SYNTHESIS OF SOME FLAVONE & PYRAZOLINE DERIVATIVE AND THEIR ANTIMICROBIAL AND PHYSICOCHEMICAL STUDY

V.D. Mane[1], Shaikh Azeem[2], P.S. Pande[3], M.O. Malpani[4], H J Kharat[5]
[1,2,3,4]Department of Chemistry, [5]Department of physics
Shankarlal Khandelwal Arts, Science and Commerce College, Akola.(MS)

ABSTRACT

In the present study the flavone derivatives have been synthesized by the cyclisation of hydroxyl chalcones by I_2 in DMSO. The required hydroxyl chalcone have been prepared by condensation of substituted acetophenone with different aromatic aldehydes. Synthesized flavones and pyrazoline have been tested for antimicrobial activity against, *Escherichia coli, S.aureus* by using pour plate method. Flavone have been studies pH metrically, proton-ligand stability constant(pk) and matal-ligand stability constant at 0.1M ionic strength has been determined at 30 ˚c in 1,4 dioxane. The pH metric study shows the 1:1 and 1:2 complex formation takes place. The study of antimicrobial activity showed the good to moderate active compounds.

Keywords: Chalcone, flavone, pyrazoline, pH metric, antimicrobial activity.

INTRODUCTION

Heterocyclic compound is a cyclic compound in which one or more atoms of the ring are hetero atoms. In other words the cyclic compound containing one or more hetero atoms is known as Heterocyclic compound. In which the different variety of hetero atom such as Nitrogen, sulphur , oxygen as can be incorporated into ring structure[1]. Hetero cyclic make up an exceeding important class of compound more than half of known organic compound are heterocyclic. The hetero cyclic compound contain one or more atoms and possessing huckle number of pi electron i.e (4n+2). Almost all the compound we know as drugs and most vitamins and many other natural products[2].

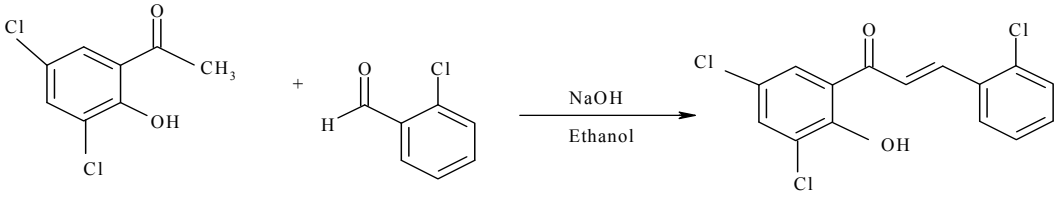
Flavone(2-phenylchromone) are natural and synthetic occurent compound belong to Flavanoid group[3]. Flavone (Flavas mean yellow) are comes from class flavanoid[4]. The flavones, pyrazoline are most useful for their various biological activities such as anti-asteoparotic, anticancer[5],anti-inflammatory[6], anti-diabetic. There are well known by their biological properties such as antibacterial, antifungul[7],antioxidant[8], antiallergic, antimicrobial and antiviral.

EXPERIMENTAL PART:-

General procedure for synthesis of flavone & pyrazoline

The synthesis of 2-Hydroxy chalcone :-

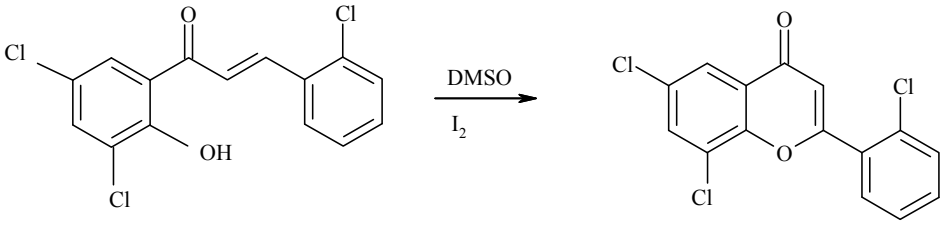
A mixture of 0.01 mole of 2, 4 dichloro hydroxy ketone and 0.01 moles of p-chloro benzaldehyde was desolved in 25 ml ethanol and 40% NaOH was added in reaction mixture with continuously stirrering. The precipitate formed was dried and weighed.



