

Synthesis, Tautomeric Structure, Dyeing Characteristics, and Antimicrobial Activity of Novel 4-(2-Arylazophenyl)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-ones

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(Received September 6, 2011; Accepted December 9, 2011)

ABSTRACT. Novel azopyrazolin-5-one dyes **4a-f** were synthesized by the regioselective reaction of phenylhydrazine with 2,3,4-chromantrione-3-arylhydrazones **2a-f**. The acid dissociation constants pK_a for the series prepared were determined and correlated by the Hammett equation. The results of such correlation together with the spectral data indicated that the studied compounds exist predominantly in the hydrazone keto structure, (**D**) as the *Z*-configuration. The dyes were applied to polyester fabrics, affording orange-yellow shades and assessments of their dyeing performance are considered. Further, the compounds **4a-f** were screened for their antimicrobial activity against various microorganisms.

Key words: Azo-hydrazone tautomerism, Disperse dyes

INTRODUCTION

The growing interest in the azopyrazolones chemistry lies in designing new synthetic approach, theoretical calculations, and applications of newer spectroscopic techniques. Much attention has been devoted towards the usage of many azopyrazolone derivatives due to their interesting structural properties and applications in divers area.¹⁻¹² On the other hand, they are of particular importance in dye chemistry,^{13,14} because many of them were used as good fastness dyestuffs for almost all kind of fabrics, whether natural or synthetic.

Molecules with 3-(2-hydroxyphenyl)-2-pyrazolin-5-ones possess peculiar properties and their facile preparation is one of most challenge goals for synthetic chemistry. Hence, progress in the chemistry of it has been inhibited in part because well defined derivative are lacking or difficult to prepare,¹⁵ poor yield,^{16,17} and complicated by numerous competing reactions.¹⁵⁻¹⁹ There is a growing demand for development of an alternative technologies. In view of these facts and in continuations of our studies designed to prepare heterocyclic azodyes with better fastness properties,²⁰⁻²³ it was considered worthwhile to synthesize the title compounds, determine their tautomeric structures, and exploring their application in dyeing polyester fabrics and their antimicrobial activity were studied.

EXPERIMENTAL

Materials and instrumentation

All the chemicals used in dye synthesis were of laboratory grade and were further purified by crystallization and distillation. Elemental analyses were performed at the Microanalytical Center, Cairo University, using CHNS-932 (LECO) Vario Elemental Analyzers and the results were within the accepted range (± 0.40) of the calculated values. All melting points were measured on a Gallenkamp electrothermal melting point apparatus and were uncorrected. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrometer. Electronic absorption spectra were recorded on Perkin-Elmer Lambda 40 spectrophotometer. ¹H NMR spectra were measured on a Bruker WP 300 in CDCl₃, DMSO as a solvent, using TMS as an internal standard, chemical shifts are expressed as δ /ppm, and *J* values are given in Hz. Mass spectra were recorded on a Finnigan MAT 212 instrument. Thin-layer chromatography (TLC) analysis was carried out on plates coated with silica gel 60 F₂₅₄. The dyeing assessment, fastness tests, and color measurements were carried out at El-Nasr Company for Spinning and Weaving, El-Mahalla El-Kubra, Egypt. The colorimetric measurements for the dyed polyester fabrics were carried out using a reflectance spectrophotometer (GretagMacbeth CE 7000a). Fastness to washing was carried out using the automatic launder Rotadyer

(sponsored by the British Standard Institute-Society of Dyers and Colourists), fastness to perspiration was assessed according to the test sponsored by the (BSS), fastness to rubbing was carried out according to the standard method of testing (BSS) using Crockmeter of Electric Hungarian FD-17 type, fastness to sublimation was carried out using the Electric Japanese Thermotester T-10 type and fastness to light was carried out using the "Weather-o-meter" (Atlas Electric Devices Co. USA), AATCC standard test method. 4-hydroxycoumarin (**1**),²⁴ 2,3,4-chromantrione-3-arylhydrazones **2a-f**,^{25,26} and 1-phenyl-3-(2-hydroxyphenyl)-2-pyrazolin-5-one (**3**)¹⁶ were prepared according to literature procedure.

Synthesis and spectroscopic characterization

General procedure for the synthesis of 4-(2-Arylazophenyl)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-ones **4a-f**

Method A: A mixture of 2,3,4-chromantrione-3-arylhydrazones **2a-f** (0.1 mol) and phenyl hydrazine (0.3 mol) in absolute ethanol (100 ml) was refluxed for 4-7 h then the solvent was evaporated under vacuum. The solid left was collected and crystallized from the appropriated solvent to give the respective compound **4a-f**. The physical constants of the compounds prepared **4a-f** are given below.

