereal layer after washing with water and drying over anhydrous sodium sulphate was evaporated to dryness. The residue was crystallized from a mixture of petroleum ether and diethyl ether to give greenish needles of VI ‘a’ (0.4g, 27.39%) m. p. 180-82° which were found to be TLC pure (irrigant ‘a’). It gave a positive test for nitrogen on sodium fusion test.
Synthesis of \( N-(3\text{-acetyl}-4\text{-hydroxy benzyl}) \text{ morpholine (VII) from (I)} \)

A mixture of morpholine \( (0.47g = 0.46ml; 5.4 \text{ m mol}) \), sodium bicarbonate \( (3.0g) \) and water \( (5.0 \text{ ml}) \) was heated as in other cases discussed above. To this hot mixture was added \( I \) \( (1.0g, 5.4 \text{ m mol}) \) in small portions during a period of fifteen minutes with continuous stirring. Stirring was continued further for another five hours. After completion of the reaction, the mixture was extracted with ether and the ethereal layer after washing with water and drying over sodium sulphate was evaporated off completely. The residual mass was dissolved in a mixture of methanol and acetone and filtered. The filtrate on concentrating and leaving at room temperature gave a low melting cream colored compound VII \( (0.3g, 23.62\%) \) m. p. 28-30° which was found to be TLC pure (irrigant 'a'). It gave a positive test for nitrogen on sodium fusion test.

Synthesis of \( N-(3\text{-acetyl}-4\text{-hydroxy benzyl})-\text{o-nitroaniline (VIII) from (I)} \)

To a mixture of o-nitroaniline \( (0.74g, 5.4 \text{ m mol}) \) sodium bicarbonate \( (3.0g) \) and water \( (5.0 \text{ ml}) \) was added \( I \) \( (1.0g, 5.4\text{ m mol}) \) in small portions with continuous stirring during a period of ten minutes. The reaction conditions were maintained exactly the same as described earlier. Stirring was continued for another four hours. After completion of the reaction, the contents were extracted with ether. Processing of the ethereal extract in the usual way gave a residual mass, which was crystallized from methanol to give an orange colored crystalline compound VIII \( (1.2g; 77.41\%) \) m. p. 112-16°. It gave a positive test for nitrogen by sodium fusion method and was TLC pure (irrigant 'a').

Synthesis of \( N-(3\text{-acetyl}-4\text{-hydroxy benzyl})-\text{m-nitroaniline (IX) from (I)} \)

To a mixture of m-nitroaniline \( (0.74g; 5.4 \text{ m mol}) \) sodium bicarbonate \( (3.0g) \) and water \( (5.0 \text{ ml}) \), was added \( I \) \( (1.0g, 5.4 \text{ m mol}) \) in small portions during a period of ten minutes with continuous stirring keeping the same reaction conditions as in other cases stated above. Stirring was continued for another two hours after completion of the reaction, the contents were extracted with ether and ethereal layer evaporated to dryness as usual. The residue in the flask was crystallized from methanol to give yellow crystals of IX \( (0.8g; 51.61\%) \) m. p. 106-08°. It was TLC pure (irrigant 'a') and gave a positive test for nitrogen by sodium fusion test.
Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-tolidine (X) from (I)

To a mixture of o-tolidine (0.57g; 2.7 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was added I (1.0g, 5.4 m mol) gradually in small portions during a period of fifteen minutes with continuous stirring maintaining reaction conditions as already mentioned in other cases. Stirring was continued for further five hours. A solid mass so obtained was filtered, washed with water to remove sodium bicarbonate and crystallized from methanol to give buff colored compound X (0.5g; 36.31%) m. p. 120-22° (decomp.). It was TLC pure (irrigant 'a') and gave a positive test for nitrogen by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-p-bromoaniline (XI) from (I)

To a mixture of p-bromoaniline (0.93g; 5.4 m mol) sodium bicarbonate (3.0g) and water (5.0 ml) was added I (1.0g, 5.4 m mol) gradually in small portions with continuous stirring over a period of ten minutes maintaining the same reaction conditions as stated above. Stirring was continued further for another three hours. A solid mass so obtained was filtered, washed with water to remove sodium bicarbonate and dried in the air. It was crystallized from methanol and dichloromethane mixture (4:1) to give colorless crystals of XI (1.2g; 69.3%) m. p. 100-02°. It was TLC pure (irrigant 'a') and gave a positive test for nitrogen and bromine by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl) norfloxacin (XII) from (I)

A mixture of norfloxacin (1.72g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated maintaining the temperature condition as stated above. To this hot mixture was added I (1.0g, 5.4 m mol) in small portions at a time with continuous stirring over a period of ten minutes. Stirring was continued for another four hours. A solid mass so obtained was filtered, washed with water to remove sodium bicarbonate and dried in the air. It was crystallized from benzene and methanol mixture to give buff colored compound XII (1.2g, 47.4%) m. p. > 350°C which was TLC pure (irrigant 'c') and tested positive for nitrogen on sodium fusion test.

Synthesis of N-(3-acetyl-4-hydroxy benzyl) sulfadoxine (XIII) from (I)

A mixture of sulfadoxine (1.68g; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated while maintaining similar temperature conditions as mentioned above. To this hot mixture was added I (1.0g, 5.4 m mol) in small portions at a time with continuous stirring, over a period of ten minutes. Stirring was continued for another three hours. A compound so
obtained was filtered, washed with water to remove sodium bicarbonate and air dried. It was crystallized from a mixture of methanol and dichloromethane to give brown colored crystals of XIII (0.7g, 28.2%) m. p. 176-80°, which was TLC pure (irrigant 'a') and gave a positive test for sulphur and nitrogen by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-2-aminobenzothiazole (XIV) from (I)

To a mixture of 2-aminobenzothiazole (0.81 g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was added I (1.0g, 5.4 m mol) in small portions at a time during a period of ten minutes with continuous stirring under the same reaction conditions as already mentioned in other cases. Stirring was continued for another two hours. The contents after cooling to room temperature were extracted by ether and the ethereal layer evaporated to dryness. The residue left in the flask was dissolved in methanol and filtered which on leaving at room temperature gave a crystalline compound m. p, 144-46°. It gave two spots on TLC examination (irrigant 'a') and gave a positive test for sulphur and nitrogen by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-aminothiophenol (XV) from (I)

A mixture of o-aminothiophenol (0.67g = 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated under the temperature conditions as stated above. To this hot mixture was added I (1.0g, 5.4 m mol) in small portions during a period of ten minutes with continuous stirring. Stirring was continued further for two hours. The reaction mixture was then extracted with ether and the ethereal layer evaporated off to dryness. The residue left in the flask after evaporation of ether was dissolved in methanol and filtered. The filtrate on keeping at room temperature gave yellowish crystals of XV (0.75g; 51.02%) m. p. 148-50° which were found to be TLC pure (irrigant 'a') and gave a positive test for sulphur and nitrogen by sodium fusion method.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-o-aminophenol (XVI) from (I)

A mixture of o-aminophenol (0.59g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated while maintaining similar temperature conditions as stated above. To this hot mixture was added I (1.0g, 5.4 m mol) gradually in small portions during a period of ten minutes with continuous stirring. Stirring was continued further for another three hours. A solid mass so
obtained was filtered, washed with water to remove sodium bicarbonate and dried in the air. It was crystallized from petrol and ether mixture to give compound XVI (0.8g, 57.5%) m. p. 194-96° (decomp.) which was TLC pure (irrigant 'a') and gave a positive test for nitrogen on usual testing.

Synthesis of N-(3-acetyl-4-hydroxy benzyl) anthranilic acid (XVII) from (I)

A mixture of anthranilic acid (0.74g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as above. To this hot mixture was added I (1.0g, 5.4 m mol) in small portions at a time over a period of ten minutes with continuous stirring. Stirring was continued further for another three hours. A solid mass so obtained was filtered, washed with water and dried. It was crystallized from ethanol to give a TLC (irrigant 'a') pure compound XVII (1.25g, 80.9%) m. p. 198-200°, which gave a positive test for nitrogen on usual testing.

Synthesis of N-(3-acetyl-4-hydroxy benzyl) sulfanilamide (XVIII) from (I)

To a hot mixture of sulfanilamide (0.93g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was added I (1.0g, 5.4 m mol) gradually in small portions over a period of ten minutes with continuous stirring while keeping parallel temperature and reaction conditions as stated above. Stirring was continued for another three hours. A product so obtained was filtered, washed with water and dried. It was crystallized from a mixture of methanol and acetone to give a colorless compound XVIII (1.3g; 75%) m. p. 194-96° (decomp.) which was TLC pure (irrigant 'd') and gave a positive test for sulphur and nitrogen on sodium fusion test.

Attempted synthesis of N-(3-acetyl-4-hydroxy benzyl) diclofenac (XIX) from (I)

A mixture of diclofenac (1.60g; 5.4 m mol), sodium bicarbonate (5.0g) and water (5.0 ml) was heated on a hot plate as described in other cases mentioned above. To this mixture was added I (1.0g, 5.4 m mol) in small portions with continuous stirring over a period of ten minutes. Stirring was continued further for two hours. After evaporation of the reaction the contents were extracted with ether and ethereal layer evaporated to dryness. TLC examination (irrigant 'a') of the compound matched with that of the starting ketone.
Synthesis of N-(3-acetyl-4-hydroxy benzyl) nimesulide (XX) from (I)

A mixture of nimesulide (1.66g; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as above. To this hot mixture was added I (1.0g; 5.4 m mol) in small portions with continuous stirring over a period of ten minutes. Stirring was continued for another two hours. A solid mass, which separated out was filtered, washed with water and crystallized from a mixture of methanol and acetone to give XX (0.9g; 36.4%) m. p. 116-18°. It gave a single spot on TLC examination (irrigant 'a') and a positive test for sulphur and nitrogen on sodium fusion test.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-2-amino pyridine (XXI) from (I)

A mixture of 2-aminopyridine (0.50g, 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as above and to this hot mixture was added I (1.0g, 5.4 m mol) in small portions over a period of ten minutes with continuous stirring. Stirring was continued for another two hours. A product so obtained was filtered, washed with water and crystallized from methanol to give a crystalline compound XXI (0.90g; 68.7%) m. p. 128-30° which were TLC pure (irrigant 'a') and gave a positive test for nitrogen on sodium fusion test.