1a

The synthesis of flavone derivatives from the 2-Hydroxy chalcone :-

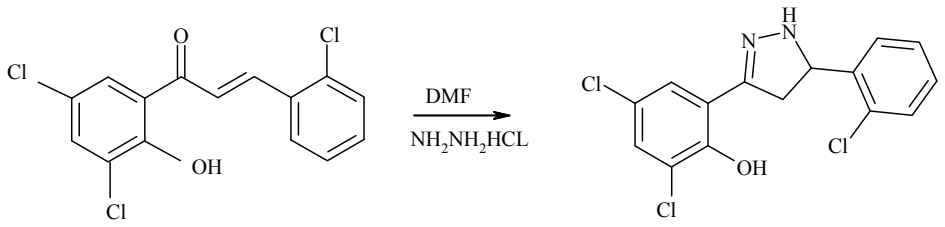
A mixture of 2-hydroxy chalcon, DMSO and I_2 as a catalyst and ethanol were kept in round bottom flask was reflux for about 3.5 hours the resulting solid was filtered and washed by ethanol, the compound recrystallized from ethanol and dry.



2a

The synthesis of pyrazoline derivative from 2-hydroxy chalcone :-

A mixture of 0.01 moles of 2-hydroxyl chalcone and hydrazine and DMF were taken in round bottom flak and to this mixture 25 ml ethanol was added and reaction mixture was refluxed for 3.5hours the product obtain after cooling was separate out, dried and weighed.



3a

PHYSICOCHEMICAL STUDY:

Physicochemical study by pH metric titration method

Experimental: The ligands i.e. flavones and pyrazoline derivative have been prepared by known reported method. Systronic make pH-meter with accuracy ±0.01 unit with combined glass electrode and saturated calomel was used for the measuring pH. The instrument was calibrated by using buffer solution of pH 4.00 and 9.20 at 30°C.

The following reagents were used for the experiment

i) Nitric acid :- (0.1M) A. R. grade
ii) Potassium nitrate (1M):- A. R. grade
iii) Sodium hydroxide (0.1N):- A. R. grade
iv) Oxalic acid(0.1M) :-A. R. grade
v) Metal ions solutions:-Cu (II) and Fe(III) were used to prepare 0.01 m solutions.

All solutions were prepared by dissolving requisite amount of substances in distilled water.

0.01m solutions of the ligands were prepared in dioxane.

Titrations:- The procedure of the experiment is involved following titration

1. Free acid titration
2. Free acid and ligand titration.
3. Free acid, ligand and metal ion titration against standard NaOH solution

The above titrations were carried out in 100 ml pyrex glass beaker maintained at constant 30^0C temperature in 70% dioxane-water mixture at 0.1M ionic strength. The reading of the pH meter was recorded at every addition of 0.5ml NaOH solution from the burette with constant stirring.

The graph of pH against volume of alkali added were plotted as

1. Acid curve (A)
2. Ligand titration curve (A+L)
3. Metal ligand titration (A+L+M)

The metal-ligand stability constant have been calculated by half integral method. The metal ligand formation number value indicates that the formation of 1:1 and 1:2 complexes[11].

Method for the determination of the stability constants

i) Calculation of proton-ligand stability constant (pK values)

The proton-ligand stability constant (pK) is defined as the pH at which half of the amount (concentration) of the ligand is ionized i.e conc. of ionized and unionized forms of the ligand are equal.

The value of pK for various ligands are estimated by half integral method by calculating the value of proton-ligand formation number **(η^-_A)** at various pH by using Irving and Rossotii equation.

$$\eta^-_A = \gamma - [(E^o + N)\,\Delta V/(V_o + V_1)\,T^o_L] \text{------ (1)}$$

Where

V_0 = Initial volume of solution

N =Normality of sodium hydroxide

T^o_L=Concentration of ligand in the solution

E^0= Initial con. of free acid (HNO_3)

γ = number of dissociable protons from ligand

$(V_2-V_1) = \Delta V$ = Difference in volumes of alkali consumed by acid and ligand on the same p H.