Method B: The aniline or its *p*-substituted derivatives (6 mmol) was dissolved in glacial acetic acid (10 ml) containing concentrated hydrochloric acid (4 ml). This solution was cooled in an ice bath and then diazotized with the solution of 0.38 g NaNO₂ (6 mmol) in 2 ml H₂O. The cold diazonium solution was poured into a mixture of 1-phenyl-3-(2-hydroxyphenyl)-2-pyrazolin-5-one **3** (6 mmol) and sodium acetate (10 g) in water (100 ml). The addition of diazonium salt solution took 30 min while stirring the reaction mixture. After the addition was completed, the mixture was stirred for further 30 min and left overnight in ice-chest. The separated colored product was collected, washed with dilute methanol, and recrystallized from suitable solvents to give the corresponding compounds **4a**, **4b**, and **4d** which was found identical in all respects with that produced by method A.

(*Z*)-4-(2-Phenylhydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (**4a**)

Red crystals, Yield: 87%; m.p. 198 °C; UV-Vis (λ_{\max} in dioxane): 240, 360, 400 nm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3434, 3285, 1658, 1623, 1592, 1548, 1488, 1342, 1268 cm^{-1} . ¹H NMR (DMSO-*d*₆): δ/ppm : 7.01-8.04 (m, 14H, ArH), 10.22 (s, H, OH, D₂O exchangeable), 13.75 (s, H, NH, D₂O exchangeable). MS, m/z (%) 356 (M⁺, 100), 340 (13), 279 (16), 223 (19), 145 (12), 132 (33), 120 (9), 102 (26),

99 (15), 84 (12), 73 (33). Anal. Calcd. for C₂₁H₁₆N₄O₂ (356.13): C, 70.77; H, 4.53; N, 15.72. Found: C, 70.88; H, 4.58; N, 15.89.

(*Z*)-4-(4-Methylphenyl)hydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (**4b**)

Red crystals, Yield: 70%; m.p. 202 °C; UV-Vis (λ_{\max} in dioxane): 247, 370, 414 nm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3444, 3295, 2945, 1650, 1617, 1581, 1546, 1317, 1258 cm^{-1} . ¹H NMR (CDCl₃): δ/ppm : 2.42 (s, 3H, CH₃), 7.01-8.04 (m, 9H, Ar-H), 7.94 (d, *J* = 9.6Hz, 2H, Ar-H), 8.47 (d, *J* = 9.6Hz, 2H, Ar-H), 10.35 (s, H, OH, D₂O exchangeable), 14.42 (s, H, NH, D₂O exchangeable). MS m/z (%) 370 (M⁺, 100), 278 (8), 223 (14), 171 (23), 145 (6), 132 (8), 120 (8), 102 (26), 84 (35), 73 (20). Anal. Calcd. for C₂₂H₁₈N₄O₂ (370.14): C, 71.34; H, 4.90; N, 15.13. Found: C, 71.56; H, 5.11; N, 15.36.

(*Z*)-4-(4-Methoxyphenyl)hydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (**4c**)

Reddish-violet crystals, Yield: 78%; m.p. 158 °C; UV-Vis (λ_{\max} in dioxane): 243, 364, 416 nm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3444, 3200, 2950, 1656, 1622, 1590, 1548, 1490, 1342, 1249 cm^{-1} . ¹H NMR (CDCl₃): δ/ppm : 3.88 (s, 3H, OCH₃), 7.02-7.52 (m, 9H, Ar-H), 7.94 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.47-8.5 (d, *J* = 8.7 Hz, 2H, Ar-H), 10.52 (s, H, OH, D₂O exchangeable), 14.46 (s, H, NH, D₂O exchangeable). MS m/z (%) 387 (M⁺, 40), 385 (M⁺-2, 31), 384 (M⁺-1, 30), 335 (29), 334 (58), 332 (43), 331 (100), 269 (11), 187 (43), 171 (23), 173 (16), 161 (11), 160 (52), 144 (49), 132 (8), 120 (58), 98 (26), 84 (30), 73 (20). Anal. Calcd. for C₂₂H₁₈N₄O₃ (386.14): C, 68.38; H, 4.70; N, 14.50. Found: C, 68.2; H, 4.88; N, 14.60.

(*Z*)-4-(4-Chlorophenyl)hydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (**4d**)

Red crystals, Yield: 66%; m.p. 215 °C; UV-Vis (λ_{\max} in dioxane): 243, 368, 418 nm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3426, 3205, 1658, 1619, 1590, 1546, 1488, 1342, 1224, 756 cm^{-1} . ¹H NMR (DMSO-*d*₆): δ/ppm : 7.06-7.59 (m, 9H, Ar-H), 7.99 (d, *J* = 7.9Hz, 2H, Ar-H), 8.35 (d, *J* = 7.9Hz, 2H, Ar-H), 10.22 (s, H, OH, D₂O exchangeable), 14.20 (s, H, NH, D₂O exchangeable). MS m/z (%) 392 (M⁺+2, 60), 391 (M⁺+1, 100), 373 (11), 370 (18), 356 (44), 279 (22), 223 (11), 144 (16), 132 (9), 120 (9), 108 (12), 84 (10), 73 (19). Anal. Calcd. for C₂₁H₁₅ClN₄O₂ (390.09): C, 64.54; H, 3.87; N, 14.34. Found: C, 64.83; H, 3.49; N, 14.67.

