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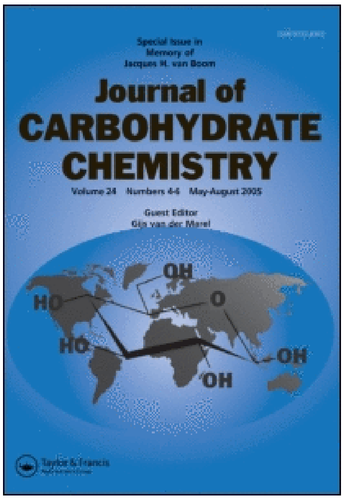

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El Sayed H. El Ashry[a]; Yeldez El Kilany[a]; Ahmed Mousaad[a]
[a] Chemistry Department, Faculty of Science Alexandria, University, Alexandria, Egypt

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PERSPECTIVES AND MECHANISTIC ASPECTS OF THE ACTION OF HYDRAZINES ON KOJIC ACID[1]

El Sayed H. El Ashry,* Yeldez El Kilany and Ahmed Mousaad

Chemistry Department
Faculty of Science
Alexandria, University
Alexandria, Egypt

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ABSTRACT

The mode of action of arylhydrazines on kojic acid was investigated. Some novel types of compounds were isolated, and their structures were determined. The mechanism of the reactions is discussed.

INTRODUCTION

The antimicrobial activity of some kojic acid derivatives has been recognized for a relatively long time.[2] Recently, a phenylhydrazone derivative of kojic acid was found to be active against Trypanosoma cruzi, but inactive against Tetrohymena pyriformis, Euglena gracilis, and Astasia chattoni. The compound gives rise to significantly decreased ^{3}H-thymidine and ^{3}H-uridine incorporation in the cells of T. cruzi, while ^{14}C-leucine incorporation is not affected.[3] The product of the reaction of kojic acid (1) with phenylhydrazine has been formulated, as reported by Corbett,[4] to be 1,5-anhydro-4-deoxy-hex-4-enitol-2,3-diulose-2,3-bis-(phenylhydrazone) (2). On the other hand, this reaction was studied by El Ashry,[5] Ichimoto et al.,[6] as well as by Thomas and

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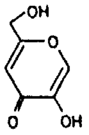
*To whom correspondence should be addressed.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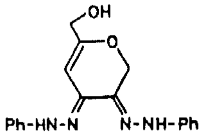
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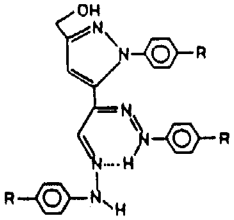
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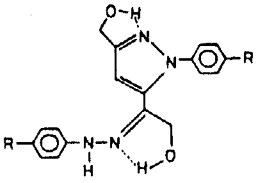
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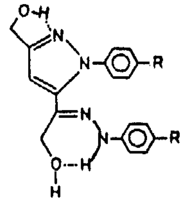
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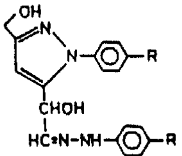
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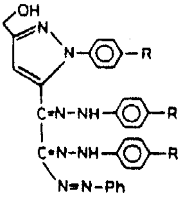
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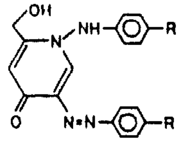
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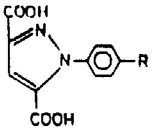
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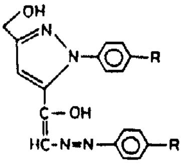
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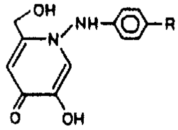
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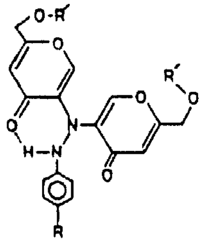
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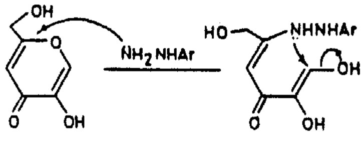
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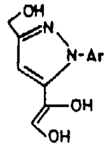
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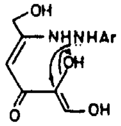
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a) R = H b) R = Br c) R = Cl

Marker,[7] and they all found that the product was a mixture (structures **3a** - **8a**, **10a**, and **11a** were proposed) and that none of its components could be assigned to structure **2**. Consequently, it is of interest to evaluate which structures in such a mixture are responsible for the biological activity.[3] The mode of reaction of kojic acid with arylhydrazines was investigated, and the possible mechanistic aspects of the reaction are discussed in this paper.