The proton-ligand formation curve was drawn by plotting graph of η^-_A against pH. The pH at which $\eta^-_A = 0.50$ gave value of proton-ligand stability constant (pk) of the ligand. The robustness of these values were confirmed by calculating the average pK value by point-wise calculation in the η^-_A range of 0.2 to 0.8, using the following equation.

$$\mathbf{pK - pH = \log \eta^-_A /1- \eta^-_A \text{ ----------(2)}}$$

Where η^-_A value corresponds to given pH. The values of pK calculated by point-wise calculation are presented in table

ii) Calculation of metal-ligand stability constant (log k value). This involves calculation of metal-ligand formation number at various pH, from the equation.

$$\eta^- = (N + E^0)\,\Delta v /(V_0 + V_2)\;\eta^-_A\,Tm^0 \text{ --------(3)}$$

Where V_0 = Initial volume of solution

N= Normality of NaOH

T^0_M=Conc. of metal ions

η^-_A=Proton-ligand formation no.

E_0= Initial conc. of free acid (HNO_3)

$(V_3-V_2) = \Delta V$=Difference in volumes of NaOH consumed by ligand and metal ions at the same pH

The metal-ligand formation curve was constructed by plotting η^- values against pH.

The free ligand concentration (pL) at $\eta^- = 0.5$ and 1.5 gives the values of metal ligand stability constant $\log k_1$ and $\log k_2$ respectively. The PL values were calculated by using Irving-Rossotti equation.

$$PL = \text{Log } K = \log [\ H^+/ (K.\ (\ T^o_L - T_m{}^o \text{ x } n^-) \text{ x } V_o + V_3 / V_o]$$

Where $[H^+]$ = Concentration of the hydrogen ion at $\eta^- = 0.5$ or 1.5

K = Ionization constant of the ligand

η^- = Metal-ligand formation numbers.

ANTIMIROBIAL ACTIVITY:

Antimicrobial activity by agar pour plate method:

The sterilized nutrient agar medium was poured into the petridish and allowed to solidify. The lawn of the culture was prepared by spreading the microbial suspension on the surface of the medium with the help of sterilized triangular loop. Petridishes were allowed to remain for 10 min, after which excess of nutrient broth cultures were taken out aseptically using pasture pipettes. Hole prepared in the solidified medium with the help of pre-sterilized steel cylinder of 8mm diameter. The wells were then filled with the 0.5ml stock solution of the test compound and standard drug chloramphenicol (200ug/ml) , controls were run using only DMSO solvent all the plates were the incubated at 37±2˚c for 24±hours. The zones of inhibition were recorded by using high media scale.

RESULT AND DISCUSSION:

pH metric titration data used construct the graph between NaOH and pH value and these curves are known as titration curve , ligand titration curve, metal titration curve . The proton-ligand stability constant (pk) and metal-ligand stability constant (log k) of Cu (II) and Fe(III)complex of various strength calculated by Rossotti's method[10] . The synthesized

compound evaluated for their antimicrobial activity against *Escherichia coli , S.aureus.* The zone of inhibition (mm) in the following table.

Table 1 pK values calculated by half integral method and point-wise calculation method.

Ligand	Half integral method	Point wise method
L1	11.00	11.04
L2	5.50	5.45

Table 1. Log K values of Metal-ligand complexes.

Name of Ligand	Metal ion	Log K_1	LogK_2
$L_{1(2a)}$	Cu^{+2}	3.0786	2.8384
	Fe^{+3}	0.1497	0.1005
$L_{2(3a)}$	Cu^{+2}	8.347	6.0245
	Fe^{+3}	10.9332	9.8029

Table 2. Antimicrobial properties of synthesized compound

Compounds	Inhibition zone in mm	
	E.coli	*S.aures*
Hydroxy Chalcone (1a)	12	09
Flavone derivative (2a)	14	12
Pyrazoline derivative (3a)	16	14

CONCLUISON

The flavone & pyrazoline derivative have been synthesized by laboratory method. The synthesized compound characterized by melting point, elemental analysis and spectral data. The synthesized compounds screened against the bacteria *E.coli & S.aures*. From the antimicrobial study it shows good to moderate effected against *E.coli & S.aures* .The synthesized compound also studies pH metrically. From pH metric studies the synthesized compound confer a good result. The pH metric study shows the 1:1 and 1:2 complex formation takes place.

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