PART - I

INTRODUCTION

CHAPTER - I

PYRAZOLINES

In 1883 Knorr\(^1\) named a five membered ring containing adjacent nitrogen atoms as Pyrazole. The dihydropyrazole is called as Pyrazoline.

\[
\begin{align*}
\text{H} & \quad \text{Pyrazole} \\
\text{H} & \quad \text{Pyrazoline}
\end{align*}
\]

Nitrogen substituted pyrazoline exhibits tautomeries. Tautomers 'A' (\(\Delta^2\)-pyrazoline) and 'B' (\(\Delta^1\)-pyrazoline) are stable. But there are no evidences for the stability of tautomer C.

\[
\begin{align*}
\text{(A)} & \quad \text{(B)} \\
\text{(C)}
\end{align*}
\]

From last 45 years the pyrazole ring has attracted much attention because of its diverse properties. Beside the traditional interest in pyrazole derivatives, which has been the base of numerous dyes and drugs, a number of pyrazole derivatives as anaesthetics have been synthesised\(^2,3\).

Pyrazolines were formerly considered only as intermediates in the synthesis of pyrazoles but these have come to notice for their use as an effective bleaching agents, and as luminescent and fluorescent**, and as oxidised forms in development of cine films³ apart from drugs.

**SYNTHESIS OF PYRAZOLINES:**

Pyrazolines can be easily synthesised by the action of hydrazine or phenylhydrazine on α,β-unsaturated carbonyl compounds like chalcones (1) and flavanones in different solvents like DMSO, acetic acid, ethanol "containing a little HCl. Hydrazones or phenyl hydrazones (1a) are formed as intermediates and are subsequently cyclised to pyrazolines⁴ (1b). The rate of cyclisation of hydrazone to pyrazoline varies with the substituents R₁ in the order

\[ R₁ = \text{Ph} > \text{Me} > \text{H}^{11,12} \]

4. Harries and Jablonski
6. Andrishchov, E. A., Boroni, E. E., Kovyraia, K. A.,
7. Hanson, G. A.
8. Nounhoeffer, O., Alsdorf, G. and Ulrich, N.,
10. Knorr, L.,
11. Raiford, L. C. and Peterson, W. S.,
Benzal acetophenone$^{13}$ (2) on treatment with an excess of hydrazine in boiling acetic acid gave N-acetyl-3,5-diphenyl pyrazoline (2a) which can be immediately converted into 3,5-diphenylpyrazoline (2b).

\[
\begin{array}{c}
\text{Ph} - \text{C} - \text{CH} = \text{CH} - \text{Ph} \\
\xrightarrow{\text{NH}_2\text{NH}_2 / \text{ACOH}} \\
\text{Ph} - \text{N} = \text{N} - \text{Ph} \\
\text{COCH}_3
\end{array}
\]

Under normal conditions, substituted hydrazines and chalcones give hydrazone derivatives. 2,4-Dinitrophenyl hydrazone of chalcone has been reported by Dhar$^{14}$ and Yaraslawsky$^{15}$. The ring closer takes place with the substituted pyrazolines from chalcone and hydrazine derivatives only under vigorous conditions.

Baker et al.$^{16}$ have reported the formation of 1-phenyl-(2-hydroxy-4-methoxy phenyl)-3-methyl pyrazoline (3a) from 2-hydroxy-4-methoxy-styryl methyl ketone (3) and phenyl hydrazone. Gheorghiu and Matei$^{17}$ have attempted to correlate the ease of formation of pyrazoline with stereoisomeric forms of phenyl hydrazone. The syn form (3b) (also called non prototropic) cannot be converted into pyrazoline. It is known that pyridine$^{18}$ does not retard the conversion of syn-form to pyrazoline.

The presence of an electron donating group such as hydroxyl, alkoxy and amino or either of the phenyl ring or benzalacetophenone makes the phenylhydrazone more labile and it can seldom be isolated\textsuperscript{19,21,22}. The electron withdrawing group like nitro and halogen stabilize the hydrazones\textsuperscript{10,20,21,22}. Unsymmetrically substituted dibenzalacetone does not yield an intermediate hydrazone and gives the corresponding pyrazoline directly\textsuperscript{23,24}.

The mechanism of the formation of pyrazoline form $\alpha,\beta$ - unsaturated ketone (4) by 1,2-addition of phenylhydrazine through an adduct intermediate has been reported by Aubaguc et al.\textsuperscript{25}. The rate determining step in the dehydration of the adduct (4a) giving hydrazone (4b) which has been supported from the activation energy calculation\textsuperscript{26} of 1,3,5-triphenyl pyrazoline (4c). The cyclisation of phenyl hydrazone is shown to be spontaneous.


Tischenko, V. G.,
Phenylhydrazine and flavanone (5) reacts to form flavanone phenylhydrazone (5a), 2-hydroxychalcone phenylhydrazone (5b) and 3-(o-hydroxyphenyl)-1,5-diphenyl pyrazoline (5c). This phenyl hydrazone undergoes thermal rearrangement to pyrazoline (5c).

Borkhade et al.\textsuperscript{30} have synthesised 3,5-diaryl-1-phenyl pyrazolines by the action of phenyl hydrazine hydrochloride in pyridine medium. It has been shown that pyridine has no effect on the rate of cyclisation of the hydrazine to pyrazoline\textsuperscript{31}.

The synthesis of 3,5-diaryl-1-phenyl pyrazoline (6a) from chalcone dibromide (6) and phenyl hydrazine hydrochloride in DMF has been reported by Joshi and Wadodkar\textsuperscript{31}.

\[
\begin{array}{c}
\begin{array}{c}
\text{Ph}\text{NH\textsubscript{2}HCl} \\
\text{D.M.F.}
\end{array} \\
\begin{array}{c}
\text{Ph}\text{N}\text{H\textsubscript{2}HCl} \\
\text{D.M.F.}
\end{array}
\end{array}
\]

Pyrazolines\textsuperscript{32} and phenyl pyrazolines\textsuperscript{33} (7b) can be obtained from flavanones (7) also, but the completion of the reaction depends upon the pH, temperature and the quantity of hydrazine added other wise flavanoneazine (7a) is formed.

\[
\begin{array}{c}
\begin{array}{c}
\text{Flavanoneazine} \\
\text{Phenyl pyrazoline}
\end{array} \\
\text{Ph}
\end{array}
\]

The synthesis of 3, 5-diaryl-1-phenyl pyrazolines (7e) from 2-hydroxychalcone (7c) and flavanones (7d) in pyridine medium by the action of phenyl hydrazine hydrochloride has been reported by Borkhade and Marathey\textsuperscript{34}.

\textsuperscript{34} Borkhade, K.T. and Marathey, M.G. \textit{Indian J. Chem.}, 10 (1972), 48
The above reaction has also been carried out using phenyl hydrazine hydrochloride in DMF and DMSO.

Jamode reported the formation of 2-hydroxy chalcone phenyl hydrazone (8a) by the action of phenyl hydrazine hydrochloride on flavanones (8) using ethylene diamine in ethanol solvent and its subsequent conversion to 3,5-diaryl-1-phenyl pyrazoline (8b).