(*Z*)-4-(4-Bromophenyl)hydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (**4e**)

Red crystals, Yield: 68%; m.p. 212 °C; UV-Vis (λ_{\max} in dioxane): 253, 369, 420 nm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3440, 3180, 1656, 1611, 1596, 1544, 1492, 1350, 1238, 766 cm^{-1} . ¹H NMR (CDCl₃): δ/ppm : 7.04-7.63 (m, 9H, Ar-H), 7.92

(d, $J = 7.8\text{Hz}$, 2H, Ar-H), 8.40 (d, $J = 7.8\text{Hz}$, 2H, Ar-H), 10.75 (s, H, OH, D₂O exchangeable), 14.35 (s, H, N-H, D₂O exchangeable). MS m/z (%) 437 ($M^+ + 2$, 41), 435 (M^+ , 64), 426 (7), 370 (100), 356 (28), 279 (21), 223 (11), 144 (17), 132 (8), 120 (9), 84 (15), 73 (21). Anal. Calcd. for C₂₁H₁₅BrN₄O₂ (434.04): C, 57.95; H, 3.47; N, 12.87. Found: C, 58.17; H, 3.11; N, 13.15.

(Z)-4-(4-Nitrophenyl)hydrazono)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-one (4f)

Redish brown crystals, Yield: 88%; m.p. 228 °C; UV-Vis (λ_{max} in dioxane): 255, 372, 426 nm. IR (KBr) ν_{max} /cm⁻¹: 3423, 3195, 1666, 1624, 1577, 1544, 1488, 1430, 1340, 1245 cm⁻¹. ¹H NMR (DMSO-d₆): δ /ppm: 7.05-7.63 (m, 9H, Ar-H), 7.95 (d, $J = 7.8\text{Hz}$, 2H, Ar-H), 8.41 (d, $J = 7.8\text{ Hz}$, 2H, Ar-H), 10.66 (s, H, OH, D₂O exchangeable), 14.33 (s, H, NH, D₂O exchangeable). MS m/z (%) 401 (M^+ , 51), 370 (37), 356 (18), 279 (29), 223 (100), 144 (24), 132 (6), 120 (11), 84 (14), 73 (24). Anal. Calcd. for C₂₁H₁₅N₅O₄ (401.11): C, 62.84; H, 3.77; N, 17.45. Found: C, 62.89; H, 3.87; N, 17.67.

pK_a Determination of compounds 4a-f

The acid dissociation constants of compounds 4a-f were determined potentiometrically in 80% dioxane-water mixture at 27±0.1 °C and ionic strength (KNO₃) 0.1 μ. A Metrohm 686 titroprocessor equipped with 665 Dosimat was used. The electrode and the titroprocessor were calibrated with standard Beckman buffer solutions of pH 4.01 and 7.00. The pH meter reading B recorded in dioxane-water solution was converted to hydrogen ion concentration [H⁺] by means of the relation of van Uitert and Hass²⁷ namely:

$$-\log [H^+] = B + \log U_H$$

where log U_H is the correction factor for the solvent composition and ionic strength used for which B is read. The value of log U_H was found to be 0.48. A carbonate-free sodium hydroxide titrant was prepared and standardized against potassium hydrogen phthalate solution. The experimental procedure followed in the determination of pK_a values and their calculations, by the method of least squares, from the titrant volume-pH data using the relation: $pK_a = \text{pH}_i - \log V_i/(V_e - V_i)$, is similar to that previously described.²⁸ In this equation, pH_i is the corrected pH value of the solution when the volume of the added titrant is V_i and V_e is the volume of the titrant at the equivalence point. The calculations of the pK_a values were carried out using computer program MINQUAD75.²⁹ The pK_a values obtained were reproducible to within ±

Table 1. Electronic absorption spectra of compounds 4a-f and their acid dissociation constants

Compound no.	σ_x	σ_x^-	pK ₁	pK ₂
4a	0.00	0.00	8.10	9.22
4b ^c	-0.17	-0.17	8.14	9.48
4c	-0.27	-0.27	8.15	9.72
4d	0.23	0.23	8.08	8.55
4e	0.23	0.23	8.00	8.22
4f	0.78	1.28	7.82	7.88

^cSolvent λ_{max} : acetonitrile 409, 364, 223; DMF 418, 373, 225; DMSO 416, 376, 221 nm.

0.02 pK_a unit. The results are summarized in (Table 1).