Synthesis of N-(3-acetyl-4-hydroxy benzyl)-2-mercaptobenzothiazole (XXII) from (I):

A mixture of 2-mercaptobenzothiazole (0.73g; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as above. To this hot mixture was added I (1.0g, 5.4 m mol) in small portions with continuous stirring during a period of ten minutes. Stirring was continued for another four hours. A solid mass which separated out was filtered, washed with water and crystallized from a mixture of methanol and dichloromethane to give cream colored crystals of XXII (0.8g, 54%) m. p. 214-16° which were TLC pure (irrigant 'a') and gave a positive test for nitrogen and sulphur on usual testing.
Synthesis of \( \text{N-phenyl-N'}(\text{3-acetyl-4-hydroxy benzyl})\text{-thiourea (XXIII)} \)
from \( \text{(I)} \) and phenylthiourea

A mixture of phenylthiourea (0.82g; 5.4 m mol), sodium bicarbonate (3.0g) and water (5.0 ml) was heated as above. To this hot mixture was added \( \text{(I)} \) (1.0g; 5.4 m mol) in small portions with continuous stirring over a period of ten minutes. Stirring was continued for three hours. A solid mass, which separated out was filtered, washed with water and crystallized from methanol to give crystals of XXIII (0.3g; 18.45%) m. p. 192-94°. It gave a single spot on TLC examination (irrigant 'a') and a positive test for sulphur and nitrogen on sodium fusion test.
ii) Synthesis of flavonoidal derivatives

Synthesis of 2'-hydroxy-5'-ethoxymethyl-4-methoxy chalcone (XXIV) from 5- chloromethyl-2-hydroxy acetophenone (I)

To a solution of chloromethyl acetophenone I (1.0 g; 5.4 m mol.) in oxygen free ethanol (20 ml) was added p-anisaldehyde (0.80 g = 0.71 ml; 5.7 m mol.) and a solution of potassium hydroxide (5.0 g) in oxygen free distilled water (5.0 ml.) dropwise with constant stirring. The reaction mixture was then refluxed on a water bath for two hours and after cooling to room temperature was poured onto crushed ice followed by acidification with hydrochloric acid. After extracting the acidified contents with ether four times, the combined ethereal layers were separated, washed with saturated sodium bicarbonate solution followed by cold water. It was dried over sodium sulphate. After filtering off the inorganic salt, ether was evaporated off to dryness to give a reddish yellow oily mass, which was dissolved in a mixture of petrol and ether (4:1). On slightly concentrating the solution a reddish yellow liquid separated out and settled in the bottom of the flask, which was separated by decanting. On TLC examination (irrigant 'a') it was found to be a mixture. It was redissolved in petrol and ether mixture and on leaving for sometime, a reddish yellow liquid settled down again, which was separated and dissolved in petrol and ether mixture to give the same type of reddish yellow liquid. The above process was repeated several times till a TLC (irrigant 'a') pure syrupy compound (XXIV) was obtained (1.1 ml = 1.225 g; 74.78%).

Synthesis of 2'-hydroxy-5'-ethoxymethyl-3, 5-methylenedioxy chalcone (XXV) from (I)

To a solution of chloromethyl acetophenone I (1.0 g; 5.4 m mol.) in oxygen free ethanol (20 ml) was added piperonal (0.81 g; 5.4 m mol.). A solution of potassium hydroxide (5.0 g) in oxygen free distilled water (5.0 ml.) was added dropwise to the above solution with stirring of the contents. The reaction mixture was covered with 2 mm layer of petroleum ether. Rest of operation was the same as described above. On evaporation of ether, a red semisolid mass was obtained which on crystallisation from a mixture of petrol and ether (4:1) yielded a yellow crystalline compound XXV (0.5 g; 28.29%) m.p. 65-67°. It was found to be TLC pure (irrigant 'a') and gave a red color with a drop of conc. sulfuric acid.
Synthesis of 4’-methoxy-6-ethoxymethyl flavone (XXVI) from 2’-hydroxy-5’-ethoxymethyl-4-methoxy chalcone (XXIV)

A solution of chalcone (XXIV) (0.5 ml = 0.57 g; 1.88 m mol.) in dry isoamyl alcohol (5.0 ml) was refluxed with selenium dioxide (0.2 g) on a heating mantle for forty eight hours under anhydrous conditions. The contents were then cooled to room temperature and a blackish insoluble mass, which separated out was filtered off and the filtrate evaporated to dryness. The residue was washed with a small quantity of cold diethyl ether and crystallized from a mixture of ether and chloroform to give a crystalline compound XXVI (0.2 g; 35.32%) m.p. 126-30°. It was TLC pure (irrigant ‘b’) and gave a pink color with Mg/Hydrochloric acid and showed a yellowish fluorescence under U.V. light.

Synthesis of 3’, 4’-methylenedioxy-6-ethoxymethyl flavone (XXVII) from chalcone (XXV)

To a solution of chalcone (XXV) (0.5 g = m mol.) in dry isoamyl alcohol (5.0 ml) was added selenium dioxide (0.2 g) and the mixture refluxed gently on a heating mantle under anhydrous conditions for forty eight hours. After usual work up and washing the residue with ether (4-5 ml), the insoluble mass was crystallized from a mixture of methanol and chloroform to give the flavone XXVII (0.2 g; 35.71%) m.p. 158-60°. It was TLC (irrigant ‘b’) pure and gave a yellow fluorescence in U.V. light. It gave a pink color with Mg/Hydrochloric acid and did not give any color with alcoholic ferric chloride.

Synthesis of 6-(o-toluidino methyl) flavanone (XXVIII) from N-(3-acetyl-4-hydroxybenzyl)-o-toluidine(III)

A mixture of (III) (1.0 g; 3.92 m mol.), benzaldehyde (0.62 g = 0.6 ml; 5.8 m mol.) in oxygen free ethanol (20 ml) was stirred and aqueous potassium hydroxide solution (7.0 ml) was added to it. The mixture was covered with 2 mm layer of petroleum ether and stirring was continued for two hours followed by refluxing the contents for three hours. The reaction mixture was cooled to room temperature, poured onto crushed ice and acidified with hydrochloric acid. It was extracted with ether and the ethereal layer processed as usual to give a semisolid mass, which was dissolved in a mixture of petrol and ether (4:1). On slightly concentrating the solution a reddish mass separated out and settled in the bottom of the flask, which was separated by decanting the supernatent solvent layer. It was redissolved in petrol and ether mixture and the process repeated several times till the red colored mass XXVIII (0.3 g; 22.30%) was found to be TLC pure (irrigant ‘a’). It did not solidify even at low
temperatures (0°-5°) and gave a positive test for nitrogen by sodium fusion method.

Synthesis of 4′-methoxy-6-(o-toluidinomethyl)-flavanone (XXIX) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III)

To a well stirred mixture of (III) (1.0g ; 3.92 m mol.) and p-anisaldehyde (0.8g = 0.71 ml = 5.88m mol.) in oxygen free ethanol (20 ml) was added dropwise a solution of potassium hydroxide (5.0g) in oxygen free water (5.0 ml). The mixture was covered with 2 mm layer of petroleum ether and stirring was continued for two hours followed by refluxing it on a water bath for three hours. After cooling to room temperature, the contents were poured onto crushed ice and extracted with ether after acidification with hydrochloric acid. The ethereal layer was washed with sodium bicarbonate solution (5%) followed by washing with water. It was then dried over anhydrous sodium sulphate. After filtering off the inorganic salt, ether was evaporated off. The residue was purified by column chromatography.

A column (50 cms. In length and 30 mm in diameter) after plugging with cotton was filled with petroleum ether and silica gel, (50g) was carefully poured into the column to avoid air pocket in silica gel. The crude product (1.0g) was dissolved in acetone in a porcelain crucible and silica gel 3.0 g was added to it. The solvent was evaporated on a water bath with proper mixing so that the compound was uniformly adsorbed on silica gel. It was added to the column and the column was eluted with petrol, in 50 ml fractions. Fifty such fractions were collected. A yellowish residue obtained after evaporating the petrol showed two spots on TLC examination in these fractions. Later on the column was eluted with a mixture of benzene: petrol; 10:90 and three fractions of 50 ml each was collected which did not yield any compound on evaporation. The ratio of benzene: petrol was therefore increased to 30:70 and five fractions of 50 ml each were collected. This ratio was further changed to 50:50 benzene:petrol. Ten fractions of 50 ml each were collected when a red colored semisolid compound was isolated. These fractions showed a similar TLC pattern on evaporation (0.2g; 13.67%) of a solid mass was isolated which was pure on TLC examination (irrigant 'a') and gave a positive test for nitrogen on sodium fusion test.

Further elution of the column with 70:30 benzene: petrol did not yield any compound. The column was finally eluted with 100% benzene and then with chloroform and acetone. No other compound could be isolated.
Synthesis of 4'-hydroxy-3'(3,4-dimethoxy cinnamoyl) benzyl-o-toluidine (XXX) from N-(3-acetyl-4-hydroxy benzyl)-o-toluidine (III)

To a stirred solution of (III) (1.0g; 3.92 m mol) and veratraldehyde (0.97g; 5.88 m mol.) in oxygen free ethanol (20 ml.) was added a solution of potassium hydroxide (5.0g) in oxygen free distilled water (5.0 ml). A 2 mm layer of petrol was added to the mixture. Stirring was continued for another three hours followed by refluxing the contents on a water bath for a period of five hours. After cooling to room temperature the contents were poured onto crushed ice and acidified with hydrochloric acid to gave a solid mass which separated out was filtered, washed with sodium bicarbonate (5%) solution and finally with water. It was air dried and crystallized from methanol to yield orange colored crystals XXX (0.7g; 44.29%) m.p. 102-04°, which were TLC pure (irrigant 'a') and developed a red color on adding one drop of concentrated sulfuric acid. It gave a positive test for nitrogen by sodium fusion method.

Synthesis of 4'-hydroxy-3'(3,4-methyleneoxy cinnamoyl)-benzyl o-toluidine (XXXI) from N-(3-acetyl-4- hydroxybenzyl)-o-toluidine(III)

A mixture of (III) (1.0g; 3.92 m mol) and piperonal (0.88g; 5.88 m mol) in oxygen free ethanol was stirred and refluxed as above after addition of an aqueous potassium hydroxide solution (7.0 ml) as described in earlier experiments. The mixture was poured onto crushed ice and acidified with concentrated hydrochloric acid. A solid mass, which separated out was filtered and purified using column chromatography. A silica gel column was packed as described in the above case. Elution details are given below.

