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Note

Mode of action of phenylhydrazine on kojic acid*

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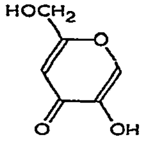
The reaction of kojic acid (1) with phenylhydrazine in acetic acid is known to yield various products. Corbett[1], who first investigated this reaction, isolated a compound, m.p. 169.5–171°, to which he assigned a 1,5-anhydro-4-deoxyhex-4-enitol 2,3-diulose 2,3-bis(phenylhydrazone) structure (2). However, this compound could not be isolated by subsequent investigators[2,3] including the present one.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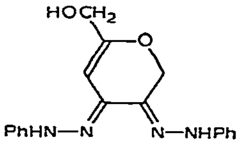
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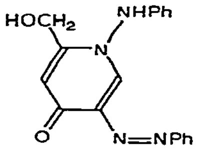
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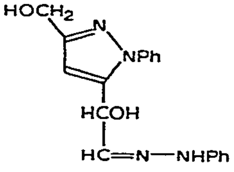
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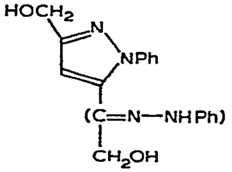
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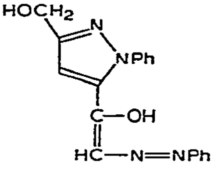
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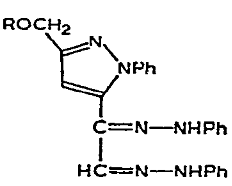
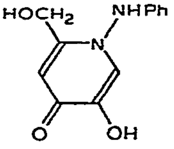

8a, R = H
8b, R = Ac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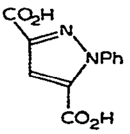
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Marxer and Thomas[2] isolated 1-anilino-2-(hydroxymethyl)-5-phenylazo-4-pyridinone (3) and the phenylhydrazone (4) of 3-(hydroxymethyl)-5-(1-hydroxy-2-oxoethyl)-1-phenylpyrazole. Later, four pyrazole derivatives were isolated[3]; these were two isomeric phenylhydrazones (5) and 6) of 3-hydroxymethyl-5-(2-hydroxy-1-oxoethyl)-1-phenylpyrazole, 3-(hydroxymethyl)-5-(1-hydroxy-2-phenylazoethenyl)-1-phenylpyrazole (7) (which is the dehydro derivative of 4), and the bis(phenylhydrazone) (8) of 5-(1,2-dioxoethyl)-3-(hydroxymethyl)-1-phenylpyrazole. In the present work, the structures, of three of these reaction products were verified by n.m.r. and mass spectrometry, and two new reaction products of kojic acid and phenylhydrazine have been isolated.

RESULTS AND DISCUSSION

Hydrazine hydrate reacts with kojic acid in the absence of acetic acid to give 1,4-dihydro-3,6-bis(hydroxymethyl)-4-oxopyridazine and 3-(hydroxymethyl)-5-(1-hydroxy-2-oxoethylpyrazole)hydrazone[4]. However, kojic acid does not react with phenylhydrazine in methanol in the absence of acetic acid; the starting acid is recovered unchanged. On the other hand, kojic acid and phenylhydrazine react readily in aqueous acetic acid, under conditions similar to those described by Corbett[1], to yield five products. Three of these products, 5, 6, and 8, have been described previously[3], whereas two (compounds 9 and 10), are described here for the first time. The first new compound (9) results from condensation of one mole of phenylhydrazine with one mole of kojic acid, and is probably a precursor of Marxer's[2] compound 3, whereas the second product proved to correspond to the condensation of kojic acid with one mole of aniline.

