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SYNTHESIS AND CHARACTERISATION OF SOME PYRAZOLONE DERIVATIVES AND THEIR FUNGICIDAL AND BACTERICIDAL STUDY-I

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A series of 4-[phenyl/substituted phenyl-p-car-boxyl alkyl aniline]-methyl-1, 3-diphenyl-2-pyrazolin-5-ones have been synthesized with variation of substituents at 4-phenyl and alkyl positions. These compounds are characterized by elemental analysis, *i.e*., n.m.r. and mass spectra-studies. The fungicidal activity was studied against the test fungi – *Pyricularia oryzae, Helminthosporium oryzae, Collectotrichum tabacum* and *Alternaria solani*. The bacteriocidal activity was studied against the standard bacteria *Salmonella typhosa* and *Staphylococcus aureus*. Some of the compounds exhibited pronounced fungicidal and bactericidal activities.

INTRODUCTION

The earlier findings show the pyrozolone and its derivatives to possess significant fungicidal and bactericidal activities [1, 2]. This prompted us to synthesize a series of 4-[phenyl/substituted phenyl-p-carboxyl alkyl aniline]-methyl-1, 3-diphenyl-2-pyrazolin-5-one derivatives. 1, 3-Diphenyl-2-pyrazolin-5-ones and alkyl-p-amino benzoate are prepared by following standard procedure [3]. These compounds were synthesized by the reflux condensation of ethanolic solution of diphenyl pyrazolones, araldehyde and alkyl-p-aminobenzoic acid taken in unimolecular proportion.

In the i.r. spectra of the above compound absorption at 3000 cm^{-1} is due to the secondary –NH stretching. Sharp band appearing at 1,700 cm^{-1} is due to the >C=O stretching. Further sharp band at 1,000 cm^{-1} with a weak complimentary bancraft 1,400 cm^{-1} arises due to the asymmetric and symmetric stretching of the >C=O group of the ester link. Other absorptions are typical of aromatic and pyrozolone nuclei.

The n.m.r. spectra shows signals at arises due to the asymmetric and symmetric stretching of the >C=O group of the ester link. Other absorptions are typical of aromatic and pyrozolone nuclei.

The n.m.r. spectra shows signals at δ 1.05, 2.1 and 4.5 for the methyl, methylene and methane protons. The multiplet at δ 7-8 is due to ring protons. A singlet appearing at δ –5.1 is for the secondary –N–H proton. No signals received at δ –3.0, –4.84 and –9.5 indicates the absence of $–NH_2$, –OH and –CHO protons.These data establish the condensation to have successfully occurred.

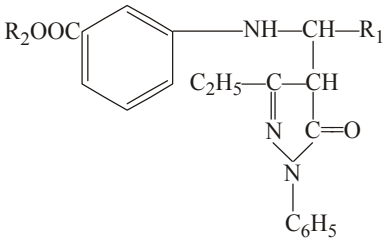
The atomic mass spectra shows the molecular ion peak (m/e) at 513 which confirms to the molecular weight of the above compound.

In all the cases cited, elemental analysis, n.m.r. and mass spectral data were consistent with the structures of the compounds.

Result and discussion

I. Fungicidal Study

The fungicidal activity was determined by the method of Montogomery and Moore [4] with slight modifications. The fungi used are *Pyricularia oryzae, Helminthosporium oryzae, Collitotrichum tabacum* and *Alternaria solani*. The fungicidal assay based upon the % non germination at 62.5, 125, 250, 500 and 1000 ppm concentrations. Time of contact is 24 hours. Table I shows the fungicidal activity of these compounds at 1000 ppm concentration. However the fungicidal activity was found to increase with concentrations against all the test fungi.



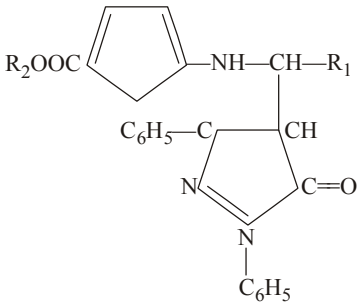
It is important to note that substituents on pyrazolone (1, 3-position) are always the phenyl group. R_2 varies from methyl, ethyl to n-butyl groups and for each R_2 eight different substituents are taken at R_1.