35. Wadodkar, K. N.,
36. Kakade, B. S. and Jamode, V. S.,
37. Jamode, V. S.,

Ph.D. Thesis "Synthesis in heterocyclic compounds". Nagpur University (1977)
Gamil Aziz et al. has reported the synthesis of \( \Delta^2 \)-pyrazolines (9b) and corresponding isomer (9d) from furochalcones (9) and hydrazine hydrate in acetic acid and ethanol respectively. They proposed 1,2-addition mechanism in acetic acid and 1,4-addition mechanism in ethanol.

\[
\begin{align*}
\text{C—CH=CH—Ar} & \quad \text{NH}_2\text{NH}_2\text{OH} \\
\text{(9)} & \quad \text{(1,2 addition)} \\
\text{OCH}_3 & \quad \text{OH} \\
\text{H} & \quad \text{H} \\
\text{Ar} & \quad \text{Ar} \\
\text{(9a)}
\end{align*}
\]

\[
\begin{align*}
\text{C—CH=CH—Ar} & \quad \text{NH}_2\text{NH}_2\text{OH} \\
\text{(9c)} & \quad \text{(1,4 addition)} \\
\text{OCH}_3 & \quad \text{OH} \\
\text{R} & \quad \text{H} \\
\text{Ph} & \quad \text{Ar} \\
\text{(9b)} & \quad \text{(9d)}
\end{align*}
\]

Joshi et al. reported the formation of 4-bromo-3-(2-hydroxyphenyl)-1,5-diphenyl pyrazolines (10a) from 3-(2'-hydroxyphenyl)-1,5-diphenyl pyrazolines (10) and NBS in CCl₄.

\[
\begin{align*}
\text{R}_1 & \quad \text{OH} \\
\text{R}_2 & \quad \text{N}—\text{N}—\text{N}—\text{Ph} \\
\text{R}_3 & \quad \text{N}—\text{N}—\text{Ph} \\
\text{(10)} & \quad \text{R}_1, \text{R}_2, \text{R}_3 \\
\text{Br} & \quad \text{N}—\text{N}—\text{Ph} \\
\text{(10a)} & \quad \text{R}_1, \text{R}_2, \text{R}_3
\end{align*}
\]

Hammouda H. A. et al.\textsuperscript{40} have synthesised new heterocyclic compounds bearing a pyrazole moiety on isoxazoline (11b) and pyrazoline (11a) ring from 4-cinnamoyl-1,5-diphenyl-3-methyl pyrazole(11).

Chalcone on epoxidation gave epoxyketones which on reaction with NH$_2$NH$_2$, Ph NH NH$_2$ and Ph NH CO NH$_2$ to gave pyrazolines\textsuperscript{41} (12).

\begin{align*}
\end{align*}
Nair\textsuperscript{42} reported the synthesis of 4-arylo-3,5 diaryl pyrazolines (13 a-b) by the action of hydrazinehydrate and phenyl hydrazine on 3-(2'-furoyl) flavanones (13) in ethanol.

\[
\begin{align*}
\text{Nair} & \quad \text{reported the synthesis of 4-arylo-3,5 diaryl pyrazolines (13 a-b) by the action} \\
& \quad \text{of hydrazinehydrate and phenyl hydrazine on 3-(2'-furoyl) flavanones (13) in ethanol.}
\end{align*}
\]

Ozawa et al.\textsuperscript{43} reported the synthesis of pyrazoline (14) which was found to be very effective in killing house flies on contact.

\[
\begin{align*}
\text{Ozawa et al.} & \quad \text{reported the synthesis of pyrazoline (14) which was found to be very} \\
& \quad \text{effective in killing house flies on contact.}
\end{align*}
\]

Van Hes et al.\textsuperscript{44} synthesised the pyrazoline (15) which was found to have insecticidal properties.

\[
\begin{align*}
\text{Van Hes et al.} & \quad \text{synthesised the pyrazoline (15) which was found to have insecticidal} \\
& \quad \text{properties.}
\end{align*}
\]

\textbf{References:}


Kramer et al.\textsuperscript{45} also synthesised chloro substituted pyrazoline (16) which was found to be an effective insecticide against rice weevil Sitophilus oryzae dominica, S. Granarius etc.

\begin{equation}
\text{Cl} \quad \text{C} \quad \text{N}
\end{equation}

\begin{equation}
\text{O} = \text{C} \quad \text{NH} - \text{Q} - \text{Cl}
\end{equation}

(16)

Therapeutically active 3-amino-1-phenyl and substituted phenyl-2-pyrazolines\textsuperscript{46} (17) were synthesised from alkenenitriles and phenylhydrazine and found anti-inflammatory bactericidal pharmaceutical and fungicidal agents.

\begin{equation}
\text{NH} \quad \text{C} \quad \text{N} \quad \text{R}
\end{equation}

\begin{equation}
\text{R} , \text{NH} - \text{C} - \text{NH} - \text{R}
\end{equation}

(17)

Suratkumar and Rastogi\textsuperscript{47} reported the anti-implantation activity of 4,5-trans/cis-1-acetyl-3,5 diaryl-4-phenylpyrazolines. (18).

\begin{equation}
\text{R} \quad \text{C} \quad \text{N}
\end{equation}

\begin{equation}
\text{O} = \text{C} - \text{CH}_3
\end{equation}

(18)

\textbf{References:}


Ankhiwala et al.\textsuperscript{48} prepared substituted pyrazolines (19) (tested for bacterial activity), by cyclocondensation of $R \text{C}_6\text{H}_4 \text{CH} : \text{CHCO-C}_6\text{H}_2 (\text{NO}_2) (\text{OBu}) - \text{OH}$-5, 4, 2.

\begin{equation}
\text{(19)}
\end{equation}

(R = H, 2-Br, 4-Me, N)

Ayoub, Mikdodt\textsuperscript{49} reported some substituted 3-aryl-5-methyl-(or 4,5-dimethyl)-2-pyrazolines (20a) from chromones (20) with hydrazine.

\begin{equation}
\text{(20)}
\begin{array}{c}
\text{R}_1 \\
\text{R}_2 \\
\text{R}_3
\end{array}
\end{equation}

\begin{equation}
\text{(20a)}
\end{equation}

Tayade V. B. and Jamode V. S.\textsuperscript{50} have prepared 3,5-diaryl-1-phenyl pyrazoline (21a) by the reaction of 2-hydroxy acetophenone (21) with phenyl hydrazine in alcoholic medium.

\begin{equation}
\text{(21)}
\begin{array}{c}
\text{R}_1 \\
\text{R}_2
\end{array}
\end{equation}

\begin{equation}
\text{(21a)}
\end{equation}

In the same way 3,5-diaryl-1-pyridoyl pyrazoline\textsuperscript{50} (22a) were prepared by the reaction of 2-hydroxy acetophenone(22) with isoniazide in alcoholic medium with a drop of piperidine.

\begin{itemize}
\item [48.] Ankhiwala, M. D., Naik, H. B. \hfill J. Inst. Chem. (India), 1988, 60 (6), 235-6 (Eng).
\item [49.] Ayoub, Mikdodt. \hfill Chem. abstr. 112 (1990), 55611n.
\end{itemize}
Uthale P. S. et al.\textsuperscript{51} had prepared 1-corboxamido-3-(substituted-2-hydroxy phenyl)-5-aryl-\(\Delta^2\)-pyrazolines (23a) by reacting 2-hydroxy-5-chlorochalcone (23) with semicarbazide hydrochloride in alcohol medium.