Dyeing and fastness determinations

Preparation of dye dispersion

The required amount of the dye (2% shade) was dissolved in acetone and added dropwise with stirring to a solution of Setamol WS, an anionic dispersing agent of BASF (sodium salt of a condensation product of naphthalene sulfonic acid and formaldehyde). The dye was precipitated in a fine dispersion ready for use in dyeing after evaporation of the solvent by warming.

Dyeing of polyester fabrics

The dye bath (1: 20, good to dye liquor ratio) in a sealed stainless steel dye pots of 250 ml capacity in "Galvanin-Marino VI-Italy" dyeing machine. Additional dispersing agent (0.5-1.0 g/l) was added and the pH of the bath adjusted to 5.5 using glacial acetic acid. Dyeing carried out by raising the dye bath temperature from 20 to 130 °C at a rate of 3 °C/min and holding at this temperature for 60 min before rapidly cooling to 50 °C at 9.9 °C/min. The dyed fabrics were then rinsed with cold water, reduction-cleared using sodium hydroxide (2 g/l) and sodium hydro-sulphite (1 g/l) and soaped with 2% nonionic detergent and ammonia (pH 8.5) at 50 °C for 30 min to improve washing fastness.

Color fastness tests

The color fastness of dyeing was evaluated using the standard method³⁰ and given in Table 3. The fastness to light, sublimation and perspiration was assessed in accordance with AATCC-15 (1985). The rubbing fastness test was carried out with a crockmeter (Atlas) in accordance with AATCC-88 (1988) and the wash fastness test in accordance with IS: 765-1979.

Color assessment

The colorimetric parameters (Table 3) of the dyed polyester fabrics were determined on a reflectance spectrophotometer (GretagMacbeth CE 7000a), equipped with a D65/108 source and barium sulphate as standard blank,

Table 2. Fastness properties of the synthesized dyes **4a-f** on polyester fabrics

Compound no.	Washing	Perspiration		Rubbing		Sublimation fastness		Light (80h)
		Acid	Alkali	Dry	Wet	180 °C	210 °C	
4a	4	4-5	4-5	4-5	4	4	3-4	6
4b	4-5	4-5	4-5	3	3	3-4	3	6
4c	4	4-5	4-5	4	4	4	4	6
4d	4-5	4-5	4-5	3-4	4	3-4	3	6
4e	4-5	4	4-5	4	5	4	3	7
4f	4-5	4-5	4-5	4	4	4	3-4	7

Table 3. Optical measurements of dyes **4a-f** on polyester fabrics

Compound no.	K/S	L*	a*	b*	C*	H*	Δa^*	Δb^*	ΔC^*	ΔL^*	ΔH^*
4a	38.5	65.2	33.2	77.3	84.2	66.8	--	--	--	--	--
4b	39.9	76.9	16.2	76.1	77.7	78.0	-17.0	-1.2	-6.5	11.7	11.2
4c	40.5	63.6	41.8	80.8	78.4	62.6	8.6	3.5	-5.8	-1.6	-4.2
4d	39.8	80.7	5.8	67.3	67.6	85.1	-26.4	-10	-16.6	15.5	18.3
4e	40.0	74.4	17.9	76.4	90.9	76.8	-15.3	-0.9	6.7	9.2	10
4f	40.4	62.4	32.8	71.6	79.1	64.9	-0.4	-5.7	-5.1	-2.8	-1.9

UV excluded, specular component included and three repeated measurements average settings.

***In vitro* antimicrobial activity**

Antimicrobial evaluation

The disks of Whatman lter paper were prepared with standard size (5.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized lter paper disks impregnated with a solution of the test compound in DMF (1 mg/ml) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10⁶ CFU/ml (Colony Forming U/ml) and 10⁴ CFU/ml were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of lter paper were inoculated in each plates. The utilized test organisms were: *S. aureus* (ATCC 25923) as example of Gram-positive bacteria and *E. coli* as example of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *F. oxysporum* fungal strains. Cephalothin and Cycloheximide were used as standard antibacterial and antifungal agents, respectively. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 24 h at 37 °C for bacteria and 48 h at 37 °C for fungi. Compounds that showed significant growth inhibition zones (>14 mm) using the twofold serial dilution technique,³¹ were further evaluated for their

minimal inhibitory concentrations (MICs).