When R_2 is n-butyl and R_1 = m-, p- or o-nitrophynyl groups the inhibition of spore germination is recorded maximum (85-90%) against all the test pathogens except *Alternaria solani*. The order of activity of R_2 when R_1 = m-, p- or o-nitrophenyl groups is represented as n-butyl ethyl methyl groups. Fungicidal activity is uniformly high against all the pathogens when R_1 = cinnamalidine group (70-80%). Generally it is observed that compounds toxic against *Pyricularia oryzae* are mostly toxic against *Helminthosporium oryzae*. Surprisingly compounds with R_1 = phenyl or chlorophenyl groups should minimum inhibition.

II. Bactericidal study :

The bactericidal activity of all these compounds were shown in Table-I against the test bacteria Salmonella typhosa and Staphylococcus aureus at 100, 125, 250, 500 and 1000 ppm concentrations. Time of contact is 10 minutes at 37°C. Variation of the 4-arylidene substitutent (R_1) has a marked effect on the bacteriostatic activity of these compounds. No additional toxicity is brought about by introducing or varying the amino-phenyl-carboxylate group. The bactericidal activity was maximum for the compounds 8, 16 and 24 when R_1 = p-Cl C_6H_5 group and the potency extended up to 125 ppm. Compounds with R_1 = phenyl, o-hydroxy phenyl or p-nitro phenyl group recorded uniform bactericidal activity against both Salmonella typhosa and Staphylococcus aureus at 1000 ppm. The bactericidal activity was nil when R_1 = p-methoxy phenyl or cinnamyl groups.

Table I. Fungicidal and bactericidal results



Sl. No.	Nature of R_1	Nature of R_2	% spore germination inhibition at 1000 ppm				Bactericidal activity	
			P. oryzae	H. oryzae	Coll. Tabacum	Alt. solani	Salm. Typhosa	S. aureus
1.	$-C_6H_5$	$-C_2H_5$	45	58	52	60	-	-
2.	p-CH_3O-C_6H_4	$-C_2H_5$	80	75	72	58	+	+
3.	o-OH-C_6H_4	$-C_2H_5$	85	74	61	52	-	-
4.	m-NO_2-C_6H_4	$-C_2H_5$	74	89	81	70	+	-
5.	-CH=CH-C_6H_4	$-C_2H_5$	80	74	71	72	+	+
6.	p-NO_2-C_6H_4	$-C_2H_5$	75	85	80	80	-	-
7.	o-NO_2-C_6H_4	$-C_2H_5$	72	83	81	75	-	-
8.	p-Cl-C_6H_4	$-C_2H_5$	48	68	60	72	-	-
9.	$-C_6H_5$	$-CH_3$	40	58	42	85	+	+
10.	p-CH_3O-C_6H_4	$-CH_3$	74	78	68	86	+	+
11.	o-OH-C_6H_4	$-CH_3$	76	74	62	40	-	-
12.	m-NH_2-C_6H_5	$-CH_3$	62	84	71	45	+	-
13.	-CH=CH-C_6H_4	$-CH_3$	74	72	62	82	-	-
14.	p-NO_2-C_6H_4	$-CH_3$	60	86	80	72	-	-
15.	o-NO_2-C_6H_4	$-CH_3$	58	83	80	46	-	-
16.	p-Cl-C_6H_5	$-CH_5$	42	67	52	55	-	-
17.	$-C_6H_5$	-n-Butyl	60	64	54	75	-	-
18.	p-CH_3O-C_6H_4	-n-Butyl	85	89	79	60	-	-
19.	o-OH-C_6H_4	-n-Butyl	86	86	72	55	-	-
20.	m-NO_2-C_6H_4	-n-Butyl	80	87	71	62	+	-
21.	-CH=CH-C_6H_4	-n-Butyl	84	88	72	82	+	+
22.	p-NO_2-C_6H_4	-n-Butyl	82	91	82	80	-	-
23.	o-NO_2-C_6H_4	-n-Butyl	80	85	80	54	-	-
24.	p-Cl-C_6H_4	-n-Butyl	62	75	69	71	-	-

(+) Sign indicates bacterial growth. (–) Sign indicates prevention of growth.