Deshmukh M. S. et al.\textsuperscript{52} had synthesised some chloro substituted - \(\Delta^2\)-pyrazoline (24a) from 2'-hydroxy 3'-5'- dichlorochalcone (24) and phenyl hydrazine in DMSO.

Kadu V. B. et al.\textsuperscript{53} have prepared 3-(substituted 3,4-benzophenyl)-4-(substituted phenyl) pyrazolines (25) and its derivatives (25a, b, c) from 2-hydroxy-3,4-benzo substituted chalcones and hydrazine hydrate in alcoholic medium.

Raghuwanshi P. B. et al.\textsuperscript{54} had reported the synthesis of some nitro pyrazolines \((26, 26a)\) by the action of \(\text{CH}_3\text{COOH}\) (ethanol) and \(\text{C}_6\text{H}_5\text{COCl}\) (pyridine).

Barot V. M.\textsuperscript{55} have prepared 1-H-3-(2'-hydroxy-4'-ethoxy-5'-bromo phen-1'-yl) -5-substituted phenyl -2-pyrazolines \((27)\) and their derivatives \((27a-d)\) from 2'-hydroxy-4' -ethoxy-5'-bromo-4-substituted phenyl chalcone on hydrazine hydrate in alcoholic medium.

Ankhiwala, M.D. et al.\textsuperscript{56} had reported 1-phenyl-3, 5-diaryl-1,2-pyrazoline (28) from C\textsubscript{6}H\textsubscript{5}NH.NH\textsubscript{2} and 1, 3-diaryl chalcones.

CHAPTER -II
PYRAZOLES

Pyrazole (29A) is a class of organic compounds, which consist of 5 membered ring containing adjacent nitrogen atom. It can also be consider as an isoxazole nucleus (29B) in which -O- has been replaced by -NH- grouping. The name pyrazole was proposed by Knorr.  

Pyrazoles (30b) are commonly synthesis by the action of hydrazine or substituted hydrazines on 1,3-dicarbonyl compounds (30). It has been reported that monohydrazone is the intermediate in this reaction. These monohydrazone (30a) have been converted by heat or acid treatment into corresponding pyrazoles.  

\[ \text{R—C—CH}_2—C—R} \xrightarrow{\text{NH}_2\cdot\text{NH}_2} \text{R—C—CH}_2—C—R \xrightarrow{\text{Acid}} \text{R—C—C}—\text{NH}_2 \xrightarrow{\text{Heat}} \text{R—C—C}—\text{N}—\text{NH}_2 \]

Isomeric pyrazoles (31a, 31b) have been reported during the reaction of monosubstituted hydrazine and unsymmetrical diketones (31) but only in few cases\textsuperscript{60,68,69} attempt have been made to determine the identity of products (31a) or (31b).

\[
\begin{align*}
R_1 - \overset{\text{O}}{\text{C}} - \overset{\text{O}}{\text{CH}_2} &\quad \overset{\text{R}_2 \text{NH.NH}_2}{\rightarrow} \quad \overset{\text{R}_2}{\text{N}} \quad \overset{\text{R}_3}{\text{C}} - \overset{\text{O}}{\text{CH}_2} - \overset{\text{R}_1}{\text{C}} \quad (31) \\
&\quad \overset{\text{R}_3}{\text{N}} \quad \overset{\text{R}_2}{\text{C}} \quad \overset{\text{O}}{\text{CH}_2} - \overset{\text{R}_1}{\text{C}} \quad (31a) \\
&\quad \overset{\text{R}_3}{\text{N}} \quad \overset{\text{R}_2}{\text{C}} \quad \overset{\text{O}}{\text{CH}_2} - \overset{\text{R}_1}{\text{C}} \quad (31b)
\end{align*}
\]

Benzyl acetone (32) with methyl hydrazine gives a mixture of 1, 3-dimethyl-5-phenyl pyrazole (32a) and 1, 5-dimethyl-3-phenyl pyrazole (32b) but with phenyl hydrazine only 1, 5-diphenyl-3-phenyl pyrazole (32c) had been reported\textsuperscript{68,69}

\[
\begin{align*}
\text{Ph} - \overset{\text{O}}{\text{C}} - \overset{\text{O}}{\text{CH}_2} &\quad \overset{\text{CH}_2\text{NH.NH}_2}{\rightarrow} \quad \overset{\text{CH}_3}{\text{N}} \quad \overset{\text{CH}_3}{\text{C}} - \overset{\text{O}}{\text{CH}_2} - \overset{\text{CH}_3}{\text{C}} \quad (32)
\end{align*}
\]

\[
\begin{align*}
\text{PhNH.NH}_2 &\quad \Downarrow \quad \text{Ph} \quad \overset{\text{CH}_3}{\text{N}} \quad \overset{\text{CH}_3}{\text{C}} - \overset{\text{O}}{\text{CH}_2} - \overset{\text{CH}_3}{\text{C}} \quad (32a) \\
&\quad \Downarrow \quad \text{Ph} \quad \overset{\text{CH}_3}{\text{N}} \quad \overset{\text{CH}_3}{\text{C}} - \overset{\text{O}}{\text{CH}_2} - \overset{\text{CH}_3}{\text{C}} \quad (32b)
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C} - \overset{\text{N}}{\text{C}} - \overset{\text{Ph}}{\text{Ph}} &\quad (32c)
\end{align*}
\]

It has been observed that the reaction of $\text{NH}_2$-$\text{NH}_2$ on acetylenic carbonyl compound is the direct method for the synthesis of pyrazole$^{70}$. The reaction of hydrazine on $\alpha, \beta$-acetylenic carbonyl compounds (33), generally gives 1, 3 or 1, 5 disubstituted pyrazoles (33b). Morrachi et al.$^{71}$ have isolated an intermediate hydrazone (33a) which on treatment with triethylamine gave pyrazole (33b).

The formation of substituted pyrazoles from 1, 3-dicarbonyl compounds and substituted hydrazines had been received by Jacobs\(^\text{72}\) and also by Finar and Simond\(^\text{73}\). It has been reported that, the formation of isomeric pyrazoles depends on steric hindrance electromeric effect of groups and also on the pH of reaction medium\(^\text{74-78}\).

\(\alpha\)-substituted - \(\beta\)-diketones with phenyl hydrazine are reported to give 4- substituted pyrazoles. Thus 2-hydroxy -1-phenyl 1, 3-butadiene (34) with phenyl hydrazine gives 4-hydroxy -3- methyl 1, 5- diphenyl pyrazole\(^\text{79}\) (34a). 4-Bromo pyrazole have also been reported from \(\alpha\)-bromo \(\beta\)-diketone\(^\text{80,81}\).

\[
\begin{align*}
\text{Ph} &- C - \text{CH} - C - \text{CH}_3 + \text{Ph - NH - NH}_2 \rightarrow \\
&\begin{array}{c}
\text{N} \\
\text{Ph} \\
\text{OH} \\
\text{Ph}
\end{array}
\end{align*}
\]

(34)

(34a)

Cyclic \(\beta\)-diketone with substituted phenylhydrazine are also reported to give two isomeric pyrazole derivatives. Thus 2-acetylcyclohexanone with DNPH give two isomeric pyrazole derivatives. It has been suggested that DNPH reacts primarily on the oxo-group or the enol form of it\(^\text{82}\).

\(\beta\)-substituted \(\alpha\), \(\beta\)-unsaturated corbonyl compounds (35) which may be regarded as the enol form of \(\beta\)-dicarbonyl compounds are reported\(^\text{83}\) to give substituted pyrazoles (35a) on reaction with hydrazine or substituted hydrazine.