The column was eluted with 15 fractions of 50 ml each. There was no indication of any compound on TLC examination. It was then eluted with 10:90, benzene: petrol mixture (5 fractions of 50 ml each) and then with 30:70; benzene: petrol mixture (10 fractions of 50 ml each) when a TLC pure compound (irrigant 'a') was isolated (0.25g; 16.47%) m. p. 128-30°. It gave a red color on treatment with one drop of conc. sulfuric acid and a positive test for nitrogen on sodium fusion test.

Further elution of column with 70:30 benzene: petrol mixture led to a separation of a compound (0.2g) m. p. 224-26° which was TLC pure and later found to match TLC pattern and m. p. of piperonilic acid.
Synthesis of 1-[2-hydroxy-5-\{o-toluidino methyl\}\-phenyl]-3-\beta\-thenyl-2-
propen-1-one (XXXII) from N-(3- acetyl-4-hydroxybenzyl)-o-toluidine (III)

To a stirred solution of (III) (1.0g; 3.92 m mol) and thiophene-2-
aldehyde (0.65g = 0.53ml; 5.88m mol) in oxygen free ethanol was added
dropwise a solution of potassium hydroxide (5.0g) in water (5.0ml). The
mixture was covered with a 2 mm layer of petroleum ether. Stirring was
continued for two hours and the mixture refluxed for another five hours on a
water bath. The contents were worked up as usual and after acidification with
hydrochloric acid, extracted with ether. The ethereal layer was washed with
sodium bicarbonate solution (5%) followed by cold water and
dried over sodium sulphate. After filtering off the inorganic salt, ether was evaporated to
dryness to give a reddish oily mass which was dissolved in a mixture of petrol
and ether (4:1). On slightly concentrating the solution a red colored liquid
separated out and settled down in the bottom of the flask, which was
separated by decanting. It was redissolved in petrol and ether mixture and the
process repeated several times till the red colored liquid mass XXXII (0.5 g;
36.53%) was found to be TLC pure (irrigant 'a'), and gave a red color with
concentrated sulfuric acid. It was found to contain nitrogen by lassaigne
method.

Attempted synthesis of 4'-hydroxy-3'-cinnamoyl-benzyl-o-anisidine
(XXXIII) from N-(3 acetyl-4-hydroxy benzyl)-o-anisidine (V)

To a solution of (V) (1.0g; 3.69 m mol) and benzaldehyde (0.58g = 0.5
ml; 5.53 m mol) in oxygen free ethanol (20 ml) was added a solution of
potassium hydroxide (5.0g) in water (5.0 ml) dropwise and with stirring. The
mixture was covered with a 2 mm layer of petroleum ether. Stirring was
continued for three hours followed by refluxing on a water bath for another five
hours. The mixture was cooled to room temperature and poured into ice cold
water. After acidifying with hydrochloric acid, it was extracted with ether and
the ethereal extract processed as usual to give a brownish red colored mass,
which was purified by repeated petrol and ether mixture extraction treatment
as described in similar cases above to give a semisolid mass, which was
found to be a mixture on TLC examination (irrigant 'a') which could not be
further purified.
Attempted synthesis of 4'-hydroxy-3'-(p-methoxy cinnamoyl)-benzyl-o-anisidine (XXXIV) from N-(3-acetyl-4-hydroxybenzyl)-o-anisidine (V)

To a solution of (V) (1.0 g; 3.69 m mol) and anisaldehyde (0.75 g = 0.67 ml; 5.53 m mol) in oxygen free ethanol (20 ml) was added a solution of potassium hydroxide (5.0 g) in water (5.0 ml) dropwise and with stirring. The mixture was covered with a 2 mm layer of petroleum ether. Stirring was continued for two hours followed by refluxing the contents on a water bath for five hours. The reaction mixture was cooled to room temperature and poured onto crushed ice. After acidifying with hydrochloric acid, it was extracted with ether and the ethereal extract processed as usual to give a colored mass which was purified by repeated petrol and ether mixture extraction treatment as described in similar cases above to give a semisolid mass, which was found to be a mixture on TLC examination (irrigant 'a') that could not be further purified.

Synthesis of 4'-hydroxy-3'-(3,4-dimethoxy cinnamoyl) benzyl-o-anisidine (XXXV) from N-(3-acetyl-4-hydroxy benzyl)-o-anisidine (V)

To a solution of (V) (1.0 g; 3.69 m mol) and veratraldehyde (0.91 g; 5.53 m mol) in oxygen free ethanol (20.0 ml) was added a solution of potassium hydroxide (5.0 g) in water (5.0 ml). It was stirred and refluxed as stated above. The mixture was poured over crushed ice and extracted with ether as usual. A TLC (irrigant 'a') pure compound XXXV (0.4 g; 25.87%) m. p. was obtained by usual work up as mentioned in earlier cases. It gave a red color on treatment with one drop of concentrated sulfuric acid and a positive test for nitrogen by sodium fusion method.

Synthesis of 4'-hydroxy-3'(3,4-methylenedioxy cinnamoyl)-benzyl o-anisidine (XXXVI) from N-(3-acetyl-4-hydroxy benzyl)-o-anisidine (V)

A solution of potassium hydroxide (5.0 g) in water (5.0 ml) was added dropwise to a stirred solution of (V) (1.0 g; 3.69 m mol) and piperonal (0.825 g; 5.53 m mol) in oxygen free ethanol. The reaction mixture was covered with petroleum ether (2 mm) layer and stirred for two hours followed by refluxing for another four hours. After cooling to room temperature the contents were poured onto crushed ice. On acidifying, a solid mass separated out which was filtered, washed with sodium bicarbonate solution and finally with water. It was crystallized from methanol to give TLC pure (irrigant 'a') brown crystals of XXXVI (0.5 g; 33.62%) m.p. 88-90° and a red color on treatment with concentrated sulfuric acid. It tested positive for nitrogen when subjected to Lassaignes test.
Attempted synthesis of 1-[2-hydroxy-5-(o-anisidino methyl)-phenyl]-3-β-thienyl-2-propen-1-one (XXXVII) from N-(3-acetyl-4-hydroxybenzyl)-o-anisidine (V)

A solution of potassium hydroxide (5.0 g) in water (5.0 ml) was added dropwise to a stirred solution of (V) (1.0 g; 3.69 m mol) and thiophene-2-aldehyde (0.61 g = 0.50 m; 5.53 m mol) in oxygen free ethanol. The mixture was covered with 2mm layer of petroleum ether and stirred for two hours followed by refluxing for another five hours. After completion of the reaction, the contents were cooled to room temperature, poured onto crushed ice, acidified with hydrochloric acid and extracted with ether. The ethereal extract was washed with sodium bicarbonate solution followed by cold water. The ethereal layer was dried over sodium sulphate and after removal of the salt evaporated to dryness. On TLC examination (irrigant ‘a’) it was found to have the same Rf. value as that of (V).
(b) Exploitation of \(\omega\)-Bromoacetophenone nucleus

i) Synthesis of phenacylamine derivatives

Synthesis of \(\omega\)-Bromoacetophenone (XXXVIII):

Bromine (62g; 20 ml) was added gradually dropwise to a mixture of acetophenone (43.1 ml; 0.32 mole) and acetic acid (66.4 ml) contained in a stoppered conical flask with constant stirring. After addition of each lot, sufficient heat was generated. The contents were then allowed to cool to room temperature and poured onto crushed ice with stirring and left in an ice bath for an hour. A solid mass, which separated out was filtered, washed with water, dried and crystallized from ethanol to give a colorless crystalline compound XXXVIII (44.0g; 60%) m. p. 50° (lit. m. p. 50°). On TLC examination it showed a single spot (irrigant 'b')

Synthesis of N-phenacylaniline (XXXIX) from (XXXVIII):

A mixture of aniline (0.46g = 0.45 ml; 5.0 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was heated on a hot plate at 50-60°. To this hot mixture was added XXXVIII (1.0g; 5.02m mol) in small portions over a period of ten minutes with stirring which was continued for another two hours. The reaction mixture was then extracted with ether three times. The ethereal layer was washed with water and dried over sodium sulphate. After filtering off the inorganic salt, ether was evaporated off to dryness and residue crystallized from ethanol to give yellow crystals (0.5g; 47.16%) m. p. 180-82°. It gave two spots on TLC examination (irrigant 'a') and gave a positive test for nitrogen on Lassaigne's test.

Synthesis of N-phenacyl-o-toluidine(XL) from (XXXVIII):

To a hot mixture of o-toluidine (0.53g = 0.52 ml; 5.0 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was added XXXVIII (1.0g, 5.02 m mol) gradually over a period of ten minutes with continuous stirring as stated above. After continuing stirring for another two hours the reaction mixture was extracted with ether which on usual work up gave a solid mass which on crystallization from methanol gave yellow crystals of XL (0.7g; 61.51%) m. p. 166-68°. It was found to be TLC pure (irrigant 'a') and gave a positive test for nitrogen by Lassaigne's test.
Synthesis of N-phenacyl-p-toluidine (XLI) from (XXXVIII):

To a mixture of p-toluidine (0.53g; 5.02 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was added XXXVIII (1.0g; 5.02 m mol) maintaining the same reaction conditions throughout as given above. The reaction mixture was processed as usual and finally the contents were extracted as usual followed by crystallization from methanol to give a crystalline compound XLI (0.3g; 26.54%) m. p. 158-60°. It was found to be TLC pure (irrigant ‘a’) and gave a positive test for nitrogen on usual testing.

Attempted synthesis of N-phenacyl-o-anisidine (XLII) from (XXXVIII):

To a mixture of o-anisidine (0.61 g = 0.57 ml; 5.02 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was added XXXVIII (1.0g; 5.02 m mol) maintaining same reaction conditions as in other cases. Finally the reaction mixture was processed as usual and the residue on crystallization from methanol gave yellow needles (0.5g; 41.32%) m. p. 136-38°, however, it was found to be a mixture of two components on TLC examination (irrigant ‘a’). It could not be purified and hence further studies were abandoned.

Synthesis of N-phenacyl-p-anisidine (XLIII) from (XXXVIII):

Compound XXXVIII (1.0g; 5.02 m mol), was added to a mixture of p-anisidine (0.61g; 5.02 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) maintaining similar reaction conditions of heating etc. as mentioned above in other cases. Usual processing followed by crystallization from methanol gave a crystalline compound XLIII (0.3g; 24.79%) m. p. 170-74°. It was found to be TLC pure (irrigant ‘a’) and gave a positive test for nitrogen.