Compound 9 ($C_{12}H_{12}N_2O_3$) gave a violet coloration with ferric chloride, indicating that it exists in the enolic form. Its n.m.r. spectrum (Table I) showed the presence of one methylene group, which resonated at τ 4.89, in addition to one imino proton (τ 0.83). The i.r. spectrum of 9 showed a hydroxyl and an imino band at ν 3300 and 3100 cm^{-1} respectively, in addition to two absorptions at ν 1600 and

TABLE I

N.M.R. SPECTRAL DATA

Compound		*Chemical shifts (τ)[a] and assignments*			
5		4.97 (H_2-3)	5.32 (H_2-5)[c]	2.15–2.4 and 2.6–2.9 (2Ph+H)	0.33 (N-H)[b]
6		4.92 (H_2-3)	5.06 (H_2-5)	2.3–2.9 and 3.05–3.25 (2Ph+H)	−0.47 (N-H)[b]
8	1.84 (H-5)	4.87 (H_2-3)	5.05 (OH)[b]	2.1–3.1 (3Ph+H)	−1.5 and −3.0 (2N-H)[b]
9[d].		4.89 (H_2-2)		2.5–3.73 (Ph+2H)	0.83 (N-H)
10		5.11 (H_2)[c]		2.00–2.96 (1Ph+2H)	

[a]Chemical shifts after addition of D_2O: [b]Chemical shifts before addition of D_2O: [c]Also includes OH signal before addition of D_2O, [d]Measured in deuterated Me_2SO on a Varian T-60 instrument.

TABLE II

MICROANALYTICAL DATA[a]

Compound	M.p. (degrees)	Found %			Molecular formula	Calculated (%)		
		C	H	N		C	H	N
5	172–74	66 83	5.74	17.50	$C_{18}H_{18}N_4O_2$	67.06	5.63	17.38
6	162–64	67.04	5.63	17.38	$C_{18}H_{18}N_4O_2$	67.04	5.62	17.45
8a	168–70	69.84	5.18	20.90	$C_{24}H_{22}N_6O$	70.22	5.40	20 48
8b	146–48	69.16	5 58	18.46	$C_{26}H_{24}N_6O_2$	69.00	5.40	18.60
9	247–50	61.59	5.22	12.34	$C_{12}H_{12}N_2O_3$	62.06	5.21	12.06
10	262–64	66.52	4.00	6.40	$C_{12}H_9NO_3$	66.97	4.22	6.51

[a] Analytical samples were recrystallized from methanol.

1635 cm^{-1}, due to the C=C of the unsaturated heterocyclic ring and to carbonyl stretching[5]. The mass spectrum showed, in addition to a molecular-ion peak at *m/e* 232, fragments corresponding to the loss of 31 mass units (CH_2OH) at *m/e* 201 and other peaks (Fig. 1) expected from structure **9**.

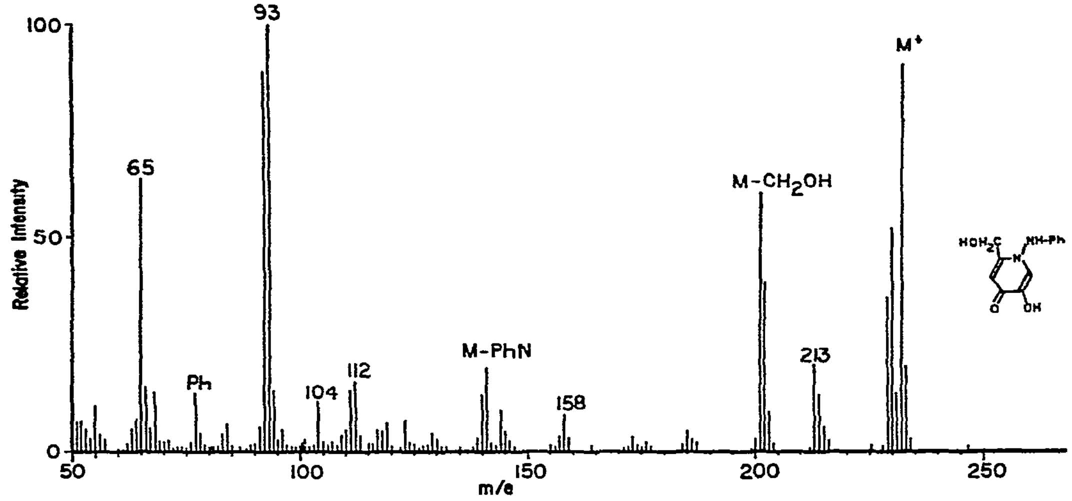

Fig. 1. Mass spectrum of 1-anilino-5-hydroxy-2-(hydroxymethyl)-4-pyridinone (9).