RESULTS AND DISCUSSION

When kojic acid was allowed to react with p-bromophenylhydrazine in methanol, the starting acid was recovered unchanged. On the other hand, kojic acid was found to react readily with p-bromo- or p-chlorophenylhydrazine in aqueous acetic acid to yield a mixture of various products. The mixture from the reaction with each hydrazine was separated, and the structures of the products were determined.

1-(Aryl)-5-[1-(arylhydrazono)-2-(hydroxy)eth-1-yl]-2-(hydroxy-methyl)pyrazole (4). These types of compounds do not give the characteristic coloration of kojic acid when treated with ferric chloride, indicating the absence of the enolic hydroxyl group. Elemental analysis indicated that these products arose from the reaction of one mole of kojic acid (**1**) with two moles of the corresponding hydrazine. Their infrared spectra indicated the absence of bands in the carbonyl frequency region. The ^{1}H NMR spectrum fo **4b** showed a four-proton doublet at δ4.50 for two methylene groups. The two hydroxyl protons appeared as two triplets at δ5.13 and 5.45 whose attachment to the two methylene groups was confirmed by deuteration (see experimental). The singlet at δ6.67 was assigned to H-4 of the pyrazole ring. The NH proton of the hydrazone residue appeared at δ9.80, indicating that it does not participate in hydrogen bonding. These data agree with the structure **4b** rather than **5b** as compared with the data shown for the phenyl derivative[5] (two separate signals shown for two methylene groups and the NH proton at δ 10.47). The structure of **4b** was further confirmed by its oxidation with potassium permanganate to afford 1-(p-bromophenyl)-pyrazole-3,5-dicarboxylic acid (**9b**), indicating that **4b** was a 3,5-disubstituted pyrazole derivative. Furthermore, **4b** failed to give a formazan raction, and upon treatment with p-bromophenylhydrazine, it yielded the bis(hydrazone) **3b** which gave a positive formazan reaction due to the formation of **7b**. This behavior confirmed the location of the hydrazone residues in **4b** and **3b**. The p-chlorophenyl derivative **4c** showed similar spectral data to **4b**, and, consequently, a similar hydrogen-bonded structure was assigned for it.

1-(Aryl)-5-[1,2-bis(arylhydrazono)glyoxalyl]-3-(hydroxymethyl)pyrazole (3). These types of compounds have a yellow color, and they are products of the reaction of three molecules of the hydrazine with one molecule of **1**. The ^{1}H NMR spectrum of **3b** showed a two-proton doublet at δ 4.50 and a one-proton triplet at δ 5.19 corresponding to the presence of a hydroxymethyl group. The H-4 of the pyrazole ring appeared as a singlet at δ 6.63. The H-C=N of the glyoxalyl residue appeared as a singlet at δ 7.91, while the two NH protons appeared as two singlets at δ 10.91 and 12.10. The difference in their chemical shifts may be attributed to the involvement of the latter in chelation between the hydrazone residues, as in **3b**, in a manner similar to that described for sugar osazones.

1-(p-Chloroanilino)-5-hydroxy-2-(hydroxymethyl)-4H-pyridin-4-one (11c). This product gave a characteristic coloration with ferric chloride, indicating the presence of an enolic hydroxyl group. Its ^{1}H NMR spectrum showed a singlet δ 4.31 for a methylene group. The two singlets which appeared at δ 6.31 and 7.25 were due to H-3 and H-6, respectively. The signal of H-6 appeared at a higher magnetic field than the corresponding proton (ca. δ 8.0) of either kojic acid or its derivatives. This shielding effect on H-6 could be attributed to the difference in the electronegativity of the nitrogen and the oxygen atoms of the rings. The NH proton appeared at δ 9.33. Consequently, structure **11c** was assigned to this product.

1-(Aryl)-2,2-di[2-(hydroxymethyl)-4H-pyran-4-one-5-yl]-hydrazine (12). These types of compounds did not give a characteristic coloration with ferric chloride, indicating the absence of the enolic hydroxyl group. The ^{1}H NMR spectrum of **12b** showed a four-proton doublet at δ 4.50 and a two-proton triplet at δ 5.75, indicating the presence of two hydroxymethyl groups. The singlet at δ 6.43 and the doublet at δ 8.03 were due to the two H-3 and two H-6 protons, respectively. A multiplet of four aromatic protons appeared at δ 7.50, and a hydrogen-bonded NH proton appeared at δ 12.20. The infrared spectrum of **12b** showed the presence of absorptions due to the C=O, NH, and OH groups. The p-chlorophenyl derivative **12c** showed similar spectral features. Furthermore, acetylation of **12c** with acetic anhydride in pyridine afforded the corresponding acetyl derivative **13c** whose ^{1}H NMR spectrum showed a singlet at δ 2.20 due to two acetyl groups. The singlet at δ 5.12 was assigned to two methylene groups, whose downfield position indicated their attachment to the acetoxyl groups. The H-3 and H-6 appeared as resonances at δ 6.58 and 7.89, respectively, and the intensities agreed with the presence of two pyronyl rings. The aromatic protons appeared at δ 7.50, and the NH proton appeared at δ 11.0. Consequently, structures **12** and **13** may be assigned for this product and its acetyl derivative, respectively.