72. Jacobs, T.L.,
73. Finar, I.L. and Simond, A.B.,
74. Knorr, L.,
75. Brady, O.L.,
76. Rotherberg, R.V.,
77. Joshi, S.S. and Gambhir, I.R.,
78. Garg, H.G. and Joshi, S.S.,
80. Keller, H. an Halbeen H.V.,
81. Garg, H.G. and Joshi, S.S.,
82. Estert, B. and Wessendorf, R.,
83. Von Auwers,K.and Dietrich,K.,

Heterocyclic compounds, edited by R.C. Elder field, (1957), 50.
An interesting synthesis of pyrazoles was (36a-e) reported from epoxides of α, β-
unsaturated ketones (36) with hydrazine or phenylhydrazine.

Chalcone dibromides (37) when reacted with hydrazine or substituted hydrazine gave
substituted pyrazoles (37a) of unequivocal structures.

Wadodkar and Doifode\textsuperscript{91} reported the formation of 1-(2,4-dinitrophenyl)-5-(2-hydroxy-4-methoxy phenyl)-pyrazole by the intermediate of 2'-hydroxy-4-methoxy dibromide methane and DNPH acetic acid containing a little concentrated H\textsubscript{2}SO\textsubscript{4}.

The reaction of hydrazine on chromone (38), thiochromone (38a) or flavone (38b) is one of the best method for the synthesis of 3-o-hydroxy phenyl -5-alkyl or 5-aryl pyrazole\textsuperscript{92,93}\textsuperscript{c and d}.

Formerly the product obtained from the above reaction was assumed to be chromonephenyl hydrazone\textsuperscript{94}, but were later found to be 3, 5-disubstituted pyrazoles\textsuperscript{92,93,95}. The exact mechanism is still uncertain but it is believed that the first step is the addition of Ph.NH.NH\textsubscript{2} on 2, 3 double bond of chromone or flavone\textsuperscript{92}.

Substituted pyrazoles and their salts\textsuperscript{96} were prepared by diazotization of p-toludine and treatment with MeCOCHCl CO\textsubscript{2}Et, with \alpha-marpolino styrene followed by heating.

\begin{itemize}
\item 95. Koangs, E. and Fround, J., Chem. Ber., 80 (1947), 143.
\item 96. Arhens., Chem. abstr. 88 (1978), 152615j.
\end{itemize}
with dioxane and HCl to gave an intermediate, later was reduced and treated with HBr, HCN and hydrolysed to gives  pyrazoles (39). \((R_3 = \text{CH}_2\text{OH})\).

\[
\begin{align*}
\text{Ph} & \quad \text{N} \\
\text{N} & \quad \text{Me} \\
\text{R}_3 & \quad \text{N}
\end{align*}
\]

(39)

Thakar et al.\(^9^7\) have synthesised some 4-aroyl-3- aryl-5-(O-hydroxyphenyl) - pyrazole (40a) by the condensation of 2-aroyl chromones (40) with \(\text{NH}_2\text{NH}_2\text{H}_2\text{O}\) in methanol.

\[
\begin{align*}
\text{O} & \quad \text{R}_2 \\
\text{COR}_3 & \quad \text{MeOH} \\
\text{NH}_2\text{NH}_2 & \quad \text{OH} \\
\text{COR}_1 & \quad \text{N} \\
\text{Ph} & \quad \text{R}
\end{align*}
\]

(40)

(40a)

Fateen\(^9^8\) reported the preparation of pyrazoles (41a) from pyrazolines (41) with \(\text{POCl}_3\).

\[
\begin{align*}
\text{N} & \quad \text{NPh} \\
\text{R} & \quad \text{H} \\
\text{CON} & \quad \text{Me, Ph; } R_1 = \text{Cl}
\end{align*}
\]

(41)

(41a)

Borkhade and Marathey\(^9^9\) have synthesised pyrazoles (42b) from o-hydroxy dibenzoyl methanes (42) or chalcone dibromide (42a) and phenyl hydrazine in pyridine medium.

\[\text{References}\]

Krentzberger\textsuperscript{100} have synthesised aminocyanopyrazoles (43, 43a) by reaction of EtOCH\(\text{C}(\text{CN})_2\) with R\(_n\)C\(_{6-8}\)H\(_{5-n}\)NH NH\(_2\), cyclised with guanidine. Both pyrazoles inhibit the growth of E. coli.

Chincholkar and Jamode\textsuperscript{101} reported the synthesis of 3-aryl -4-aroyl -5-(2-hydroxy phenyl) -1-phenyl pyrazoles (44a) by the condensation of 3-aroyl flavanones (44) and Ph.NH.NH\(_2\) in methanol.

Chincholkar and Jamode\textsuperscript{102} have reported the synthesis of 3-(2-hydroxy aryl) -4- aroyl -5-aroyl -1-phenyl pyrazoles (45a) by the interaction of 3-aroyl flavanones (45) and Ph.NH.NH\(_2\) HCl in pyridine medium.

\textsuperscript{100} Krentzberger, Chem. Abstr. 92 (1980), 198346h.
Chloromethyl pyrazoles\(^{103}\) (46a) and their substituted analogs were prepared by refluxing pyrazolines (46) with AgCl and Ca (OH)\(_2\) in dioxane and reported to have antibacterial activity.

Ishihara Sangyo Kaisha Ltd\(^{104}\) prepared pyrazoles (47a) by rearrangement of (47) in the presence of alkali metal or alkaline earth metal hydroxide or carbonates.

Gaggad\(^{105}\) synthesised 1-phenyl-3-(2-hydroxy phenyl)-5-styryl pyrazoles (48b) from 2-hydroxy benzoyl cinnamoyl methanes (48) and phenylhydrazine in ethanol. The same pyrazoles could also be prepared from styryl chromones (48a) with phenyl hydrazine hydrochloride in pyridine medium.

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103. Kumai Chemical.,
104. Ishihara Sangyo Kaisa Ltd.,
105. Gaggad, H.L.,
Chem. Abstr. 94 (1981), 47319S
Sankyo Co. Ltd.\textsuperscript{106} synthesised chloropyrazoles (49a) by rearrangement of (49) in presence of $\text{AlCl}_3$ or $\text{Me}_3\text{COK}$ imidazole mixture.

![Chemical structure of 49 and 49a](image)

Nair\textsuperscript{107} has synthesised new pyrazoles (50a) from 1-(2'-furyl)-3-(2"-hydroxyphenyl)-1, 3-propanediones (50).

![Chemical structure of 50 and 50a](image)

Paranjape\textsuperscript{108} have reported 3-(2-hydroxy-3, 4-benzophenyl)-5-phenyl pyrazole (51a) from 2-hydroxy-3, 4-benzodibenzoyl methanes (51).