The structure of the second new compound (**10**, $C_{12}H_9NO_3$) has not been completely elucidated. Its n.m.r. spectrum showed the presence of one methylene group (τ 5.11) in addition to the phenyl protons; its i.r. spectrum showed hydroxyl absorption at ν 3270, and its mass spectrum showed a molecular ion peak at *m/e* 215, in addition to peaks at 198 (M – OH), 186 (M – CHO), 170 (M – OH – CO), 159, 141 131, 115, 107, 104, 103, 102, 94, 84, 78, 55, and 50. This compound appears to be a reaction product of aniline and kojic acid.

In addition to compounds **9** and **10**, three of Ishimoto's products (**5**, **6** and **8**) were isolated and the structures proposed for these compounds were confirmed by spectroscopic as well as by the following evidence.

(*a*) Isomers **5** and **6** failed to give formazan reactions and, upon treatment with phenylhydrazine, they yielded compound **8**, which gave a positive formazan reaction. This was taken to indicate that the phenylhydrazone residue in **5** and **6** was not terminal as in Marxer's product as, the later would give a positive formazan reaction[6].

(*b*) Oxidation of **5**, **6**, and **8** with potassium permanganate afforded the same 1-phenylpyrazole-3,5-dicarboxylic acid (**11**), as that obtained[3] by the oxidation of the reduction products of **5**, **6**, and **8** with potassium permanganate. This observation was taken as evidence that they were 3,5-disubstituted 1-phenylpyrazole derivatives.

(*c*) On acetylation with acetic anhydride in pyridine, compound **8a** gave a monoacetyl derivative, 3-acetoxymethyl-5-(1,2-dioxoethyl)-1-phenylpyrazole bis(phenylhydrazone) (**8b**). Its i.r. spectrum showed *O*-acetyl absorption at ν 1740cm^{-1} and its n.m.r. spectrum showed the presence of an acetate methyl group (τ 7.93` and a one methylene group (τ 4.90).

(*d*) The isomers **5** and **6** gave almost identical mass spectra, showing a molecular-ion peak at *m/e* 322, followed by peaks at *m/e* 293 (M$-$CHO), 261 (M$-$CH$_2$OH$-$CHOH), and 216, due to the removal of PhNHN. The base peak appeared at *m/e* 199 in **5** and 200 in **6**.

(*e*) Compound **8** showed a molecular-ion peak at *m/e* 411, followed by a base peak at *m/e* 318, and the fragmentation after loss of the extra hydrazone residue was