Acetyl-arylhydrazines. 2-Acetyl-1-arylhydrazines were isolated from the reaction of kojic acid with either the p-bromo or p-chlorophenylhydrazine.

Mechanistic aspects. It can be concluded from the present results, as well as from those previously reported,[5,8,9] that the reaction of hydrazines with kojic acid is very sensitive to the molar ratio of the reactants, the acid catalyst, and the duration of the reaction. The various products obtained from the reactions of hydrazine or arylhydrazines with kojic acid draw attention towards their mode of formation. Electrophilic substitutions on kojic acid take place exclusively at the 6-position, owing to the presence of the 5-hydroxyl group.[2] Consequently, it has been concluded that nucleophilic attack will be at the 2-position. This conclusion is in agreement with the present results. Thus the attack of hydrazine on the 2-position, followed by a ring opening and shift of electrons may give **14**, which cyclized to the pyridone **11**.

Intermediate **14** seems to be the most important one because of its possible cyclization in various ways. Cyclization can be achieved by considering any of its various conformers. Thus, **14** is cyclized to the pyrazole **15**. The latter pyrazole possesses an enediol system as a side chain which can be easily attacked by the hydrazine to give the hydrazone derivative **4**. However, upon further reaction with the arylhydrazine, **4** gave the corresponding bis-arylhydrazone 3 via a mechanism similar to that reported for the formation of sugar arylosazones from the corresponding arylhydrazones. It should be noted that similar formation of bishydrazones by using hydrazine is not anticipated because of the unique oxidizing character of the arylhydrazine compared with that of hydrazine.

When **4** reacted with the arylhydrazine, two moles of the arylhydrazine were required, and a mole of the aromatic amine was produced as a byproduct which may in turn react with kojic acid (**1**) to give the corresponding pyridone.

Another conformer of **14** could be **16**, which upon cyclization affords pyridazine derivatives. This pathway seems to play an important role in the case of using hydrazine, as verified experimentally,[9] to give the corresponding pyridazine as a major product. However, in case of arylhydrazines, no product of this type could be isolated. Reaction of **16** with hydrazine may afford **12**. Although, the formation of such compounds seems improbable, it was verified experimentally by the isolation of the corresponding p-bromo- and p-chlorophenyl derivatives.

However, no such compound has been isolated from the reaction with phenylhydrazine.

Consequently, the results accumulated from these studies indicate that the reaction proceeds through various pathways, and efforts should be directed towards the isolation of other products that can be expected from the possible reaction of the intermediates. Most importantly, structure 2, as formerly postulated to be a direct reaction product, does not exist.

EXPERIMENTAL

General procedures. Melting points were determined with a Kofler-block apparatus and are uncorrected. Infrared spectra were recorded with a Pye Unicam SP 200 spectrometer. ^{1}H NMR spectra were measured with an EM-390 NMR spectrometer for solutions in DMSO-$\underline{d}_6$ or in $CDCl_3$ using tetramethylsilane as external or internal standard, respectively. Chemical shifts are given on the δ-scale. TLC was performed on "Baker-flex" silica gel 1B-F (2.7-7.5 cm^{-1}) plates. Elemental analyses were performed in the Unit of Microanalysis, Faculty of Science, Cairo University, Cairo, Egypt.

Reaction of kojic acid (1) with p-bromophenylhydrazine. A solution of kojic acid (1.42 g, 1 mmol) in water (20 mL) was treated with p-bromophenylhydrazine hydrochloride (8.94 g, 4 mol), sodium acetate (3.2 g, 4 mol), and acetic acid (3 mL). The reaction mixture was heated for 1 h on a boiling water bath and then cooled, whereby a red oil separated out. The aqueous layer was decanted and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to give colorless crystals of 2-acetyl-1-(p-bromophenyl)-hydrazine (0.2 g): mp 163-164 °C (lit.[10] mp 167 °C). IR (KBr): 3300 and 3120 (NH), 1660 and 1640 cm^{-1} (OCN); ^{1}H NMR (DMSO-$\underline{d}_6$): δ9.61, 8.93 (2s, 1 H, 5:1, NH), 8.10, 7.80 (2s, 1H, 1:5, NH), 7.30, 6.67 (2d, 4H, aromatic protons), and 1.93 (s, 3H, Ac).