Attempted synthesis of N-phenacyl-o-nitroaniline (XLIV) from (XXXVIII):

To a mixture of o-nitroaniline (0.69g; 5.02 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was added compound XXXVIII (1.0g; 5.02 m mol) in small portions with continuous stirring over a period of ten minutes keeping similar reaction conditions as in other cases above. On usual processing the reaction mixture followed by crystallization from methanol gave a compound m. p. 75-77° was obtained. It showed two spots on TLC (irrigant ‘a’) examination and gave a positive test for nitrogen. Inspite of several attempts it could not be purified and hence further studies were abandoned.
Synthesis of N-phenacyl-m-nitroaniline (XLV) from (XXXVIII):

m-Nitroaniline (0.68g; 5.02 m mol), sodium bicarbonate (2.0g) and water (5.0 ml) was heated on a hot plate at a temperature between 90-100°. To this hot mixture was added compound XXXVIII (1.0g; 5.02 m mol) in small portions with continuous stirring over a period of ten minutes. Stirring was continued further followed by processing the contents as usual resulting in a residual mass which on crystallization from methanol gave compound XLV (0.3g; 23.43%) m. p. 62-64°. It gave a single spot on TLC examination (irrigant 'a') and answered positive to a test for nitrogen by Lassaigne’s method.
A) Anti-inflammatory activity

Inflammation is basically a part of the host defense mechanism and is defined as a tissue reaction to injury in higher animals. The condition is a complicated biological phenomenon and is clinically characterized by redness and swelling with heat and pain leading to subsequent reduction in cellular functions. An inflammatory response is a culmination of events beginning with release of various chemical mediators in response to tissue injury.

Clinically, anti inflammatory agents are judged by their effect on pain, stiffness and swelling of the affected part, the action on swelling being the most objectively observable and therefore the most important.

The anti-inflammatory activity of several of the compounds reported was carried out by the method of Winter, C. A., et al. (1962). Pedal inflammation in albino rats was induced by carageenan in rat hind paw and the oedema volume was measured by mercury displacement in a plethysmograph.

Plethysmograph

It is a simple apparatus containing mercury having two arms 'A' and 'B'. The level of mercury is the same in both the arms. The paw of the animal is dipped into arm 'A' causing displacement of mercury from 'A' which leads to increase in mercury level in arm 'B'.
A third arm 'C' is fused in such a way that the mercury displaced by dipping of the paw is allowed to rise in arm 'C' until the level of mercury is same in arms 'A' and 'B'. The flow of mercury was regulated by stop cock.

Animals: The studies were carried out on healthy rats (male) weighing between 120-200 g, divided in groups of six animals each and housed in polypropylene cages. They were fed on standard pellet diet, water ad libitum.

Drugs, their doses, dosage form and routes of administration: The drugs used as standards were Indomethain and Diclofenac in dose of 10 mg/kg body weight and 20 mg/kg body weight respectively. The dose of test compounds was 20 mg/kg body weight. The standard and test compounds were administered through intraperitoneal route in the form of (0.5% CMC) suspensions. The control group of animals were administered 0.2 ml of normal saline intraperitoneally. Carrageenan was injected subcutaneously, 0.1 ml of a 1% w/v carrageenan suspension (in 0.5% CMC) to each of the rats.

Experimental procedure:

Rats were weighed and marked/numbered. A mark was made on the left hind paw near tibio-tarsus junction so that every time the paw was dipped in the mercury column up to that fixed mark to ensure constant paw volume. Initial paw volume of all the animal groups was recorded. The test and standard drugs were administered intraperitoneally. Carrageenan was injected subcutaneously into the subplantar region of left hind paw of all animals, thirty minutes after the test drugs and standard had been injected. The paw volume was measured by mercury displacement (plethysmograph) at 1, 2 and 3 hours after carrageenan injection.

Thus the oedema volume in control group (Vc) and oedema volume in groups treated with test compounds (Vt) was measured and the percentage inhibition of oedema was calculated using the formula.

\[
\% \text{ inhibition} = \frac{(V_c - V_t)}{V_c} \times 100
\]
B) Analgesic activity:

Analgesic activity was carried out by tail clip method [Palanichamy, S. and Nagarajan, S. J. Ethanopharmacol. 29, 73 (1990)]. A clip was placed on the base of tail of mouse and the time when the mice tried to remove the clip was recorded.

Mice (male) weighing 25-30 g were divided into groups of five animals each and housed in polypropylene cages and fed on standard pellet diet, water ad. libitum. The test compounds and standard (Aspirin) were administered intraperitoneally at a dose of 25 mg/ kg body weight.

Experimental procedure:

The test drugs and standard were given in the form of suspensions (0.5% CMC). Initially the basal reaction time was recorded by placing the clip on the base of tail. The time after which the mouse tried to remove the clip was taken as the end point. Any animal not responding within 3-5 sec was rejected from the study.

Each group was administered different test compound and one of the group was given standard drug intraperitoneally. Immediately after administration of drug and at intervals of 15, 30 and 60 minutes, the reaction time was recorded. As the reaction time reached ten seconds, it was considered maximum analgesia and the clip was removed from the tail to avoid tissue damage.

Results and Discussion

A) Anti-inflammatory activity:

Among the synthesized compounds, eleven derivatives of chloromethyl hydroxy acetophenone and seven flavonoids were screened for anti-inflammatory activity by carrageenan induced rat paw oedema method. The percentage inhibition in oedema (Table-1) after two hours indicated that compound no. XVI, XX and XXII exhibited good anti-inflammatory activity ranging from 74-83%. The others showed inhibition in the range of 30-68%.

The mean reduction ± standard deviation (S ± SD) in paw volume is given in Table-2.

The chloro group in chloromethyl hydroxy acetophenone was substituted by reacting with different aromatic amines. The replacement of chloro group by aniline showed an increase in activity.

Incorporation of methyl group at position 2 of the aniline ring did not change the activity significantly however, if the methyl function was located at para position or methoxyl function was located at ortho position there was
complete loss of activity. Substitution at ortho position of the aniline moiety with OH, SH or COOH leads to a significant increase in activity.

Substitution of chloro group in chloromethyl hydroxy acetophenone either by mercaptobenzothiazole or nimesulide moiety showed significant increase in activity.

In flavone derivatives, compound XXXI was found to be comparable in activity to indomethacin while the rest were moderate in their action.

B) Analgesic activity:

Among the chloromethyl hydroxy acetophenone analogs tested for their anti-inflammatory action, five derivatives were found to have good activity. These compounds were selected for screening their analgesic activity also.

Analgesic activity was tested by tail flick method using aspirin (25 mg/kg, bw) as standard for comparison. The pain threshold was calculated and is presented in Table-3.

Compound XVI and XVII showed good analgesic activity, which was at par with aspirin whereas the rest of the compounds were moderate to low in their action.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment (compound no.)</th>
<th>Per cent inhibition at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Hour</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>I</td>
<td>7.86517</td>
</tr>
<tr>
<td>3.</td>
<td>II</td>
<td>21.3483</td>
</tr>
<tr>
<td>4.</td>
<td>III</td>
<td>15.579</td>
</tr>
<tr>
<td>5.</td>
<td>IV</td>
<td>-7.8652</td>
</tr>
<tr>
<td>6.</td>
<td>V</td>
<td>-10.112</td>
</tr>
<tr>
<td>7.</td>
<td>XV</td>
<td>37.0787</td>
</tr>
<tr>
<td>8.</td>
<td>XVI</td>
<td>73.0337</td>
</tr>
<tr>
<td>9.</td>
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<td>61.7978</td>
</tr>
<tr>
<td>10.</td>
<td>XX</td>
<td>57.3034</td>
</tr>
<tr>
<td>11.</td>
<td>XXI</td>
<td>-21.348</td>
</tr>
<tr>
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<td>XXII</td>
<td>68.5393</td>
</tr>
<tr>
<td>13.</td>
<td>XXVI</td>
<td>18.47</td>
</tr>
<tr>
<td>15.</td>
<td>XXIX</td>
<td>1.1236</td>
</tr>
<tr>
<td>16.</td>
<td>XXX</td>
<td>29.2135</td>
</tr>
<tr>
<td>17.</td>
<td>XXXI</td>
<td>34.8315</td>
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<tr>
<td>18.</td>
<td>XXXII</td>
<td>57.3034</td>
</tr>
<tr>
<td>19.</td>
<td>Indomethacin</td>
<td>64.0449</td>
</tr>
<tr>
<td>20.</td>
<td>Diclofenac</td>
<td>38.5768</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Treatment</td>
<td>Mean reduction ± Std. Deviation (S±S.D.) at 1 hour</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.225±0.04193</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>0.205±0.05</td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>0.175±0.03416</td>
</tr>
<tr>
<td>4</td>
<td>III</td>
<td>0.1165±0.0104</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>0.22±0.037</td>
</tr>
<tr>
<td>6</td>
<td>V</td>
<td>0.245±0.025</td>
</tr>
<tr>
<td>7</td>
<td>XV</td>
<td>0.14±0.016</td>
</tr>
<tr>
<td>8</td>
<td>XVI</td>
<td>0.095±0.041</td>
</tr>
<tr>
<td>9</td>
<td>XVII</td>
<td>0.085±0.025</td>
</tr>
<tr>
<td>10</td>
<td>XX</td>
<td>0.095±0.025</td>
</tr>
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<td>11</td>
<td>XXI</td>
<td>0.27±0.026</td>
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<td>XXVIII</td>
<td>0.19±0.047</td>
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<td>15</td>
<td>XXIX</td>
<td>0.22±0.036</td>
</tr>
<tr>
<td>16</td>
<td>XXX</td>
<td>0.1575±0.063</td>
</tr>
<tr>
<td>17</td>
<td>XXXI</td>
<td>0.145±0.05</td>
</tr>
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<td>18</td>
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<td>19</td>
<td>Indomethacin</td>
<td>0.08±0.04</td>
</tr>
<tr>
<td>20</td>
<td>Diclofenac</td>
<td>0.136±0.035</td>
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</table>
TABLE 3: ANALGESIC ACTIVITY: Effect of test compounds on pain threshold in tail-clip induced pain.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Mean Reaction Time 60 min after drug treatment (± S.D)</th>
<th>Level of Significance</th>
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<tr>
<td>1</td>
<td>CONTROL</td>
<td>1.5 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>XV</td>
<td>2.5 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>XVI</td>
<td>11.75 ± 5.63</td>
<td>P&lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>XVII</td>
<td>12.0 ± 5.2</td>
<td>P&lt; 0.001</td>
</tr>
<tr>
<td>5</td>
<td>XX</td>
<td>8.0 ± 7.0</td>
<td>P&lt; 0.01</td>
</tr>
<tr>
<td>6</td>
<td>XXII</td>
<td>1.5 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>ASPIRIN</td>
<td>11.75 ± 5.63</td>
<td>P&lt; 0.001</td>
</tr>
</tbody>
</table>
C) Anti-microbial Studies:

The potential of microorganisms is tremendous as far as their beneficial effects are concerned. Several microorganisms however, are detrimental and perhaps outweigh the beneficial effects with respect to health of human beings. The increased resistance of microorganisms to various drugs is also a cause of alarm. Several research and development units are therefore in search of such drugs which shall be susceptible to different microorganisms and also be active against a broad spectrum of microbes. The generation of antibiotics produced, are a proof of this concern.