![Chemical structure of 51 and 51a](image)

Sharma et al.\textsuperscript{109} has used lead tetraacetate in benzene as oxidising agents to convert 1-phenyl -3-(2-hydroxy phenyl) -5- arylpyrazolines (52) to corresponding pyrazoles (52a).

\begin{itemize}
\item \textsuperscript{106} Sankyo Co. Ltd., Chem. Abstr. 97 (1982), 182410s.
\item \textsuperscript{107} Nair, S.B. and Wadodkar, K.N., Indian J. Chem., 21B (1982), 573 -A.
\item \textsuperscript{108} Paranjape, M.V., "$\alpha$- naphthyl analogs of flavonoids in the synthesis of 'O' and 'N' hetrocyclics", Ph.D. Thesis, Amravati University (1986).
\end{itemize}
3, 5-Diphenyl or substituted 1,3,5-triphenyl pyrazolines on oxidation yield corresponding pyrazoles. Chloromic oxide\(^{100}\), bromine\(^{100a}\), \(\text{Kmno}_4\)^{100b}, \(\text{AgNO}_3\)^{100c}, Potassium ferricyanide\(^{100d}\), lead oxide\(^{100e}\) and \(\text{MnO}_2\)^{111} have been used as oxidising agents in this reaction.

Many drugs and dyes contain the pyrazole nucleus, so this class of compounds has been widely studied and continues to attract even today much attraction. Pyrazoles are known for their physiological activities\(^{112-118}\). Herbicidal composition containing pyrazole derivatives like, acylaminopyrazoles were prepared by Seki et al.\(^{119}\) (53)

\[\text{R} - \text{C} - \text{NH} - \text{C} - \text{H} (53)\]

\[\text{(CH}_3\text{)}_2\text{C} - \text{NH} - \text{C} - \text{O} - \text{F} (53)\]

100. Von Auwers, K and Heimke, P.R., Liebig's Ann., 458 (1927), 186.
100b. Strauss, F., Miffat, C. and Heitz, W., Ber., 51 (1918), 1547.
100c. Buchner, E. and Dessamer, H., Ber., 26 (1893), 258.
Trifluromethyl -1-arylpyrazoles have been reported to be analgesic, antipyretic and anti-inflammatory agents\textsuperscript{120}

Asahi Chem. Industry\textsuperscript{121} Jpn prepared chloropyrazoles (54) by esterification with ROH. It showed biological activities against E.Coli, S. Juncoide, Sagattaria etc.

\[
\begin{align*}
\text{Me} & \quad \text{N} & \quad \text{COCH}_2 \quad \text{OR} & \quad \text{Cl} \\
\text{Me} & \quad \text{N} & \quad \text{R} & \quad \text{Cl}
\end{align*}
\]

(54)

Nissan Chem. Ind.\textsuperscript{122} Synthesised 4-[2, 4-dichlorodibenzoyl -1-methyl -5- (2-benzothiazole) methoxy] pyrazole (55) by adding 2-(chloromethyl) benzothiazole to 4-(2, 4-dichlorobenzoyl) -1-methyl -5-hydroxy pyrazole and Et\textsubscript{3}N in C\textsubscript{6}H\textsubscript{6}. It was found active against E.Coli.

\[
\begin{align*}
\text{O} & \quad \text{Cl} & \quad \text{Me} & \quad \text{N} & \quad \text{OCH}_2 \\
\text{Me} & \quad \text{N} & \quad \text{C} & \quad \text{Cl} & \quad \text{Cl}
\end{align*}
\]

(55)

Mitsubhisi Petrochem. Co.Ltd. Jpn\textsuperscript{123} prepared 1, 3-dimethyl -4- (2, 4-dichloro -3-methyl benzyl) -5- hydroxypyrazole (56a) by condensation of pyrazoline (56), 2, 6-Cl\textsubscript{2} C\textsubscript{6}H\textsubscript{3} Me and (COCl\textsubscript{2})\textsubscript{2} followed by hydrolysis of the condensation product.

\[
\begin{align*}
\text{Me} & \quad \text{N} & \quad \text{CO} & \quad \text{Me} & \quad \text{C} & \quad \text{O} & \quad \text{Cl} & \quad \text{Me} \\
\text{Me} & \quad \text{N} & \quad \text{Cl} & \quad \text{Me} & \quad \text{OH}
\end{align*}
\]

(56)

\[
\begin{align*}
\text{2,6-Cl}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{(COCl)}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{2,6-Cl}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{2,6-Cl}_2 \text{C}_6 \text{H}_3 \text{Me} \\
\text{2,6-Cl}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{(COCl)}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{(COCl)}_2 \text{C}_6 \text{H}_3 \text{Me} & \quad \text{(COCl)}_2 \text{C}_6 \text{H}_3 \text{Me}
\end{align*}
\]

\[
\begin{align*}
\text{Me} & \quad \text{N} & \quad \text{Cl} & \quad \text{Me} & \quad \text{N} & \quad \text{Cl} & \quad \text{Me} \\
\text{Me} & \quad \text{N} & \quad \text{Cl} & \quad \text{Me} & \quad \text{N} & \quad \text{Cl} & \quad \text{Me}
\end{align*}
\]

(56a)

\textsuperscript{121} Asahi Chem. Industry Co. Ltd., Chem. abstr. 98 (1983), 126072s.
\textsuperscript{122} Nissan Chem. Ind., Chem. abstr. 100 (1984) 51569h.
\textsuperscript{123} Mitsubhi petrochemi. Co. Ltd., Chem. abstr. 103 (1985), 22583g.
Nissan Chem. Ind. Ltd. Jpn\textsuperscript{124} synthesised a compound containing 5-tertbutyl-3- (2, 4-dichloro 5-isopropylphenyl) -1,3,4 oxadiazolines -2- on and pyrazoles (57) (R = halo, H, NO\textsubscript{2} or alkyl; n = o or 1, 5-integer, z = alkyne) These were tested as herbicides.

![Chemical structure of compound 57]

Faucher\textsuperscript{125} prepared (phosphodithioacetamido) phenyl pyrazoles (58) which were shown to act as insecticides.

![Chemical structure of compound 58]

Nihon Nohyaku Co. Ltd. Jpn\textsuperscript{126} reported the synthesis of chloropyrazole derivatives (59,59a) with XCH\textsubscript{2}NRCO\textsubscript{2}R\textsubscript{1} (X = halo).

![Chemical structures of compounds 59 and 59a]

Kobayashi et al.\textsuperscript{127} reported the synthesis of chloropyrazoles (60) as herbicides.

\textsuperscript{124} Nissan Chem. Ind. Ltd., Chem. abstr. 102 (1985), 2153w.
\textsuperscript{125} Faucher, L.W., Chem. abstr. 98(6) (1983) 89671p.
\textsuperscript{126} Nihon Nohyaku Co. Ltd., Chem. abstr., 102 (1985).
\textsuperscript{127} Kobayashi, Hisafumi, Kato, Motto, Nitani, Fumio., Chem. abstr., 106 (1989), 297008g.
Mittal and Singhal\textsuperscript{128} reported antimicrobial activity of substituted pyrazoles (61).

Tayade V.B. and Jamode V.S.\textsuperscript{129} have prepared 3, 5-diaryl-1-pyridoyl pyrazoles (62a) by the reaction of 2-aryloxy acetophenones (62) and isoniazide in alcoholic medium.

Tayade V. B. and Jamode V.S.\textsuperscript{130} have also prepared 3, 5-diaryl-1-phenyl pyrazoles (63a) by the reaction of 2-aryloxyacetophenones (63) and phenyl hydrazine hydrochloride in alcoholic medium.