The red oil that that been separated earlier was dissolved in hot chloroform and allowed to cool whereby the following compounds could be fractionally crystallized.

(a) 1-(p-Bromophenyl)-2,2-di[2-(hydroxymethyl)-4H-pyran-4-one-5-yl]hydrazine (12b). Compound **12b** was crystallized from ethanol to give colorless needles (0.3 g, 10% yield): R_f 0.36 (4:1, chloroform-methanol): mp 320 °C. IR (KBr): 3380 (OH), 3180 (NH), 1650 (CO) and 1640 cm^{-1} (C=C); ^1H NMR (DMSO-d_6): δ 12.20 (s, 1H, NH), 8.03 (d, 2H, J <1 Hz, 2H-6), 7.50 (m, 4H, aromatic protons), 6.43 (s, 2 H, 2H-3), 5.75 (bt, 2H, 2 OH), and 4.50 (d, 4H, $2CH_2$). Addition of D_2O caused the disappearance of the NH and OH signals and the appearance of a resonance at δ 4.50 as a singlet. Anal. Calcd. for $C_{18}H_{15}BrN_2O_6$: C, 49.67; H, 3.47; N, 6.44. Found: C, 49.9; H, 3.7; N, 6.5.

(b) 1-(p-Bromophenyl)-5-[1-(p-bromophenylhydrazono)-2-(hydroxy)eth-1-yl]-3-(hydroxymethyl)pyrazole (4b). Compound **4b** was recrystallized from ethanol to give colorless needles (0.1 g, 2% yield): R_f 0.70 (4:1 chloroform-methanol); mp 176 °C. IR (KBr): 3350 and 3320 (OH), 1600 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ 9.80 (s, 1H, NH), 7.66, 6.40, 7.33, and 7.16 (4d, 8H, aromatic protons), 6.67 (s, 1H, H-C=), 5.45 (t, 1H, OH), 5.13 (t, 1H, OH), and 4.50 (d, 4H, $2CH_2$). Addition of D_2O caused the disappearance of the resonances of the NH and OH groups and the appearance of a singlet at δ 4.50. Anal. Calcd, for $C_{18}H_{16}Br_2N_4O_2$: C, 45.03; H, 3.36; N, 11.67. Found: C, 45.3; H, 3.4; N, 11.3.

(c) 5-[1,2-Bis(p-bromophenylhydrazono)glyoxalyl]-1-(p-bromophenyl)-3-(hydroxymethyl)pyrazole (3b). Compound **3b** was isolated after the separation of the above two compounds and was recrystallized from ethanol to give yellow needles (0.3 g, 5% yield): R_f 0.5 (4:1 chloroform-methanol); mp 222-223 °C. IR (KBr): 3400 (OH), 3260 (NH), 1585 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ 12.10 (s, 1H, NH), 10.91 (s, 1H, NH), 7.91 (s, 1H, H-C=), 7.65, 6.60, 6.95 and 7.50 (3d, and m, 12H, aromatic protons), 6.63 (s, 1H, H-4), 5.14 (t, 1H, OH), and 4.5 (d, 2H, CH_2). The addition of D_2O caused the disappearance of the resonances at δ 12.1, 10.91, and 5.19, as well as the transformation of the signal at δ 4.50 into a sharp singlet. Anal. Calcd. for $C_{24}H_{19}Br_3N_6O$: C, 44.54; H, 2.96; N, 12.99. Found: C, 44.2; H, 3.1; N, 12.6.

Formazan test. Compound **3b** in a mixture of pyridine (1 mL) and methanol (1 mL) was treated with benzenediazonium chloride[11] at 0-5 °C, and a red formazan color developed immediately.

Reaction of kojic acid (1) with p-chlorophenylhydrazine. This reaction was processed as previously described, and the following compounds were isolated.

(a) 2-Acetyl-1-(p-chlorophenyl)-hydrazine. Colorless crystals of (0.1 g) were obtained: mp 152-153 °C (lit.[12] mp 154 °C). IR (KBr): 3290, 3100 (NH) and 1660 cm^{-1} (OCN); ^1H NMR (DMSO-d_6): δ 9.63 and 8.93 (2s, 1H, 8:1, NH), 8.10, 7.80 (2s, 1H, 1:8, NH), 7.15, 6.70 (2d, 4H, aromatic protons), and 1.90 (s, 3H, Ac).