The microbiological testing of the synthesized compounds was done by agar diffusion method (cup plate method) against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* NCTC 8236, *Escherichia coli* and *Candida albicans*. The medium used was meat peptone agar medium.

Washed spores of these organisms were added into sterile and cooled media at 45° and these seeded media were poured into plates and allowed to solidify. Stainless steel cylinder of 8 mm dia (presterilized) was used to bore the cavities. All the thirty seven compounds (100 μg/ml) mentioned in Table-4 serially were placed in the cavities with the help of micropipettes and allowed to diffuse for one hour. These plates were incubated at 37° for twenty four hours. Solvents only were poured as control. Norfloxacin (10 μg) was used as a reference drug.

The plates were observed after twenty four hours. The plates showing zones of inhibition have been indicated with +ve sign in Table-4.

**Minimum Inhibitory Concentration**

Minimum Inhibitory Concentration (MIC) is the highest dilution, which fails to show microbial growth. MIC of twenty four compounds (Table-5) was determined against *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* NCTC 8236 by turbidity method, as described in Indian Pharmacopeia, 1985, for microbiological assay of antibiotics and MIC method as described in Medical Microbiology by R. Cruick Shank.

**Method:**

Dilutions of the compounds from stock solution of 1 mg/ml to 1:50 (20 μg/ml), 1: 100 (10 μg/ml), 1:200 (5 μg/ml), 1:500 (2 μg/ml) were prepared. To each of a series of sterile stoppered test tubes a standard volume of nutrient broth medium was added. A solution of different compounds as mentioned in Table-5 was prepared in broth and a series of doubling dilutions prepared with sterile pipettes. A control tube containing no anti-microbial agent was included. The inoculum consisting of an overnight broth culture of
both *Bacillus subtilis* and *Staphylococcus aureus* was added to separate tubes. The tubes were incubated at 37° for 24 hours and examined for turbidity. The tube with the highest dilution showing no turbidity was the Minimum Inhibitory Concentration (bacteriostatic).

**Discussion**

**Anti-microbial activity:**

The compounds tested for anti-microbial activity included N-(3-acetyl – 4-hydroxy benzyl) anilines and miscellaneous derivatives, phenacylamine derivatives and flavonoids. Introduction of nitrogen atom along with other features imparted or enhanced various pharmacological actions associated with the parent molecule. Flavonoids have been known to possess anti-microbial activity and hence these studies with simple molecule and after introduction of a nitrogen atom were undertaken.

The anti-microbial activity was determined by observing zones of inhibition against the micro-organisms viz. *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* at a concentration of 100 µg/ml of the test substance using cup plate method (Table-4).

Candidates inhibiting growth of micro-organisms at 100 µg/ml were selected for determining their Minimum Inhibitory Concentration (MIC). The MIC was established against *Staphylococcus aureus* and *Bacillus subtilis* (Table-5). The compounds XXVI and XXXI showed good activity and were found to have MIC at 2 µg/ml against *S. aureus* while Compound No. III, VIII, IX, XII, XXVII, XXIX and XXXVI were found to have MIC at 5 µg/ml against the same organism.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound (Treatment)</th>
<th>Activity against</th>
<th>Solvent</th>
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<td></td>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>+</td>
<td>+</td>
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<tr>
<td>3</td>
<td>III</td>
<td>+</td>
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<td>IV</td>
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<td>-</td>
<td>-</td>
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<td>7</td>
<td>VII</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>26</td>
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<td>28</td>
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Table-5: Minimum Inhibitory Concentration

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<th>Sl. No.</th>
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<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
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<tr>
<td>4</td>
<td>IV</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>VIII</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
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<td>XXII</td>
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</tr>
<tr>
<td>14</td>
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</tr>
<tr>
<td>15</td>
<td>XXVI</td>
<td>+++</td>
</tr>
<tr>
<td>16</td>
<td>XXVII</td>
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<tr>
<td>24</td>
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+ = 20 μg/ml; ++ = 10 μg/ml; +++ = 5 μg/ml; ++++ = 2 μg/ml; - = above 20 μg/ml
SUMMARY

Three series of compounds have been synthesized for testing their biological actions. All the compounds have been characterized based on modern analytical techniques, which include UV, IR, NMR and Mass spectrometry.

The first series is based on chloromethylation of o-hydroxy acetophenone followed by condensation with various amino compounds. Twenty one compounds were synthesized, variations in the aniline ring include the following substituents at different positions of the benzene ring CH₃, OCH₃, NO₂, Cl and sulfonamides. Besides, norfloxacin, nimesulide, 2-mercaptobenzothiazole and 2-aminopyridine were also condensed with the above chloromethyl derivative. The new compounds as given below were synthesized and screened for their biological actions.

Compound No, I, II, III, IV, V, VI VII, VIII, IX, X, XI, XII, XIII, XV, XVI, XVII, XVIII, XIX, XX, XXI, XXII, and XXIII.
Eleven of the above compounds I, II, III, IV, V, XV, XVI, XVII, XX and XXII were tested for anti-inflammatory activity. Five of these compounds, XV, XVI, XVII, XX and XXII were found to have good activity, comparable to indomethacin, the standard reference drug. The percent inhibition in edema has been calculated and given in Table 1.

Five of the compounds i.e. XV, XVI XVII, XX and XXII were tested for analgesic action also when two compounds XVI and XVII showed an action comparable with aspirin, the standard drug (Table-3). Anti-microbial studies were carried out on all the twenty one compounds against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*. Thirteen compounds I, II, III, IV, V, VIII, IX, X, XI, XII, XIII, XXI and XXII inhibiting growth of one or more of the above micro-organisms at a concentration of 100 μg/ml were further tested for Minimum Inhibitory Concentration (MIC) against *S. aureus* and *B. subtilis*. Compound Nos. III, VIII, IX, XII had MIC-5 μg/ml, while compounds I, IV and V had MIC = 10 μg/ml against *S. aureus*. Compound No. III, had MIC- 10 μg/ml against *B. subtilis*.

Second series of compounds are based on further elaboration of the above substituted o-hydroxy acetophenone moiety with chalcone, flavanone and flavone derivatives; variations in the additional aryl ring include OCH₃ and methylenedioxy groups. Besides thiophene –2- aldehyde was also condensed with the acetyl group of the acetophenone system. During the chalcone condensation it was observed that the chloro group of the chloromethyl function got converted to ethoxymethyl group, however, in such cases where the chloro group had been replaced by the amino function, the expected chalcone/flavanone derivatives were obtained.

The new compounds of this series are compound nos.: XXIV, XXV, XXVI, XXVII, XXVIII, XXIX, XXX, XXXI XXXIII, XXXV and XXXVI were synthesized.

Six of these compounds were tested for anti-inflammatory action. An edema inhibition ranging from 20-63% was observed (Table –1).

Anti-microbial studies were carried out on all these compounds against *S. aureus*, *E. coli*, *B. subtilis* and *C. albicans*. Eight of these compounds numbering XXV, XXVI, XXVII, XXIX, XXXI, XXXII, XXXV and XXXVI which, inhibited the growth of one, two, three or all the four micro-organisms (Table 4) at 100 μg/ml were tested for Minimum Inhibitory Concentration. Two compounds XXVI and XXXI had MIC = 2 μg/ml while compound nos. XXVII, XXIX and XXXVI had MIC-5 μg whereas XXXV had MIC = 10 μg/ml against *S. aureus*. Compound Nos. XXVI and XXXI had MIC- 10 μg/ml against *B. subtilis*.
The third series of compounds XL, XLI, XLIII and XLV are based on 1β-
Bromo acetophenone which were derived by condensation with different
aromatic amines like aniline, toluidine, anisidine and nitro aniline.

These compounds were tested for anti-microbial activity against S.
aureus, B. subtilis, E. coli and C. albicans.

Three compounds XL, XLV were found to be active at 100 μg/ml and
therefore were tested for MIC against S. aureus and B. subtilis They had MIC-
20 μg/ml against S. aureus.
II) SUBSTITUTED AZOBENZENES DERIVED FROM 4, 6-DIACETYL RESORCINOL
It has been stated by Venkataraman that during the course of attempts to synthesize quinine, Perkin in 1856 discovered mauveine, the first synthetic dye to be manufactured and used for practical dyeing. From then on synthetic dyes have been prepared in bewildering number and variety and the possibilities of further synthesis are unlimited.

Dyes, in addition to their use in textile industry, form a major tool in the hands of research chemists, biologists, histologists etc for their use in chromatography and electrophoresis for identification of proteins, in chemistry as indicators for analysis and in medicine as diagnostic agents and as antimicrobials.

Azodyes form the largest group of all synthetic colorants and play a prominent part in almost every type of application including medicine.

The aim of this study was to synthesize azodyes using 4, 6 - diacetyl resorcinol and (coupling it with) different diazonium salts of aromatic amines, preferably sulfonamides. These drugs were expected to have a broad
spectrum of anti-microbial activity comparable or better to their parent compounds; sulfonamide derivatives.

B) THEORETICAL
In 1856, W. H. Perkin discovered Mauve, the first synthetic dye to be manufactured and used for practical dyeing. He had obtained Mauve by the action of potassium dichromate and sulfuric acid on crude aniline, which had a certain affinity for silk fibres and dyed them purple.

Simultaneously, with developments in the dyestuff industry and as a direct result, there has been a continual progress in the chemical technology of textiles. Dyes however are not mere agents for imparting color but they have a special purpose in biology, medicine, chemistry and allied fields. Histologists, cytologists and other workers in the field of biology and medicine therefore frequently require dyes whether they are engaged in industry or in the academic sphere.