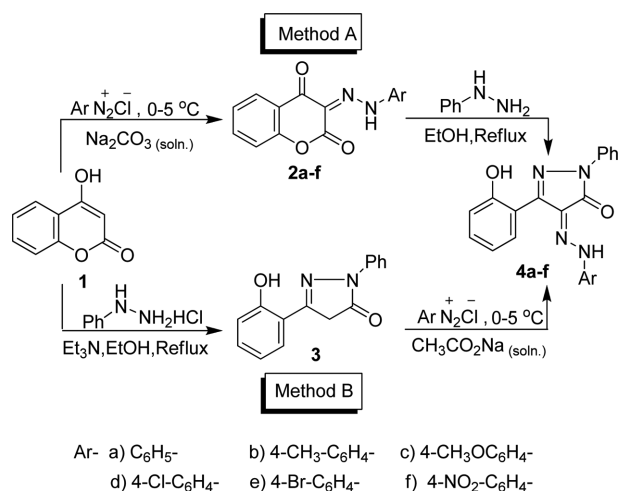
Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müllere-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, Cephalothin and cycloheximide were prepared in DMF at concentration of 1000 mg/ml. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-3.125 mg/ml 10 ml of the broth containing about 10⁶ CFU/ml of test bacteria was added to each well microtiter plate. The sealed microplates were incubated 24 h at 37 °C for antibacterial activity and 48 h at 37 °C for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

RESULTS AND DISCUSSIONS

Synthesis and tautomeric structure

The starting 2,3,4-chromantrione-3-aryl hydrazones **2a-f** were prepared according to published methods.^{25,26} Treatment of **2a-f** with phenyl hydrazine in ethanol under reflux afforded the respective 4-(2-arylazophenyl)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-ones **4a-f** in good yields (*Scheme 1*). The structures of the latter derivatives **4a-f**



Scheme 1. Synthesis of the studied compounds **4a-f**.

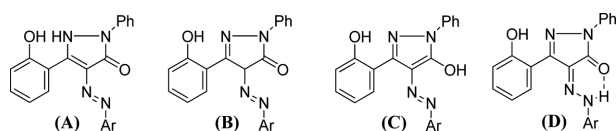


Fig. 1. The tautomeric structures of 4-(2-arylazophenyl)-3-(2-hydroxyphenyl)-1-phenyl-2-pyrazolin-5-ones (**A-D**).

were elucidated on the basis of their spectroscopic (UV, ¹H NMR, IR and MS) data and elemental analyses (see Experimental).

Azo-hydrazo tautomerisms have an importance to the dyestuff manufactures because of they have different properties eg.: light fastness. Azodyes **4a-f** may have one or more of four tautomeric structures as shown in Fig. 1. Spectral studies of these compounds revealed that such compounds have the structure **D**; no evidence for the other tautomeric forms could be obtained.

In order to elucidate the actual tautomeric form(s) of these compounds **4a-f**, their electronic absorption spectra were first studied. The spectra of the compounds in dioxane showed in each case three absorption bands in the regions 240–250, 362–377, and 398–426 nm. This absorption pattern is analogous to that reported for the hydrazo chromophore of pyrazolin-5-one dyes.^{32,3} Moreover, absorption bands attributed to azo structure between 332–360 nm^{33,34} and at above 430 nm³ are missing. This excludes the possibility of azo structures (**A-C**), and provides support for the hydrazo structure (**D**) as the most plausible structure tautomeric form. Also, the electronic absorption spectra of **4b**, taken as a typical example of series prepared, in different solvents showed little, if any, shift (Table 1). This result supports that only one tautomer in solution phase and the

relatively small difference observed are assumed to be caused by solute-solvent interaction.³⁵

Corroborative evidence that compounds **4a-f** exist in the tautomeric form (**D**) comes from inspection of the infrared spectral data. The presence of a strong band due to C=O group appears at 1666–1650 cm⁻¹³⁶ rules out the tautomeric structure (**C**) with no carbonyl group from consideration. The absence of tangible band associated with a conjugation of C=O with the C=N (attached to the hydrazo group) at 1720 cm⁻¹³⁷ has also omitted the existence of structures (**A-C**) which have incompletely conjugated chromophores, and leaves structure (**D**) as the possible tautomeric form. The pyrazolinones investigated possess the hydrazo moiety; therefore, all compounds exhibit a weak and broad NH stretching band in the region 3295–3180 cm⁻¹.³⁸ The strong decrease in this band is attributed to the intramolecular H-bond structure.^{39–41} The absorption bands between 3444–3423 cm⁻¹ region are attributed to the phenolic OH group. Also, the prominent band occurs around 1544 cm⁻¹ assigned to the benzene ring C=C skeletal vibration associated with the -NH-N= linkage in the 4-position on the pyrazolinone ring.⁴¹ In the region 1624–1611 cm⁻¹, an exo-type C=N stretching mode of the -NH-N= in the 4-position on the pyrazolinone ring is also expected.^{42,43} On the other hand, the band around 1500 cm⁻¹ is, in general, fairly intense because of the conjugation between the benzene and the pyrazolinone rings. The stretching vibration of the C_{arom}-N linkage show band between 1350–1337 cm⁻¹ in their spectra.⁴⁴ The band at 1260–1224 cm⁻¹ was also related to the N-N stretching mode.^{45,46}

In addition, the ¹H NMR spectra showed that the conspicuous absence of a proton at C-4 signal at about 6.4 ppm³⁶ that rules out the existence of structures (**B**). the highly deshielded singlet at 13.40–14.33 ppm was assigned to the presence of hydrazone-NH proton.^{36,47–49} This down field position of N-H proton is in evidence of chelation with the carbonyl group. Other down-field singlet at around 10.5 ppm was assigned to the phenolic OH group^{16–18} and peaks for aryl protons at 7.01–8.50 ppm. These results were compatible with a conclusion of such compounds **4a-f** have the structure (**D**).