(b) 1-(p-Chloroanilino)-5-hydroxy-2-(hydroxymethyl)-pyridin-4-one (11c). The aqueous layer, after extraction with chloroform, was cooled overnight, whereby the title compound was obtained as colorless crystals (0.1 g, 4% yield), in addition to some unchanged kojic acid (**1**, 0.1 g). The product was recrystallized from ethanol: R_f 0.3 (4:1 chloroform-methanol); mp 235 °C (dec). IR (KBr): 3250 (OH), 1640 cm^{-1} (CO); ^1H NMR (DMSO-d_6): δ 9.33 (s, 1H, NH), 7.30, 6.50 (2d, 4H, aromatic protons), 7.25 (s, 1H, H-C=), 6.31 (s, 1H, H-C=), and 4.31 (s, 2H, CH_2). The resonance at δ 9.33 disappeared upon deuteration. Anal. Calcd. for $C_{12}H_{11}ClN_2O_3$: C, 54.05; H, 4.16; N, 10.50. Found: C, 53.7; H, 4.0; N, 10.2.

(c) 1-(p-Chlorophenyl)-2,2-di-[2-(hydroxymethyl)-4H-pyran-4-on-5-yl]hydrazine (12c). The red oil that had been isolated earlier was dissolved in hot chloroform and then allowed to cool, whereby the title compound separated out. It was recrystallized from ethanol (0.2 g, 5% yield): R_f 0.62 (4:1 chloroform-methanol); mp 304-305 °C. IR (KBr): 3300 (OH), 1645 (CO), 1620 cm^{-1} (C=C); ^1H NMR (DMSO-d_6): δ 12.2 (bs, 1H, NH), 7.90 (d, 2H, J <1 Hz, 2H-6), 7.54 (m, 4H, aromatic protons), 6.43

(s, 2H, 2H-3), 5.77 (s, 2H, 2OH), and 4.54 (s, 4H, $2CH_2$). The NH and OH signals disappeared upon addition of D_2O. Anal. Calcd. for $C_{18}H_{15}ClN_2O_6$: C, 55.33; H, 3.87; N, 7.17. Found: C, 55.5; H, 3.7; N, 6.9.

1-(p-Chlorophenyl)-2,2-di-[2-(acetoxymethyl)-4H-pyran-4-on-5-yl]-hydrazine (13c). A solution of **12c** (0.4 g, 1.0 mmol) in pyridine (5 mL) was treated with acetic anhydride (5 mL), and the reaction mixture was kept overnight at room temperature. The mixture was poured onto crushed ice, and the product that separated out was filtered, washed with water, and recrystallized from ethanol to give colorless needles (0.3 g, 62% yield): R_f 0.7 (4:1 chloroform-methanol); mp 237-238 ^{o}C. IR(KBr): 1760, 1720 (OAc), 1660 (CO), 1630 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ11.0 (bs, 1H, NH), 7.89 (d, 2 H, J <1 Hz, 2H-6), 7.50 (m, 4H, aromatic protons), 6.58 (s, 2H, 2H-3), 5.12 (s, 4H, $2CH_2$), and 2.20 (s, 6H, 2 AcO). Anal. Calcd. for $C_{22}H_{19}ClN_2O_8$: C, 55.63; H, 4.03; N, 5.90. Found: C, 56.0; H, 3.6; N, 5.9.

(d) 1-(p-Chlorophenyl)-5-[1-(p-chlorophenylhydrazono)-2-(hydroxy)eth-1-yl]-3-(hydroxymethyl)pyrazole (4c). The mother liquor from **12c** was concentrated to give the title compound that was recrystallized from ethanol to give colorless needles of **4c** (0.1 g, 3% yield): R_f 0.08 (4:1 chloroform-methanol), mp 173-174 ^{o}C. IR (KBr): 3300 (OH), 1580 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ 9.77 (s, 1H, NH), ~7.5 (m, 4H, aromatic protons), 7.02, 6.40 (2d, 4H, aromatic protons), 6.67 (s, 1H, H-C=), 5.40 (bt, 1H, OH), 5.12 (bt, 1H, OH), and 4.48 (d, 4H, $2CH_2$). The resonances at δ9.77, 5.40, and 5.12 disappeared upon addition of D_2O, whereas the doublet at δ4.48 collapsed to a singlet. Anal. Calcd. for $C_{18}H_{16}Cl_2N_4O_2$: C, 55.26; H, 4.12; N, 14.33. Found: C, 55.7; H, 4.4; N, 14.1.

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