Almost all dyes are now derived synthetically by known chemical steps and with a few exceptions, all synthetic dyes are aromatic organic compounds. The aromatic hydrocarbons provide the molecular framework for the final dye molecule, such aromatic nuclei or other conjugated systems being essential to the structures of substances having the property of color.
Dyes may be divided into three main groups: nonionic, anionic and cationic. The molecules of ionic (anionic or cationic) dyes are composed of two main parts, one of which is a complex aryl radicle. This is the color-imparting ion. If the balance of the charge on the latter is negative then the dye is classed as anionic. On the other hand, if the balance of the charge on this ion is positive then the dye is classed as cationic.

The second part of an ionic dye molecule is an inorganic ion (or an aliphatic organic ion or, in a few cases, an aryl ion) of opposite charge to that of the color imparting aryl ion. The former is sometimes called the gegenion, and the latter the dye ion. The individual chemical, physical and tinctorial characteristics of a dye are due to its dye ions.

A non-ionic dye molecule consists of three main parts, namely. The chromogen, the chromophore(s) and the auxochrome(s). A chromogen is an uncharged aryl substance, which is colored by virtue of the fact that it contains a chromophore as part of its structure. A chromophore is a configuration, which has a group containing one or more multiple bonds. The chromophore(s) is responsible for the chromogen being colored. It does not however determine the particular shade of color. Attached to the chromogen(s) of a dye are auxochromes that can be defined as a substituent atom or group, which increases the intensity of the absorption of light due to a chromophore. Auxochromes are divided into two classes, colligators and non-colligator auxochromes. In non-ionic dyes, uncharged colligators enable the chromogen under appropriate conditions to interact with certain other substances. The main function of the non-colligator auxochromes appears to be that of color modifiers. The azodyes forms the largest group of all synthetic colorants and plays a prominent part in almost every type of application. The chromophoric system consists essentially of the azo group, \(- N = N -\), in association with one or more aromatic systems. There may be more than one azo group present in the dye molecule and thus are referred monoazo, disazo, trisazo, etc. depending on the number of azo group present in the dye molecule. A common process involving two reactions prepares them all:

1. diazotizing an aromatic primary amine and
2. coupling the diazonium salt with a phenol or aromatic amine with a free \(\alpha\)– and/or \(\beta\)–position, or with certain other components having reactive positions, such as \(\beta\)–ketonic acid arylamides.
The amine which is diazotized (A) is the first component, and the substance with which it couples is the second or end component (E), and the reaction is indicated as $A \rightarrow E$.

e.g.

\[
\begin{align*}
\text{ArNH}_2 + \text{NaNO}_2 + 3\text{HCl} & \rightarrow \text{ArN}_2\text{Cl} + \text{NaCl} + 2\text{H}_2\text{O} \\
0^\circ - 5^\circ & \rightarrow \\
\end{align*}
\]

This is represented as aniline $\rightarrow \beta$-naphthol.

Diazotization and Diazonium Salts

Diazotization, discovered by Peter Griess in 1860, is the most important reaction of aromatic primary amines and involves the reaction of amine with nitrous acid. The diazonium salts are indispensable intermediates for the preparation of azo dyes and are also useful for the replacement of an amino group by hydroxyl, halogen, cyanogen and other groups.

Methods

Diazotization proceeds as follows:

\[
\begin{align*}
\text{ArNH}_2 + \text{NaNO}_2 + 3\text{HCl} & \rightarrow \text{ArN}_2\text{Cl} + \text{NaCl} + 2\text{H}_2\text{O} \\
0^\circ - 5^\circ & \rightarrow \\
\end{align*}
\]

The experimental conditions necessary for the preparation of a solution of a diazonium salt are as follows. The amine is dissolved in a suitable volume of water containing 2.5 - 3 equivalents of hydrochloric acid (or of sulfuric acid) by the application of heat if necessary, and the solution is cooled in ice when the amine hydrochloride (or sulphate) usually crystallizes. The temperature is maintained at $0^\circ - 5^\circ$, an aqueous solution of sodium nitrite is added portion wise until, after allowing 3 - 4 minutes for reaction, the solution gives an immediate positive test for excess of nitrous acid with an external indicator, moist potassium iodide starch paper.
Sodium nitrite is employed in theoretical quantity, but the acid must be in excess in order to prevent partial diazotization and condensation of the diazonium salt with the undiazotized amine to form the diazo amino compound, \( \text{Ar} - \text{N} = \text{N} - \text{NH} - \text{Ar} \).

The molar proportion usually necessary is 2.5 - 3, but for many amines, 6 - 7 moles can be used with advantage.

The excess of acid is required to stabilize the diazonium salt solution by reducing the secondary changes to a minimum, e.g., the interaction of some of the diazonium salt with unchanged amine to form a diazo amino compound a reaction which occurs readily in neutral solution.

The amines are comparatively weak bases, so that a certain amount of free amine will be produced by salt hydrolysis unless an excess of acid is present.

The addition of sodium nitrite is carried out at 0° - 5° since the process is exothermic in character and at higher temperatures, the diazonium salt may be partially converted into the corresponding hydroxyl compound.

The readiness with which an aromatic amine may be diazotized depends on the nature and position of substituents in the nucleus as affecting the basicity of the amine.

The mechanism of diazotization was first reported by Bamberger\textsuperscript{10}. He suggested that diazotization involves the N - nitrosation of the amine.

\[
\text{Ar}-\text{NH}_2 \rightarrow \text{NO} - \text{X} \rightarrow \text{Ar}-\text{NH} - \text{NO} \leftrightarrow \text{Ar} - \text{N=NOH}
\]

\[
\text{Ar}.\text{NH}_2 \rightarrow \text{Ar} - \text{N=NO} \leftrightarrow \text{Ar}.\text{N} - \text{OH} \rightarrow \text{Ar}.\text{N} = \text{N} + \text{H}_2\text{O}
\]

The acidified nitrite solution provides a source of the nitrosonium ion which electrophically replaces the hydrogen in the primary amine group to form the N - nitroso derivative. This has a tautomeric structure, the hydroxydiazo form yielding the diazonium ion under acidic conditions.

**Coupling Reactions:**

Azo compounds are prepared by the interaction of a diazonium salt with a phenol in the presence of sodium hydroxide or with an amine in presence of sodium acetate.

Diazot coupling can be regarded as an electrophilic substitution by a diazonium cation. It follows therefore, that the positions where coupling will occur are those at which there is increased electron density and thus the
presence of an electron donating group, -OH - NH₂ in the aromatic system of
the coupling component is necessary for the coupling to take place thus the
diazonium ion reacts at the position of greatest electron availability i.e. the
position ortho or para to the electron releasing phenoxy or amino groups.

\[
\text{Ar-N=N-} \xrightarrow{-\text{H}^{+}, +\text{H}^{+}} \text{Ar-N=N-} \text{NR}
\]

Phenols are important coupling components and couples under
alkaline conditions principally in the 4 - position. In case of resorcinol, coupling
occurs first in the 4 - position and then according to the pH at which the
second coupling is done viz. pH 5 - 8, position 2; pH > 8, position 6.
Thus electron accepting or electron attracting groups in the diazonium
salt will increase its reactivity compared with unsubstituted benzene
diazonium salt. Similarly electron donating or repelling groups in the phenol or
tertiary amine will accelerate the reaction. A qualitative measure of the effect
of substituents in the diazonium salt is given by the Hammet \( \sigma_p \) relation.

Classification of Azodyes

The division of the azo dyestuff class into smaller groups, adopted in
the following treatment, takes into account (a) the number of azo groups; (b)
the presence of characteristic ring systems (c) the order of the series of
coupling reactions, and (d) special dyeing properties. The insoluble azo dyes
are grouped together as azoic dyes and treated separately.

Thus dyes are referred to as Monoazo, Mordant, Disazo, Trisazo etc.
The colour of Azo dyes

With the azo group as the characteristic chromophore, the possible variations that influence the color and the dyeing properties are,

a) The number and position of azo groups.

b) The nature of the aromatic nuclei-benzene, naphthalene etc.

c) The nature, number and positions of the substituents such as halogen alkyl, amino, hydroxyl, alkoxy and nitro groups and

d) The number and position of the sulfonic groups.

Analytical Techniques used in azodyes

The chemistry of azo compounds is intimately connected to that of azo dyes. The correlation between the absorption spectrum of a compound especially a complex dye molecule, with its chemical constitution and its resonance states is made difficult because of factors like purity of the dye molecule, the formation of cis-trans isomers and the azophenol-quinonehydrazone tautomers in the case of ortho and para hydroxy azo compounds. Inter conversion of cis and trans forms are responsible for certain azo compounds exhibiting phototropism. The presence of a hydroxy group located ortho or para to the azolink leads to tautomerism (as mentioned above). Spectroscopic methods especially ultraviolet and visible spectroscopy helps to indicate the presence of tautomerism. To determine this phenomenon a peak due to each form is observed, the relative heights varying from solvent to solvent. The formation of an isobestic point by the absorption curves measured in a variety of solvents indicates tautomerism. If one form only is present comparison of the spectrum with that of the 0-and N-methyl derivatives will often indicate the nature of that form.

The azo compounds have also been analyzed using infrared spectroscopy, although, because of the symmetry of the azo link are not well suited for analysis by IR. The weak intensity of absorption prevents the assignment of a definite wavelength for N = N stretching; the azo absorption’s are also quite variable and often obscured by phenyl ring vibrations thus the presence of weak peaks at 1410 ± 30 cm⁻¹ maybe due to the azo group. However, useful information can be had from IR spectroscopy concerned with features of the molecule other than the azo link such as the presence of a carbonyl group in a suspected quinone-hydrazone form.

Nuclear magnetic resonance spectroscopy offers a most valuable technique in structure determination. The deshielding effect of the azo group
colorant in drugs and cosmetics, orange G\textsuperscript{19-21} in biological microtechnique as a staining agent for cell and tissue substances, to demonstrate red blood corpuscles, minute blood vessel and in cytological examinations.

Several dyes have also been reported to be active against HIV\textsuperscript{22} and thus dyes which act by preventing viral binding may represent prototypes for the development of novel drugs for the treatment or prevention of AIDS.