EXPERIMENTAL

All chemicals used in the synthesis were of AnalaR grade. Melting points were taken in a Toshniwal melting point apparatus and were uncorrected. I.R. spectra were taken in a Perkin-Elmer Infrared spectrophotometer, n.m.r. spectra were taken on Varian 90D instrument with T.M.S. as internal standard and atomic mass spectra were taken in T.M.S.-D-300 (JEOL Red., Tokyo) N.B. at EI Mode at C.D.R.I., Lucknow.

Table II. Physical and Analytical data of 4-[phenyl/substituted phenyl-p-carboxy alkyl aniling] methyl-1,3-diphenyl-2-pyrazolin-5-one

Sl. No.	Nature of R_1	R_2	Molecular formula	M.P.°C	Yield %	% of Carbon		%of Hydrogen		% of Nitrogen	
						Found	Calcd.	Found	Calcd.	Found	Calcd.
1.	$-C_6H_5$	C_2H_5	$C_{31}H_{27}N_3O_3$	235	80	76.05	76.07	5.53	5.52	8.59	8.58
2.	p-CH_3O-C_6H_4	C_2H_5	$C_{32}H_{29}N_3O_4$	300	72	78.9	78.98	5.64	5.59	8.92	8.9
3.	o-OH-C_6H_4	C_2H_5	$C_{31}H_{27}N_3O_4$	86	75	73.63	73.66	5.38	5.35	8.34	8.32
4.	m-NO_2-C_6H_4	C_2H_5	$C_{31}H_{26}N_4O_5$	162	82	69.65	69.66	4.88	4.86	10.52	10.49
5.	-CH=CH-C_6H_4	C_2H_5	$C_{33}H_{28}N_3O_3$	115	60	76.87	76.82	5.66	5.63	8.18	8.16
6.	p-NO_2-C_6H_4	C_2H_5	$C_{31}H_{26}N_4O_5$	182	85	69.64	69.66	4.87	4.86	0.52	10.49
7.	o-NO_2-C_6H_4	C_2H_5	$C_{31}H_{26}N_4O_5$	200	70	69.65	69.66	4.88	4.86	10.51	10.49
8.	p-Cl-C_6H_4	C_2H_5	$C_{31}H_{26}N_3O_3Cl$	177	76	71.08	71.06	4.98	4.96	8.04	8.02
9.	$-C_6H_5$	$-CH_3$	$C_{30}H_{25}N_3O_3$	230	80	75.75	75.74	5.3	5.36	5.28	5.26
10.	p-CH_3O-C_6H_4	$-CH_3$	$C_{31}H_{27}N_3O_4$	130	62	78.65	78.66	5.4	5.39	8.35	8.32
11.	o-OH-C_6H_4	$-CH_3$	$C_{30}H_{25}N_3O_4$	136	72	73.3	73.35	5.08	5.09	8.58	8.55
12.	m-NH_2-C_6H_5	$-CH_3$	$C_{30}H_{24}N_4O_5$	158	76	69.2	69.23	4.63	4.61	10.77	10.76
13.	-CH=CH-C_6H_4	$-CH_3$	$C_{32}H_{26}N_3O_3$	165	62	76.88	76.89	5.4	5.39	8.39	8.38
14.	p-NO_2-C_6H_4	$-CH_3$	$C_{30}H_{24}N_4O_5$	197	83	69.2	69.23	4.65	4.61	10.77	10.76
15.	o-NO_2-C_6H_4	$-CH_3$	$C_{30}H_{24}N_4O_5$	190	70	69.22	69.23	4.66	4.61	10.78	10.76
16.	p-Cl-C_6H_5	$-CH_5$	$C_{30}H_{24}N_3O_3Cl$	169	85	70.63	70.64	4.75	4.71	8.25	8.24
17.	$-C_6H_5$	n-Butyl	$C_{33}H_{31}N_2O_3$	215	80	76.56	76.59	6.02	5.59	8.15	8.12
18.	p-CH_3O-C_6H_4	n-Butyl	$C_{34}H_{33}N_3O_4$	182	82	74.55	74.56	6.05	6.02	7.70	7.69
19.	o-OH-C_6H_4	n-Butyl	$C_{33}H_{31}N_3O_4$	143	65	74.27	74.30	6.04	5.81	7.84	7.86
20.	m-NO_2-C_6H_4	n-Butyl	$C_{33}H_{30}N_4O_5$	80	69	70.44	70.46	5.35	5.34	9.98	9.96
21.	-CH=CH-C_6H_4	n-Butyl	$C_{35}H_{32}N_3O_3$	153	70	77.35	77.45	6.10	6.08	7.75	7.73
22.	p-NO_2-C_6H_4	n-Butyl	$C_{33}H_{30}N_4O_5$	174	82	70.44	70.46	5.37	5.34	9.97	9.96
23.	o-NO_2-C_6H_4	n-Butyl	$C_{33}H_{30}N_4O_5$	167	72	70.44	70.46	5.36	5.34	9.98	9.96
24.	p-Cl-C_6H_4	n-Butyl	$C_{33}H_{31}N_3O_4$	165	83	71.85	71.89	5,46	5,49	7.63	7.61