The assigned structure was confirmed by an alternative synthesis of the products **4a**, **4b**, and **4d** as typical examples of the series prepared. Thus, coupling of 1-phenyl-3-(2-hydroxyphenyl)-2-pyrazolin-5-one **3** (lit. 18% yield)¹⁶ with the respective diazotized anilines gave the products **4a**, **4b**, and **4d** that proved identical in all respects (mp., mixed mp., IR and UV.) with those obtained above from

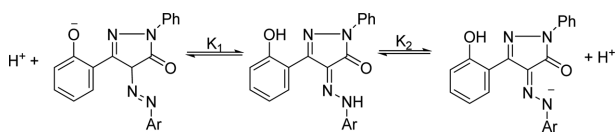


Fig. 2. Acidity constants pK_1 and pK_2 of the studied compounds **4a-f**.

the method A (Scheme 1).

To provide further evidence for the assignment of the tautomeric structure **D**, their acid dissociation constants of the series prepared were determined and their correlation with Hammett substituent constants were examined.⁵⁰ The acid dissociation constants were determined at 27 °C, potentiometrically, in 80% dioxane-water mixture (v/v). In all determinations the ionic strength μ was kept constant at 0.1. From the pH-titrant volume data, the acid dissociation constants of the compounds were calculated and the results are summarised in Table 1. These results show that each of the studied dyes behaves as a dibasic acid (H_2A) since each dye has two acidity constants pK_1 and pK_2 . The higher acidity constant K_1 was found to be independent of the nature of the substituent, whereas the lower acidity constant pK_2 is affected by such substituents (Table 1). These findings seem consistent with tautomeric structure **D** and indicate that pK_1 and pK_2 correspond to the deprotonation of phenolic OH and the hydrazone NH groups, respectively (Fig. 2).

When the pK_a values were plotted versus Hammett substituent constants σ_x ⁵⁰ all the substituents fall on the correlation line except the substituents with -R effect, namely the *p*-Cl, *p*-NO₂ and *p*-Br groups, which are capable of direct interaction with the negatively charged reaction site. However, when the pK_a values were plotted versus σ_x^- constants,⁵⁰ better correlation was obtained. The Hammett type equation of the regression line obtained is:

$$pK_2 = 9.05 - 2.30 \sigma_x^-; r^2 = 0.997; s = \pm 0.08$$

where r is the correlation coefficient and s is the standard deviation. This excellent linear correlation indicates that the parameter r^- in the Hammett-Ryan equation⁵¹

$$pK_2 = pK_2^0 + \rho \{ \sigma_x + r^- (\sigma_x^- - \sigma_x) \},$$

which gives the contribution of the resonance effect of the substituent varied, is close to unity for the series **4a-f** studied. The foregoing linear correlation between pK_a values and σ_x^- constants and the values of ρ and r^- found provided further evidence that the studied compounds **4a-f** exist predominantly in the hydrazone form (**D**). This is because the values of $\rho = 2.30$ and $r^- = 1.00$ are similar to those reported

for ionization of phenols ($\rho = 2.67$; $r^- = 1.00$) and anilinium ions ($\rho = 2.77$; $r^- = 1.00$) in 50% ethanol-water mixture.⁵² This finding indicates that the negative charge on the anion formed by deprotonation of **4a-f** is largely localized on the N-atom adjacent to the benzene bearing the substituent. This anion will take up a proton on the N-atom bearing the negative charge to give (**D**) which is also stabilized by hydrogen bonding. Furthermore, if either form (**A**), (**B**) or (**C**) were the predominant form for the studied compounds, the ρ values would be less than 2.0 and will be similar to that reported for ionization of arylazophenols ($\rho = 1.223$; $r^- = 0.286$).⁵² Since the bridge between the substituent and the deprotonation reaction site in either form **A**, **B**, or **C** is longer than in **D**.

Thus, it is not unreasonable to conclude that the observed linear correlation of the acid dissociation constants with the Hammett equation indicates that the hydrazone tautomeric form (**D**) prevails under the conditions of the pK_a measurement. Compound **4a-f** can exist in the E-structure or the Z-structure, the Z-structure of hydrazone (**D**) was preferred than the E-structure^{33-35,53-55} in which the carbonyl was involved in the H-bonded system. The preferential formation of Z-structure was supported from molecular orbital calculation using Hyperchem 7, semi-empirical method (PM3), which revealed that the heat of formation of Z-structure is lower than E-structure.