Another azodye, Evans Blue, USP\textsuperscript{23} when injected into the bloodstream combines firmly with plasma albumin. The color developed is proportional to its concentration and has been used as a diagnostic aid for blood volume determination.
REFERENCES


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12. Reynolds, W. B., Am. Dyestuff Reprtr., 32, 455 (1943); C. A., 38, 6564 (1944)


DISCUSSION

The Reagent/ Chemicals/ Solvents used during the course of these studies were obtained from Merck (India), Qualigens, BDH, s. d. fine and CDH Laboratories and were of the Laboratory grade. The solvents were purified by distillation before their use.

Silica Gel used for thin layer chromatography was of Central Drug House brand.

Iodine chamber and UV lamp were used for visualization of TLC Spots. Whatman filter papers (No. 1, England) were used for filtration (Vacuum or ordinary).

The solvent system used for thin layer chromatography were
1) Irrigant 'e' Benzene: methanol (2:1)

It has been accordingly mentioned at appropriate places.

The U.V. Spectras were recorded on Perkin Elmer, UV/VIS Spectrophotometer Lamda Bio 20

The I.R. Spectras were recorded on Hitachi IR Spectrometer model 270-30 Potassium Bromide was used for making pellets.

The NMR Spectras were recorded on 60 MHz, 90 MHz or 300 MHz instruments.

The Mass Spectras were recorded on a GC/ MS instrument.
Melting points of all the compounds were recorded on liquid paraffin bath in open capillary tubes and are uncorrected.

Detection of elements present (Nitrogen, Sulphur and Halogens)

The detection of nitrogen, sulphur and halogens was carried out by sodium fusion method (Lassaigne's test) as follows:

In a fusion test tube, a small cube of freshly cut sodium metal was introduced. The tube was heated slowly until sodium vapour rises in the test tube. The test compound (about 0.05g) was added to the molten sodium. The tube was then heated to redness for about two minutes and then allowed to cool. The tube was crushed into a crucible containing 10 ml of distilled water. Three such tubes were added to the crucible. The mixture was slightly concentrated and filtered. The filtrate was used for the various tests detailed below:

a) Test for nitrogen: To 2-3ml of the filtered fusion solution in a test tube was added 0.1-0.2g of powdered ferrous sulphate crystals. The mixture was heated gently with shaking till it boiled, then dilute sulfuric acid was added. A prussian blue color or precipitate indicated the presence of nitrogen. When sulphur was also present the mixture was boiled after addition of ferrous sulphate and then acidified with dilute sulfuric acid.

b) Test for sulphur: The fusion solution (2ml) was acidified with dilute acetic acid and a few drops of lead acetate solution was added. A black precipitate indicated the presence of sulphur.
Synthesis of 4,6-diacetyl resorcinol (I) from resorcinol

Resorcinol was taken in a conical flask and acetic anhydride was added to it followed by immediately adding zinc chloride. The mixture was heated over a sand bath for fifteen minutes at a temperature not exceeding 150°. The reaction mixture was cooled to room temperature and to it was added a mixture of water and HCl (1:1). The solid so obtained was washed with water, dried and crystallized from ethanol to give pink colored crystals of (I) m.p. 182-84°. It was found to be TLC pure.

\[
\begin{array}{c}
\text{HO} \quad \text{OH} \\
\text{acetic anhydride} \quad \text{ZnCl}_2 \\
\text{HO} \quad \text{OH} \\
\end{array}
\]

In the U.V. spectrum, it showed the following maxima at 250.43, 251.99, 321.33 nm.

In the I.R. spectrum it showed absorption peaks at 3200 cm\(^{-1}\) (OH str.), 1640 cm\(^{-1}\) (CO), 1500 cm\(^{-1}\) (C=C aromatic str.) 880, 840, 790 cm\(^{-1}\) (o-disubstituted ring C-H bend.).

The NMR spectrum of the compound showed chemical shifts in three regions at \(\delta\) 2.65, \(\delta\) 6.65 and \(\delta\) 8.25 integrating for six protons, one proton and one proton each, which could arise from the two methyl functions of the two acetyl groups, one proton ortho to the hydroxyl group and one proton ortho to the diacetyl function respectively. These data are satisfactory for the structure assigned to the compound.

Synthesis of (2, 6-dihydroxy-3, 5-diacetyl phenyl) azo benzene (II) from (I)

To a cooled solution of aniline in a mixture of conc. HCl and water was added dropwise and with stirring a cold solution of sodium nitrite to give the diazonium salt solution, which was then added slowly to a solution of 4,6-diacetyl resorcinol (I) in 20% sodium hydroxide solution with stirring. A red colored dye which separated out was filtered, washed with water and crystallised from methanol to give (II) m.p. >350°. It was TLC pure and gave a positive test for nitrogen by sodium fusion method.
In the U.V spectrum it showed the following maxima at 250.07, 288.29, 337.86, 400.67, 559.11, 633.46, 764.18 nm.

In the I.R. region it showed the followed absorption peaks, 3750 cm\(^{-1}\) (OH), 1620 cm\(^{-1}\) (CO), 1570, 1520 cm\(^{-1}\) (C=C aromatic str.) 1435 cm\(^{-1}\) (N=N str.), 1210, 960 cm\(^{-1}\) (phenyl ring C-H bend.).

The NMR spectrum showed a singlet at \(\delta\) 2.5 for two acetyl groups. A one proton singlet at \(\delta\) 8.8 possibly arises from the proton flanked by the two acetyl functions. A multiplet from \(\delta\) 7.1 to \(\delta\) 7.6 accounted for the protons of the phenyl ring, which could not be further analysed. These data are satisfactory for the above structure.

Synthesis of 2'-methyl (2, 6-dihydroxy-3, 5-diacetyl phenyl) azobenzene (III) from (I)

\(o\)-Toluidine was diazotised as described above. The diazonium salt solution was added slowly to a cold solution of (I) in 20% sodium hydroxide solution with stirring. Usual workup of the reaction mixture and crystallisation from ethanol gave a red dye (III) m.p. 234-36\(^\circ\) which was TLC pure and gave a positive test for nitrogen by sodium fusion method.

In the U.V. spectrum it showed four maxima at 253.03, 288.70, 339.2 and 408.88 nm.
The I. R. spectrum showed the presence of absorption peaks at 3740 (OH) 1660 cm\(^{-1}\) (CO), 1380 cm\(^{-1}\) (N=N, str.).

The NMR spectrum of the compound showed singlets for one methyl group and two acetyl functions located at \(\delta\) 2.7 and \(\delta\) 2.8. In the aromatic region there was a multiplet arising from four hydrogens of the o-toluidine ring located at \(\delta\) 7.5. The lone aromatic proton of the diketone system flanked by the two acetyl groups appeared as a singlet located at \(\delta\) 8.8. These data are in favour of the structure assigned to the compound.

**Synthesis of 2'-methoxy (2,6-dihydroxy-3, 5-diacetyl phenyl) azobenzene (IV) from (I)**

\(\text{o-Anisidine was diazotised and coupled with 4,6-diacyl resorcinol (I) as above. A red dye which separated out was crystallized from methanol and acetic acid mixture (5:1) to give a crystalline compound (IV) m.p. 226-28\(^\circ\)\). It was TLC pure and gave a positive test for nitrogen on sodium fusion test.**

![Diagram](image)

In the U.V. spectrum it showed the following maxima at 254.2, 288.76, 336.96, 444.56 nm.

The I.R. spectrum showed absorption peaks at 3750 cm\(^{-1}\) (b, OH), 1605 cm\(^{-1}\) (CO) 1500, 1475 cm\(^{-1}\) (C=C aromatic str.) 1380 cm\(^{-1}\) (N=N str.), 1265, 1250, 1230 cm\(^{-1}\) (o-anisidine ring C-H bend).

Due to solubility reasons the NMR spectrum could not be recorded.

**Synthesis of 4'-(5-methylisoxazole-3-amino sulphonyl)-(2, 6-dihydroxy-3, 5-diacetyl phenyl)- azobenzene (V) from (I)**

Sulfamethoxazole was diazotised as stated above. The cold diazonium salt solution was added, slowly and with stirring to a cold solution of (I) in 20% sodium hydroxide solution. The reaction mixture was processed as usual and crystallised from a mixture of methanol and acetic acid to give red colored needles of (V) m.p. 240-42\(^\circ\). It was TLC pure and answered positive to a test for nitrogen by sodium fusion method.
In the U.V. spectrum it showed absorption maxima at 255.84, 284.24, 339.17 and 403.31 nm.

The I.R. spectrum showed the following absorption peaks 3550 cm$^{-1}$ (OH), 3150 cm$^{-1}$ (NH), 1630 cm$^{-1}$ (CO), 1490, 1430 cm$^{-1}$ (C=C aromatic str.), 1390 cm$^{-1}$ (N=N str.), 1190 cm$^{-1}$ (S=O) 810, 770 cm$^{-1}$, (p – disubstituted ring C-H bend), 610 cm$^{-1}$ (O-H bend).

The NMR spectrum of the compound showed the presence of one singlet located at δ 2.1 integrating for three protons and another singlet integrating for six protons located at δ 2.65 which may account for the methyl group of sulphamethoxazole moiety and the two acetyl functions of the diacetyl resorcinol ring system. A singlet at δ 6.1 may arise from the lone proton of the oxazole ring system while the four protons of the para disubstituted phenyl ring appeared as a singlet located at δ 8.0 and the most downfield singlet located at δ 8.6 may arise from the aromatic proton located between the two carbonyl groups of the acetyl function of the diacetyl resorcinol moiety. These data indicate successful formation of the above compound.

The mass spectrum of the compound showed the molecular ion peak located at m/z 458 analysing for the molecular formula C$_{20}$H$_{18}$O$_7$ N$_4$ S. Other important peaks could be picked up at m/z 253, 221 and 194. The fragmentation pattern is as given below.
Synthesis of 4'-acetyl aminosulphonyl (2, 6-dihydroxy-3,5-diacetyl phenyl) azobenzene (VI) from (I)

Sulphacetamide was diazotised as described in earlier cases. The diazonium salt solution was added with stirring to a cold solution of (I) in 20% sodium hydroxide solution. After usual processing and crystallisation from methanol, a red colored dye was obtained (VI) m.p. > 350° which was TLC pure and gave a positive test for nitrogen by sodium fusion method.

In the U.V. spectrum it showed the following maxima at 255.89, 284.16, 340.33 and 407.00 nm.
The I.R. spectrum showed the following absorptions 3750 cm\(^{-1}\) (OH), 1590 cm\(^{-1}\) (CO), 1550, 1440 cm\(^{-1}\) (C=C aromatic str.) 1420 cm\(^{-1}\) (N=N str.), 1140 cm\(^{-1}\) (S=O), 830 cm\(^{-1}\) (p-disubstituted ring C-H bend.).