A. Synthesis of 1, 3-diphenyl-2-pyrazolin-5-one :

It was synthesized following the standard method of condensation (i) of a β-keto ester with phenyl hydrazine. Phenyl hydrazone condenses with β-ketoester to form hydrazone which finally cyclises (5).

Benzoyl acetic ester (9ml) and phenyl hydrazine (3 ml) were mixed in a dry basin. It was heated on a steam bath for 1 hour with occasional stirring and then cooled to solidify. It was then washed with diethyl ether and filtered to remove coloured impurities and then dried. The product was finally crystallized from absolute alcohol.

Yield – 80%,M.P,. 138°C, Found C,76.45,H,5.19,N,11.78;

Calculated C, 76.32, H,5.08 and N,11.87%.

B. Synthesis of Ethyl-p-aminobenzoate :

Dry HCl gas was passed into ethyl alcohol (50 ml). p-Amino-benzoic acid (g gm) was added to it and refluxed for 10 hrs. Excess ethyl alcohol was removed by distillation. Solid separated out which was crystallized from ethyl alcohol.

M.P. 91°C, Yield – 75%

C. Synthesis of n-butyl-p-amino benzoate :

In a 500 ml round bottomed flask benzoic acid (30 g), n-butyl alcohol (30 g), benzene (50 ml) and Conc. H_2SO_4 (5.4 ml) were taken and refluxed for 10 hrs. The product was poured into 250 ml of water and then extracted with ether. The ethereal extract was washed with sodium bicarbonate solution followed by washing with water. It was then dried over anhydrous $MgSO_4$. Ether and benzene were distilled off through fractionating column.

M.P. - 55°C Yield = 82%

D. Synthesis of 4-[phenyl-p-carboxy ethyl aniline] methyl-1, 3-diphenyl-2-pyrazolin-5-one:

Benzaldehyde (0.01M), ethyl-p-amino benzoate (0.01 M) and 1,3-diphenyl-2-pyrazolin-5-one (0.01 M) were dissolved in ethanol (25 ml) and refluxed for 30 minutes. It was then kept in a stoppered bottle for 20 days. The product was filtered and crystallized. Other compounds in this series were prepared simply by altering the aldehyde component of the reactants. Compounds 2, 5, 8, 11, 13, 16, 1`9, 20, 21 and 24 were crystallized from acetone-ethanol mixture (1:1) and rest others from acetone only.

The physical and analytical data of 4-[phenyl/ substituted phenyl-p-carboxy alkylanilino] methyl-1, 3-diphenyl- 2-pyrazolin-5-one are recorded in Table- II.

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