\textsuperscript{130} Tayade V.B. and Jamode V.S., Asian J. of Chemistry Vol.9, No.8 (1997); 431-434.
Virendrer Parmar\textsuperscript{131} had reported 3, 5-disubstituted pyrazoles (64).

\begin{center}
\includegraphics[width=0.3\textwidth]{image1.png}
\end{center}

Mogha A. Abdallan et al.\textsuperscript{132} had prepared 5-amino-4 (2-benzothiazolyl) pyrazoles (65).

\begin{center}
\includegraphics[width=0.5\textwidth]{image2.png}
\end{center}


CHAPTER III

STUDIES IN PROTON - LIGAND STABILITY CONSTANTS AND METAL - LIGAND STABILITY CONSTANTS OF PYRAZOLINES AND PYRAZOLE.

In the view of analytical applications, biological activities and confirmation of -OH group, substituted pyrazolines and pyrazoles have been investigated in the present work. ‘Azoles’ are well known for their enhanced biological activity. These organic heterocycles have been extensively used as drugs in pharmaceuticals since many drugs and dyes contain pyrazole nucleus. Thus, this class has wide importance.

Pyrazoles and especially aryl pyrazoles are found to possess antidiuretic, antihelmintic activities in addition to fungicidal activity\textsuperscript{131}. Different substituted pyrazoles are reported as antimicrobial agents\textsuperscript{134}, hypolipidermic agents, antidiabetics and insecticides.

Different substituted pyrazolines are also used as anti-inflammatory agents, insecticides, bactericidal and pharmaceutical fungicides. Ozawa et al.\textsuperscript{135} reported substituted pyrazolines to be very effective in killing houseflies on contact. Pyrazolines are tested for bactericidal and fungicidal activity. Literature survey reveals the importance of pyrazolines and isoxazoles as bleaching agents and luminiscent apart from their use as drugs. Pyrazolines are weakly basic and less stable than pyrazoles.

Shelke et al.\textsuperscript{136} have investigated the interaction between UO$_2^{2+}$ (II) and Cu (II) with dicarboxylic acids in dioxane - water mixture.

Narwade et al.\textsuperscript{137} have studied the equilibrium constant of Fe (III) with substituted chalcones at 0.1 M ionic strength potentiometrically.

Sawalakhe and Narwade\textsuperscript{138} have studied role of dielectric constants on substituted

Pyrazolines complexes pH metrically. Recently Sawalakhe et al.\textsuperscript{139} have studied stability constants of Cu(II) complexes with some substituted diketones at 0.1M ionic strength by pH metric technique.

Gudadhe et al.\textsuperscript{140} have studied the formation and stability constants of Thorium (IV) complex with some substituted pyrazolines. Characters of some Cobalt(II) complexes with substituted pyrazoles have been studied by Gopal Narain et al.\textsuperscript{141}

Raghuwanshi et al.\textsuperscript{142} Showed confirmation of complex formation of N, O and S containing heterocycle with Cu(II) metal ion.

Sondawale et al.\textsuperscript{143} studied the effect of ionic strength on complex equilibria with peptides.

Tayade V. B et al.\textsuperscript{144} had studied proton -ligand and metal -ligand stability constant of 3, 5-diaryl pyrazolines, pyrazoles, isoxazolines and isoxazoles potentiometrically.

**Pyrazoles and Pyrazolines as Chelating agents:**

Pyrazoles and pyrazolines fall in the class of aromatic heterocyclic compounds and the unique structural feature involving pyrrole nitrogen and pyridine nitrogen of these ligands is normally considered to be the co-ordination site, co-ordination via pyrrole nitrogen has been reported recently\textsuperscript{145}.

The metal complex formation imparts some important characteristics to the drug which are helpful in their biological activity.

\textsuperscript{141} Gopal Narain, S. Safar, Kumar Naidu Asian Jour, of Chemistry. Vol.4, No.4 (1992), ii 75.
\textsuperscript{144} Tayde V.B. and Jamode VS., Synthesis studies of hetrocyclic containing oxygen, nitrogen and sulphur hetro atom ; Ph.D. Thesis, Amravati University, Amravati.
CHAPTER IV

ANTIMICROBIAL ACTIVITY OF PYRAZOLINES AND PYRAZOLES.

Treatment of diseases with chemical substances has been known since the 1500's. The chemical substances used for the treatment of infectious diseases and diseases caused by the proliferation of malignant cells are called as chemotherapeutic agents.

Chemical agents not only provide the structural basis and energy supply of living organisms but also regulate their functional activities. The interaction between potent chemicals and living systems contribute to the understanding of life processes and provide effective methods for the treatment, prevention and diagnosis of many diseases. Chemical compounds used for this purpose are called 'drugs' and their actions on living systems is referred to as 'drug effect'. The subject of drug is as old as disease. Sickness has been man's heritage from the beginning of his existence and search for remedies to combat is perhaps equally old. Fighting disease with drug is the timeless struggle. Its beginning echoed out to pre-iceval jungle. Man's survival on this planet is dependent upon its success.

Antibiotics are substances which are synthesised by micro-organisms and are lethal to other micro-organisms in low concentrations. An effective antibiotic must be selectively toxic to the microbial pathogen with little toxicity for the human host.

Antibacterial agents have been used in folk remedies from early time. The earliest source of medicine come from 'Egypt' and two Kingdoms of 'Assyria' and 'Babylonia'. The 'Papyri' were the first written account of medical experience from Egypt and date back to 1900 B.C. The papyrus discovered by Eber in 1872 was prepared in 1500 B.C. and mentions about 700 herbal medicines including the 'Ophium'. A Baby-lonian clay-tablet (700 B.C.) has been discovered which mentions about 300 drugs.
Modern medicine is considered to date from 'Hippo-Cratus' a Greek Physician (450 B.C.) who for the first time introduced the concept of disease as a pathologic process and tried to organize the science of medicine on the basis of observation, analysis and deduction.

Till the beginning of 19th century the treatment of disease consisted of abnoxious remedies such as fresh excreta and metallic and plant preparations. James Gregory (1753-1821) was responsible for popularising heroic symptomatic treatment consisting of blood letting, larger doses of emetics and drastic purgatives often with disastrous results. Such treatment without any rational basis was called 'Allopathy' (meaning the other suffering).

When a living system is in imbalance through distortion of normal bodily processes a pathologic condition is present, it is then that the chemical stimulus, the pharmacologic agent, the drug, is administered to attempt to reestablish the organism to a normal physiologic and chemical balance. A drug is administered to alleviate symptoms and possibly cure the diseased condition. Examples will serve further to show this relationship between the sciences.

The trihydric alcohol glycerin \( C_3H_5(OH)_3 \), as a product of digestion from edible fats the biological chemical is concerned with its fate in the body. If, however, one replaces the three hydroxyl groups in the glycerin molecule with three nitrate radicals, producing glycercyldinitrate \( C_3H_5(NO_3)_3 \), the compound serves no longer as a food. The oily glycercyldinitrate, in exceedingly small doses, dilates the blood vessels, causing a fall in blood pressure. The coronary arteries share in this dilatation and hence glycercyldinitrate serves as a drug, a therapeutic agent. It is used also by the physician in the treatment of hypertension and angina pectoris.

The concept of 'Homeopathy' was first introduced in the early 19th century by 'Hanneman' who thought that 'Like curse Like' and dilution potentiates the action of drugs. Homeopathy outlines the therapy for various ailments with drugs in very high dilution.

Various animal experiments have been designed to study the effects of drugs on
living organisms and isolated tissues and these give an insight into where and how a drug act. By knowledge of the mode of action of drug, its effect on various body systems and the probable adverse effect is important.