Dyeing properties of the dyes on polyester fabrics Assessment of color fastness

The synthesized disperse dyes under investigation **4a-f** were applied to polyester fabrics at the 2% shade by high-temperature pressure technique (130 °C) and gave generally hues, ranging from yellow to orange. The dyes on polyester fabrics were evaluated in terms of their fastness properties as shown in Table 2.

Fastness to Washing

Excellent behavior is shown in the fastnesses to washing may be due to:

- 1) The absence of solubilizing groups, which renders solubility, and wash ability of the dye-out of the fabrics.
- 2) The size of the dye molecule is considered relatively big.
- 3) The good intrafiber diffusion of the dye molecules inside the fabrics.

Fastness to Perspiration: (acid and alkaline)

The fastness to acid perspiration is rather satisfactory ranging 4-5. Such results can be expected considering the fact that the prepared arylazopyrazolin-5-one dyes are relatively stable in acid medium, similar to the conditions used in this test. On the other hand, fastness to alkaline

perspiration can be considered good fastness 4-5.

Fastness to Rubbing

The rubbing fastness (wet and dry) is boarder line and this may be attributed to inadequate diffusion of dye molecule into the fabrics.

Fastness to Sublimation

The sublimation resistance of disperse dyestuffs is determined mainly by its molecular size. Consequently, the majority of these dyes were found to have good sublimation fastness 3-4 according to the international Geometric Grey Scale.⁵⁶

Fastness to Light

It is significantly affected by the nature of the substituents in the diazo component. In this study, the inclusion of the electron withdrawing (nitro or bromo) groups improves the light fastness. This is in agreement with the notion that the azo compounds appended with electron withdrawing substituents on the diazo components are less prone to photofading.⁵⁷ Generally, the prepared dyes showed satisfactory fastness to light ranging 6-7 according to the international Geometric Grey Scale.

Color assessment

The color parameters (Table 3) of the dyed polyester fabrics were measured using the Gretag Macbeth CE 7000a spectrophotometer. The assessment of color-dyed fabrics was made in terms of tristimulus colorimetry. The following CIELAB coordinates are measured, lightness (L^*) chroma (C^*), hue angle from 0° to 360° (h), (a^*) value represents the degree of redness (positive) and greenness (negative) and (b^*) represents the degree of yellowness (positive) and blueness (negative). A reflectance spectrophotometer (Gretag Macbeth CE 7000a) was used for the colorimetric measurements on the dyed samples. K/S value given by the reflectance spectrometer is directly correlated with the dye concentration on the dye substrate according to the Kubelka-Munk equation:⁵⁷

$$K/S = (1-R)^2/2R,$$

where K = absorbance coefficient, S = scattering coefficient, R = reflectance ratio. The parent dyestuff in each group is taken as the standard in the color difference calculation (ΔL^* , ΔC^* , and ΔH^*). The results were obtained using CIELAB techniques, and are given in Table 3, where ΔL^* is the lightness difference, ΔC^* is the chroma difference, and ΔH^* is the hue difference. A negative sign of ΔL^* indicates that the dyed ber becomes darker than the standard, whereas a positive sign indicates that the dyed ber becomes lighter than the standard. A negative sign of ΔC^* indicates that the dyed ber becomes duller than the

standard, whereas a positive sign indicates that the dyed ber becomes brighter than the standard. A negative sign of ΔH^* indicates that the color shifted to a red color, while a positive sign indicates that the color shifted to yellowish.

The application of the dyes **4a-f** on polyester fabrics has shown that the K/S values in this group vary from 38.5 to 40.5, which all members of this groups **4b-e** increase the strength of the K/S value of the polyester fabric compared with that of the parent **4a**. Each dye on polyester fabric is measured by comparing chromaticity difference C^* , where the introduction of methyl, methoxy, bromo, and nitro groups into dyes **4b-e**, made each of these dyes brighter than the parent dye **4a**. On the other hand, the darkness or lightness of the dye on polyester fabrics is measured by comparing the values of L^* (+ve means lightness while -ve means darkness). The L^* values given in Table 3 showed that the introduction of methyl, chloro and bromo groups made each of these dyes lighter than the parent dye **4a**. While, the introduction of methoxy, and nitro groups in dyes made each of these dyes darker than the parent dye **4a**. The negative values of a^* (red-green axis) and b^* (yellow-blue axis) indicated that the color hues of the dyes **4b**, **4d**, **4e** and **4f** on polyester fabric are shifted to the greenish and yellowish directions. these results are in line with the previously reported by Müller⁵⁸ on the effect of the substituent in the dye structure and hue.