The NMR spectrum showed a three protons singlet at \(\delta\) 1.9 arising from acetamide moiety and a six proton singlet at \(\delta\) 2.5 accounting for two acetyl functions. The aromatic region showed two doublets of two protons centered at \(\delta\) 7.6 and \(\delta\) 7.9 arising from sulphacetamide moiety and a one proton singlet at \(\delta\) 8.8 assignable to the aromatic hydrogen flanked by the two ortho acetyl functions. These data suggest that the compound is the expected product.

Synthesis of 4, 5-dimethoxy pyrimidinyl-6-amino sulphonyl (2, 6-dihydroxy 3, 5-diacetyl phenyl)-azobenzene (VII) from (I)

Sulfadoxine was diazotised and coupled with (I) in the manner described above. A red colored dye, which separated out was filtered, washed with water and dried. It was crystallized from methanol to give (VII) m. p. > 350° It was TLC pure and gave a positive test for nitrogen by sodium fusion method.

In the U.V. spectrum it showed absorption maxima at 258.52, 339.22, 406.64 nm.

The I.R. spectrum showed the presence of the following peaks at 3750 cm\(^{-1}\) (OH), 1620 cm\(^{-1}\) (CO) 1580, 1430 cm\(^{-1}\) (C=C aromatic str.) 1370 cm\(^{-1}\) (N=N str.), 870, 850 cm\(^{-1}\) (C-H bend.)

The NMR spectrum of the compound showed a singlet at \(\delta\) 2.4 for the two acetyl functions besides two singlets at \(\delta\) 3.66 and \(\delta\) 3.78 for the two methoxyl functions. In the aromatic region it showed two proton doublets located at \(\delta\) 7.5 and \(\delta\) 7.9 forming an \(A_2B_2\) system, which could arise from the protons of the para disubstituted phenyl ring. The two one proton singlets located at \(\delta\) 7.76 and \(\delta\) 8.69 can be assigned to the lone proton on the
pyrimidine ring and the proton flanked by the two acetyl functions. These data are satisfactory for the expected structure.
EXPERIMENTAL

Synthesis of 4,6-diacetyl resorcinol (I) from resorcinol

Resorcinol (12.0 g) was taken in a 250 ml conical flask and acetic anhydride (16 ml) was added followed by freshly fused zinc chloride (15 g). The contents were then heated at 142° for 15 minutes. A viscous red solution so obtained was allowed to cool to room temperature, which was then diluted with a mixture of hydrochloric acid and water in the ratio 1:1 (80 ml). On stirring the contents an orange red crystalline mass separated out, which was filtered and washed with water to remove zinc chloride and hydrochloric acid. The product after drying was crystallized from ethanol to give needles of compound I (yield 72%) m. p. 182-84°. It gave a deep violet color with alcoholic ferric chloride solution.

Synthesis of (2, 6-dihydroxy-3, 5-diacetyl phenyl) azo benzene (II) from (I)

To a cooled (0°-5°) solution of aniline (1.0 g=0.97 ml) in a mixture of concentrated hydrochloric acid (3.0 ml) and water (5.0 ml) was added dropwise and with stirring a cold solution of sodium nitrite (1.0 g) in water (5.0 ml). The cold diazonium salt solution was then added slowly to a cooled solution of 4,6-diacetyl resorcinol (I) (1.0 g; 5.15 m mol) in 20% sodium hydroxide solution (8.0 ml) with stirring. A red colored dye which separated out was filtered, washed with water and crystallized from methanol to give II.
(0.5 g; 32.67%) m. p. >350°. It was found to be TLC pure (irrigant 'e') and gave a positive test for nitrogen on sodium fusion test.

Synthesis of 2' methyl (2,6-dihydroxy-3,5-diacetyl phenyl) azobenzène (III) from (I)

o-Toluidine (1.0 g=0.99 ml) was diazotised as described above. The diazonium salt solution was added slowly to a cold solution of I (1.0 g; 5.15 m mol) in 20% sodium hydroxide solution (10.0 ml) with stirring. A red dye which separated out was filtered, washed with water and air dried. It was crystallized from ethanol to give III (0.4 g; 25%) m. p. 234-36° which was TLC pure (irrigant 'e') and gave a positive test for nitrogen on sodium fusion method.

Synthesis of 2' methoxy (2,6-dihydroxy-3, 5-diacetyl phenyl) azobenzene (IV) from (I)

o-Anisidine (1.0 g=0.91 ml) was diazotised and coupled with 4,6-diacetyl resorcinol (I) (1.0 g; 5.15 m mol) as above. A red dye which separated out was crystallized from methanol and acetic acid mixture (5:1) to give a crystalline compound IV (0.7 g; 41.42%) m. p. 226-28° which was TLC (irrigant 'e') pure and gave a positive test for nitrogen by sodium fusion method.

Synthesis of 4'-(5-methylisoxazole-3-amino sulphonyl )–(2,6-dihydroxy-3,5–diacetyl phenyl) azobenzene (V) from (I)

Sulfamethoxazole (1.0 g; 3.95 m mol) was diazotised to give the diazonium salt solution as described above. It was added, slowly and with stirring, to a cold solution of I (1.0 g; 5.15 m mol) in 20% aq. sodium hydroxide solution (10 ml). A deep red colored dye which precipitated out was filtered, washed with water and crystallized from a mixture of methanol and acetic acid to give red colored needles of V (1.3 g; 55.08%) m. p. 240-42°. It was TLC pure (irrigant 'e') and gave a positive test for nitrogen and sulphur by sodium fusion method.
Synthesis of 4'-acetyl aminosulphonyl (2,6-dihydroxy-3,5-diacetyl phenyl) azobenzene (VI) from (I)

Sulfacetamide (1.0 g; 4.67 m mol) was diazotised as described above and the diazonium salt solution added slowly and with stirring to a cold solution of I (1.0 g; 5.15 m mol) in 20% aq. sodium hydroxide (10 ml). A red colored dye which separated out was filtered, washed with water and crystallized from methanol to give VI (0.8 g; 37.20%) m. p. >350°. It was found to be TLC pure (irrigant 'e') and gave a positive test for nitrogen and sulphur by sodium fusion method.

Synthesis of 4,5-dimethoxy pyrimidinyl 6-aminosulphonyl (2,6-dihydroxy-3,5-diacetyl phenyl) azobenzene (VII) from (I)

Sulfadoxine (1.0 g; 3.22 m mol) was diazotised as described in earlier cases. The diazonium salt solution of sulfadoxine was added slowly and with stirring to a cold solution of I (1.0 g; 5.15 m mol) in 20% aq. sodium hydroxide solution (10 ml). A red colored dye which separated out was filtered, washed with water and crystallized from methanol to give VII (0.8 g; 30.8%) m. p. >350°. It was TLC pure (irrigant 'e') and gave a positive test for nitrogen and sulphur by sodium fusion method.
Anti-microbial activity:

The microbiological testing of the synthesized compounds was done by agar diffusion method (cup plate method) against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* NCTC 8236, and *Escherichia coli*. The medium used was meat peptone agar medium.

Washed spores of these organisms were added into sterile and cooled media at 45° and these seeded media were poured into plates and allowed to solidify. Stainless steel cylinder of 8 mm dia. (presterilized) was used to bore the cavities. All the synthesized compounds (100 µg/ml) mentioned in Table-6 serially were placed in the cavities with the help of micropipettes and allowed to diffuse for one hour. These plates were incubated at 37° for twenty four hours. Solvents only were poured as control. Norfloxacin (10 µg) was used as a reference drug.

The plates were observed after twenty four hours. The plate showing zones of inhibition have been indicated with +ve sign in Table-6.

**Minimum Inhibitory Concentration**

Minimum Inhibitory Concentration (MIC) is the highest dilution which fails to show microbial growth. MIC of three compounds (Table-7) was determined against *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* NCTC 8236 by turbidity method, as described in Indian Pharmacopoeia, 1985,
for microbiological assay of antibiotics and MIC method as described in Medical Microbiology by R. Cruickshank.

Method:

Dilutions of the compounds from stock solution of 1 mg/ml to 1:20 (50 µg/ml), 1:50 (20 µg/ml), 1: 100 (10 µg/ml) were prepared.

To each of a series of sterile stoppered test tubes a standard volume of nutrient broth medium was added. A solution of the compounds as mentioned in Table-7 was prepared in broth and a series of doubling dilutions prepared with sterile pipettes. A control tube containing no anti-microbial agent was included. The inoculum consisting of an overnight broth culture of both Bacillus subtilis and Staphylococcus aureus was added to separate tubes. The tubes were incubated at 37° for 24 hours and examined for turbidity. The tube with the highest dilution showing no turbidity was the Minimum Inhibitory Concentration (bacteriostatic).

Discussion

All the substituted azobenzenes synthesized were tested for antimicrobial activity against S. aureus, B. subtilis and E. coli at a concentration of 100 µg/ml. Three of the test compounds V, VI and VII which inhibited one or more of the above micro-organisms were selected for determining their Minimum Inhibitory Concentration. All the three compounds had MIC-50 µg/ml. There was however no significant improvement observed over the activity of the parent sulfonamides from which the above three compounds were synthesized.
### Table-6: Anti-microbial activity of compounds at 100 μg/ml concentration

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound (Treatment)</th>
<th>Activity against</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>VI</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>VII</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Norfloxacin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sulfamethoxazole</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Sulfacetamide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Sulfadiazine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>DMF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>CHCl₃</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table-7: Minimum Inhibitory Concentration

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound (Treatment)</th>
<th>MIC against</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>1</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>VI</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>VII</td>
<td>+</td>
</tr>
</tbody>
</table>

+= 50 μg/ml − = above 50 μg/ml
Substituted azobenzenes namely compound nos. II, III, IV, V, VI and VII were synthesized by coupling 4,6-diacetyl resorcinol with diazonium salts of aromatic amines including a few sulfonamide derivatives.

The variations in the aryl ring derived from the amine component are the substituents – CH$_3$, OCH$_3$ groups sulfonamide moieties.

The structures of all the compounds were established on the basis of UV, IR, NMR and/or Mass spectral data. Anti-microbial activity of all these compounds was tested against *S aureus*, *B. subtilis* and *E. coli*. Compound no. V, VI and VII were found to have a MIC of 50 μg/ml against *S. aureus*. 