The work, Pasteur and Koch established that, micro-organisms were the cause of infectious diseases. Paul Ehrlich was the first to propose that infections diseases might be curable by using chemicals that inhibit or kill the infectious agent but do not harm the host at the concentration employed. He discovered the famous organoarsenical compound 'Salvarson' which was active against the causative organisms of 'Syphillus'. It was he who used the term 'Chemotherapy'. According to his theory of drug action cells possesses chemicals receptors to which the drug bind. He recognised the importance of quantitative measurement to determine the drug dose, that would be effective against the causative agent and not have toxic effect on the host. He also pioneered methods for screening a large number of compounds for biological activity in relation to chemical structure. Chemical variants of effective compounds were then synthesized and tested to see whether they have improved antimicrobial activity and reduced toxicity.

The effect of ethyl alcohol, on the animal organism is intriguing and illustrates the divisions of interest among the basic medical sciences. When ingested in small quantities alcohol produces no symptoms. It is metabolized, producing carbondioxide and water and each gram gives rise to seven calories. Hence in small quantities alcohol serves merely as a food and its fate in the body is mainly a biochemical problem. In larger amounts alcohol profoundly affects the central nervous system, causing a depression of the inhibitory centers in the brain and produces a syndrome of inebriety. As a product of a gland of internal secretion the physiologist studied the effect of epinephrine on the living organisms. The action of epinephrine on the organism under stress and its effect on metabolism are of prime physiologic importance. As a product extracted from the medullary portion of the adrenal glands and available for injection as a therapeutic agent. It is a potent drug, useful in the treatment of the asthmatic spasm because it dilates the bronchioles upon injection. Its stimulation of the
myocardium has been proved useful in collapse. Indeed one can readily judge from the examples cited that there are no sharp lines of demarcation between the basic medical sciences. These sciences are the study of the human body in health and in disease, structurally, functionally and chemically. The ultimate purpose of each is the same i.e. "knowledge of life processes and the utilization of this knowledge for the health of mankind".

**Review of literature**

The world's oldest pharmacological writing come from 'India' and 'China'. The great herbal of chinese 'materia media', 'Pan Tsao' was probably written in 2735 B. C. The earliest Indian records are the 'Vedas'. Although there are medical discriptions in 'Rigveda' (2500 - 3000 B. C.) it was 'Charaka' renouned ancient Indian physician and later 'sushutra' and 'Vagbhat' who discovered various medical preparations included in 'Ayurveda', 'the science of life'. Initially these consisted mostly nonpoisonous vegetable drugs and minerals. Thus. Charaka described about 300 vegetable drugs and classified them according to their effects, mostly on symptoms into fifty groups.

Development of modern pharmacology as a science is fairly recent and probably started taking sharp following the introduction of experimental procedure by Francois Magendie (1783-1855) and claude Bernard (1813-1878).

Spectacular developments in physiology, biochemistry and organic chemistry during the recent years have greatly accelerated the advance in pharmacology.

Literature shows that much work has been done over many heterocyclic compounds of their antibacterial activities including both gram positive and gram negative bacteria.

Pyrazolines were known to have bactericidal\textsuperscript{146}, fungicidal\textsuperscript{147} and insecticidal\textsuperscript{148} properties. Some pyrazolines are also reported for anti inflammatory, antidiabetic, anaesthetic

and analgesic properties\textsuperscript{149-152}.

Ozawa et al.\textsuperscript{151} reported the synthesis of very effective pyrazolines (66) in killing houseflies on contact.

\begin{equation}
\text{RCF}_2O
\end{equation}

(66)

Van Hes et al.\textsuperscript{154} (1982) synthesised pyrazolines (67) which were found to have insecticidal properties.

\begin{equation}
\text{R}_1
\end{equation}

(67)

Therapeutically active 3-amino-1-phenyl and substituted phenyl-2-pyrazolines\textsuperscript{155} (68) were synthesised and found to be anti-inflammatory, bactericidal and fungicidal.

\begin{equation}
\text{NH}_2
\end{equation}

(68)

\begin{enumerate}
\item Garge, H. G. and Chandraprakash, J. Pharm. Sc., \textbf{14} (1971), 649.
\item Dusza, J. P., Joseph, J. P., and Bernstein, S.,
\end{enumerate}
Substituted pyrazolines have also been reported as anti bacterial and antimicrobial agents.\(^{156,157}\) It has been found that N-heterocyclic nucleus incorporated with a carbonyl group shows significant biological activity.\(^{158}\)

Roda et al.\(^{159}\) synthesised new pyrazoline derivatives which showed antimicrobial activity.

Anderson and Paolella\(^{160}\) synthesised 1-phenylpyrazole derivatives (69) and reported as an effective antidiabetics.

\[
\begin{align*}
(69) & \quad (R = \text{Me}, \text{CF}_3, \text{NH}_2, \\
& \quad n = 1, 2)
\end{align*}
\]

Feucher\(^{161}\) prepared (phosphonodithioacetamido) phenyl pyrazole (70) which were found insecticides and miticides.

\[
\begin{align*}
(70) & \quad \text{Ph—N}^+, \text{NHCOCH}_2\text{S Ph RR,}
\end{align*}
\]

Sharma et al.\(^{162}\) reported the antimicrobial activity of hydroxyaryl pyrazoles (71) obtained from hydroxyaryl pyrazolines.

\[
\begin{align*}
(71) & \quad R = \text{OR}, \\
& \quad \text{OR}
\end{align*}
\]

Alkyl pyrazole derivatives (72) have also been reported as hypolipidemic agents.\(^{153}\)

\[
\begin{align*}
\text{(72)} & \\
R_1 & - \text{C} \longrightarrow \text{N} \bigg\langle \text{N} \bigg\rangle \bigg\langle \text{N} \bigg\rangle & \text{R}_2 \\
\end{align*}
\]

Antimicrobial activity of substituted pyrazoles (73) were reported by Mittal and Singhal\(^{164}\).

\[
\begin{align*}
\text{(73)} & \\
\end{align*}
\]

The antifungal nature of 1-substituted 3-(2-hydroxyphenyl)-5-(4-nitrophenyl) pyrazoles was studied by Giri\(^{165}\), while trifluoromethyl-1-aryl pyrazoles were reported to be analgesic, antipyretic and anti-inflammatory agents\(^{158}\).

Ishihara Co. Ltd. Jpn\(^{166}\) synthesised pyrazoles (74) which were used as herbicides.

\[
\begin{align*}
\text{(74)} & \\
\end{align*}
\]

Formulation of [(4-phenoxyphenyl oxy) alkyl pyrazoles] (75) as pesticides have been reported by Kisida et al.\(^{167}\).

\[
\begin{align*}
\text{(75)} & \\
\end{align*}
\]

---

3,5-Diphenyl-1H-pyrazole derivatives\textsuperscript{168} (76) (76a) showed sedative, platelet antiaggregating anaesthetic, analgesic and antiinflammatory activities.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} & \quad \text{Ph} \\
\quad & \quad R
\end{align*}
\]

(76)

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} & \quad \text{OR} & \quad \text{CH}_2 & \quad \text{OPh}
\end{align*}
\]

(76a)

Preparation of 4,4'-dibromo-1,1'-methylenedipyrazole as a antiparasitic has been reported. Nitro derivatives of 1-phenyl-3-sulphanilamido pyrazole (77) have been reported to be antibacterial against E. Coli. and its minimum inhibition concentrations were determined.

\[
\begin{align*}
\text{H}_2\text{SO}_3 & \quad \text{NH}_2 \\
\text{R} & \quad \text{R}
\end{align*}
\]

(77)

\(R = 2 - \text{O}_2\text{N}, 3-\text{O}_2\text{N}, 4-\text{O}_2\text{N}\)

Basu et al.\textsuperscript{169} reported the synthesis of pyrazoles (78) as useful intermediate for pesticide and anticonvulsants.

\[
\begin{align*}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\text{N} \quad \text{R}_1
\end{align*}
\]

(78)


Vijay M. Barot\textsuperscript{170} had reported the antibacterial activities of 1-H-3 (2'-'hydroxy -4' -ethoxy -5' -bromo phen -1-yl) -5 substituted phenyl -2- pyrazolines against E.Coli and S. aureus.