Antimicrobial evaluation

The tested microorganisms were obtained from the culture collection at the Microbiology laboratory, National Organization for Drug Control and Research (NODCAR). The newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 25923) as example of Gram-positive bacteria and *Escherichia coli* (ATCC 14169) as example of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Fusarium oxysporum* (ATCC 16417) fungal strains.

Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Cephalothin and cycloheximide were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds showed significant growth inhibition zones (>14 mm) using twofold serial dilution

Table 4. Minimal inhibitory concentrations (MIC, mg/ml) and inhibition zone (mm) of synthesized compounds

Compound no.	MIC ^a in mg/ml, and zone of inhibition (mm)		
	Bacteria		Fungi
	Gram-positive bacteria (<i>S. aureus</i>)	Gram-negative bacteria (<i>E. coli</i>)	(<i>F. oxysporum</i>)
4a	100 (22)	100 (19)	100 (17)
4b	50 (26)	50 (24)	100 (23)
4c	100 (20)	50 (18)	100 (15)
4d	6.25 (32)	12.5 (31)	6.25 (30)
4e	3.125 (37)	6.25 (38)	25 (34)
4f	12.5 (33)	12.5 (26)	25 (24)
Reference drug			
Cephalothin	6.25 (36)	6.25 (38)	NT ^b
Cycloheximide	NT ^b	NT ^b	3.125 (43)

^aMIC: Minimal inhibitory concentration values.

^bNT: Not tested.

method.³¹ The MIC (mg/ml) and inhibition zone diameters values are recorded in *Table 4*.

The inhibition zone diameters values cited in *Table 4* between brackets are attributed to the tested original concentration (1 mg/ml) as a preliminary test. The results depicted in *Table 4* revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against fungal strains. In this study, regarding the structure reactivity relationship of the pyrazoline-5-one substituents of different sizes and electronic properties are tried on the hydrzono/azophenyl ring to see if they have any effect on antimicrobial activity. In general, most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. The results of this study with regard to antimicrobial activity have been found to be interesting against *S. aureus*.

Among the pyrazoline-5-one derivatives **4a-f**, compound **4e** (MIC 3.125 mg/ml) having a *p*-bromo group on the phenyl ring was found to be most potent antibacterial agent against Gram-positive *S. aureus* compared to the standard drug Cephalothin (MIC 6.25 mg/ml), while its activity was 50% lower than of Cephalothin against *E. coli*. Compounds **4d** and **4f** (MIC 6.25 mg/ml) having *p*-chloro and *p*-nitro substituent on phenyl ring, respectively also showed equipotent to Cephalothin in inhibiting the growth of *S. aureus* (MIC 6.25 mg/ml) but compound **4f** showed about 25% of the activity against *E. coli*. On the other hand, concerning the antibacterial activity of the compounds **4a-c** exhibited weak to moderate growth inhibitory activity against *S. aureus* and *E. coli* as revealed from their MIC values (50-100 mg/ml).

Regarding the activity of synthesized compounds, against antifungal strains, the results revealed that compound **4d** having a *p*-chloro group at the phenyl ring showed significant antifungal activity (MIC 6.25 mg/ml) against *F. oxysporum* in comparison to the standard drug Cycloheximide (MIC 3.125 mg/ml). Rests of the compounds tested were found to have good to moderate antifungal activity (25-100 mg/ml). The results of antibacterial and antifungal activities showed that compounds having electron-withdrawing group at the para position of phenyl ring were more active than compounds having electron donating groups.

CONCLUSION

2,3,4-Chromantrione-arylhydrazones **2a-f** are useful precursors for synthesis of the title azopyrazolin-5-one dyes **4a-f**. The spectroscopic data of the compounds prepared and the correlation of their acidity constants with the Hammett equation have proved that such compounds exist predominantly in the hydrazone keto structure, (**D**), in both ground and excited states. New compounds applied as disperse dyes on polyester fabrics. The dyed fabrics exhibited very good washing, perspiration, rubbing and light fastness properties with little variation in the moderate to good sublimation fastness. Furthermore, it was also observed that compounds having electron-withdrawing groups **4d-f** were more active than compounds having electron-releasing groups **4b, 4c** at the phenyl ring attached by hydrazono/azo linkage at the 4th position of pyrazolinone moieties. Preliminary study of the structure-activity relationship revealed that electron-withdrawing groups in phenyl ring had a promising effect

on the antimicrobial activity. Thus, the series provided a new opportunity for possible modification of pharmacophoric, dyes requirements and future exploitations.

Acknowledgment. The authors are very grateful to Dr. Ehab Ali, Department of Botany, Faculty of Science, Mansoura University, for performing the antimicrobial evaluation. We thank Mis. Asmaa Elgainde, El-Nasr Company for Spinning and Weaving, El-Mahalla El-Kubra, Egypt. for her help in performing dyeing and fastness tests for the dyes. Mr. Moaz M. Abdou thanks Academy of Scientific Research and Technology, ASRT, Egypt, for their grant.

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