Sonare S.S.\textsuperscript{171} have studied the antimicrobial activity of 1-H-3 (2-acetyl -4- methoxy) phenyl -5- substituted phenyl -pyrazolines.

Kadu V. B.et al.\textsuperscript{172} have reported MIC values of 3-(substituted -3, 4-benzophenyl) -4 (substituted phenyl pyrazoline and its derivatives.

Recently Sorathiya S.D.et al.\textsuperscript{173} have reported antimicrobial activities of 3- (P-(2',5' -dibromobenzene sulphonamido)-phenyl) -5- aryl -1H /acetyl /phenyl pyrazolines.

Recently Tayade V.B.et al.\textsuperscript{174} have reported the antimicrobial activities of 3, 5-diryl pyrazolines and 3, 5-diaryl pyrazoles.

Antimicrobial susceptibility testing is carried out on a large scale in clinical laboratories as a guide to antibiotic therapy when one adds to these clinical tests those done in pharmaceutical and research laboratories in screening and evaluation of new compounds. They are carried out by many different methods with variable degrees of accuracy and relevance. In the management of specific infection in a patient the test is being performed to determine whether or not the infecting organism is susceptible to a series of antibiotics that might be relevant in treatment. Antibiotic sensitivity tests are also often used as an aid in the identification of infecting organisms. All are helpful in indicating the identity of isolates from clinical material.

The broth dilution susceptibility test was among the first to be developed and still serves today as the reference method. With the advent of several new antibiotics during the 1940s, tube dilution methods were no longer practical to meet the large volume of work required. For example, in a busy microbiology laboratory, 25 or more antibiotic susceptibility tests may be performed daily. Furthermore, each organism may be tested against 10 or more different antibiotics.

In 1943, Foster and Woodruff first reported the use of antibiotic impregnated filter paper strips in the performance of susceptibility tests. The test was performed by placing a moistened strip to the surface of an agar plate that had been previously inoculated with the organism to be tested. Note the zone of growth inhibition adjacent to those strips containing the antibiotics to which the bacterium is sensitive. This procedure had no advantage over the tube dilution methods in that more than one antibiotic could be tested simply by placing multiple antibiotic impregnated strips on the same agar plate.

Vincent and Vincent introduced the use of paper discs in 1944, increasing even more the number of antibiotics that could be tested simultaneously. One year later in 1945, Morely added another dimension by demonstrating that the paper discs could be dried after applying the antibiotics solution, thereby precluding the necessity of having fresh stock solutions available each time a test was to be performed.
This discovery led shortly thereafter to the commercial manufacture of antibiotic susceptibility discs not unlike those used today. Bondi and associates in 1947 were the first to establish performance standards for the various concentrations of antibiotics to be used in different discs, from which were developed the first guidelines by which practical clinical applications could be made in the treatment of patients with infectious disease, and are now more commonly used than others such as cups (Rose and Miller 1939) tablets (Lund et al 1951), or wells cut in the medium.

Although it is possible to produce dry filter paper discs in the laboratory (Fair-brother and sherris, 1959, Leach and Wills, 1971), most laboratories consider this to be too-time consuming and subsequently rely on commercial sources.

The disc-diffusion method provides an simple, convenient, and reliable test specially applicable in routine clinical bacteriology laboratory. It consists of impregnating small discs of a standard filter paper with given amounts of a chosen range of antibiotics. These are placed on plates of culture medium previously spread uniformly with an inoculum of the bacterial isolate to be tested. After incubation the degree of sensitivity is determined by measuring the easily visible areas of inhibition of growth produced by the diffusion of the antibiotic from the discs into the surrounding medium.

The basic principle of the disc-diffusion method of antimicrobial susceptibility testing is as soon as the antibiotic impregnated disc comes in contact with the moist agar surface, water is absorbed into the filter paper and the antibiotic diffuses into the surrounding medium. The rate of extraction of the antibiotic out of the disc is greater than its outward diffusion into the medium, so that the antibiotic concentration immediately adjacent to the disc may exceed that in the disc itself.

However, as the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration until a point is reached at which the bacterial growth on the agar surface is no longer inhibited. The result is a sharply marginated zone of growth inhibition.
The so called zone Vs no zone method was used for interpreting the results, meaning that the development of a zone of growth inhibition of any size around a disc indicated that the organism was susceptible to the antibiotic contained. Resistant bacteria growth right up to the margin of the disc. The zone Vs no zone concept was abandoned in the early 1960s when Bauer introduced the high potency, single-disc technique. Early in the interpretation of these tests, it was believed that zone sizes could be correlated with the relative sensitivity of the organism, that is, it was believed that the larger the zone, the more effective the antibiotic should be therapeutically. However, it was soon learned that zone sizes depend on certain physical chemical properties, such as the molecular weight of antibiotic and the net negative charge, depth of pour, composition and pH of the culture medium, that influence the vitro diffusion rates in agar and do not necessarily correlate with in vivo antimicrobial activity.

**ISONIAZIDE**

The new pyrazolines and pyrazoles are prepared by the action of isoniazide on flavanones and flavones respectively. The isoniazide is one of the most effective antituberculous drug. The isoniazide is the hydrazide of isonicotinic acid the structural formula is as follows.

\[ \text{CONH.NH}_2 \]
**Antibacterial Activity**

Isoniazide\(^\text{175}\) is bacteriostatic for 'resting' bacilli but is bactericidal for rapidly dividing microorganisms. The minimal tuberculostatic concentration is 0.025 to 0.05μg/ml. The bacteria undergo one or two divisions before multiplication is arrested. The drug is remarkably selective for mycobacteria and concentrations in excess of 500μg/ml are required to inhibit the growth of other micro-organisms.

Isoniazide is highly effective for the treatment of experimentally induced tuberculosis in animals and is strikingly superior to streptomycin. Unlike streptomycin, isoniazide penetrates cells with ease and is just as effective against bacilli growing within cells as it is against those growing in culture media.

**Mechanism of Action:**

Although the mechanism of action of isoniazide is unknown, several hypotheses have been proposed. These include effects on lipids, nucleic acid biosynthesis, and glycolysis. Takayama and associates (1975) has suggested a primary action of isoniazide to inhibit the biosynthesis of mycolic acids, important constituents of the mycobacterial cell wall. Because mycolic acid are unique to mycobacteria, this action would explain the high degree of selectivity of the antimicrobial activity of isoniazid. Exposure to isoniazide leads to a loss of acid fastness and a decrease in the quantity of methanol extractable lipid of the microorganisms.

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