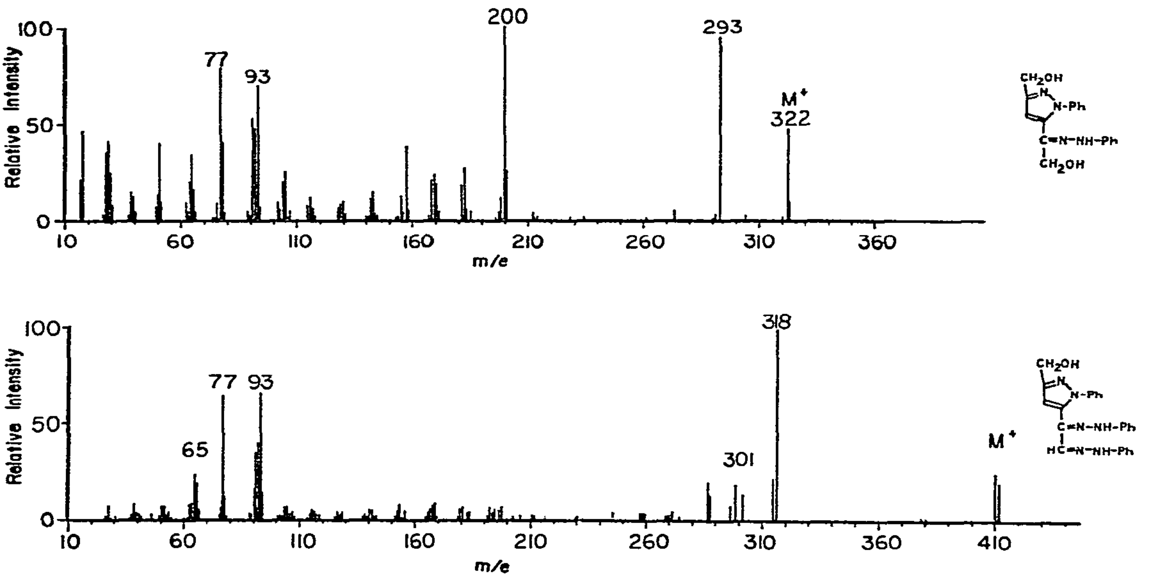

Fig. 2. Mass spectrum of 3-(hydroxymethyl)-5-(hydroxy-1-oxoethyl)phenylpyrazole phenylhydrazone (**6**) (upper) and 3-(hydroxymethyl)-5-(1,2-dioxoethyl)-1-phenylpyrazole bis(phenylhydrazone) (**8**) (lower).

nearly the same as that of **5** and **6** (Fig. 2), which further confirms that the central heterocyclic rings are the same.

EXPERIMENTAL

General methods. — Unless otherwise indicated, solutions were evaporated under diminished pressure at 40–50°. Melting points were determined with a Kofler-block apparatus and are uncorrected. I.r. spectra were recorded with a Hitachi Model EPI-GS spectrometer. N.m.r. spectra were recorded for the solutions in pyridine-d_5 with INM 4H-100 and Varian A-60 spectrometers with tetramethylsilane as the standard. Chemical shifts are given on the τ scale. Mass spectra were obtained with an M-66 instrument. Microanalyses were performed in the Chemistry Department, Tokyo Institute of Technology, Tokyo, Japan.

Reaction of kojic acid (**1**) *with phenylhydrazine.* — A solution of kojic acid (20 g) in water (400 ml) was treated with phenylhydrazine (20 ml) and acetic acid (40 ml), and the reaction mixture was heated for 1 h on a boiling water bath. A red oil separated on cooling and was decanted off. The aqueous layer was extracted with chloroform, and concentration of the extract yielded **10** (0.2 g), m.p. 262–264°. On further concentration of the chloroform, two more products could be separated by fractional crystallization from methanol, namely 3-(hydroxymethyl)-5-(2-hydroxy-1-oxoethyl)-1-phenylpyrazole phenylhydrazone (**5**), yield 0.4 g, m.p. 172–174°, and from the mother liquor, its isomer **6**, yield 0.1 g, 162–164°. The aqueous layer, after extraction with chloroform and refrigeration overnight, yielded 1-anilino-5-hydroxy-2-(hydroxymethyl)-4-pyridinone (**9**), yield 3.2 g, m.p. 247–250°, as well as another crop (50 mg) of **10** and some unchanged kojic acid (0.1 g).

The red oil that had been decanted earlier was dissolved in hot chloroform and the solution kept overnight at 0° to give more of products **6** (3 g) and **10** (20 mg). The organic mother liquor, after filtration, was concentrated to give **10** (0.2 g), **5** (0.2 g), and traces of yellow needles of 3-(hydroxymethyl)-5-(1,2-dioxoethyl)-1-phenylpyrazole bis(phenylhydrazone) (**8a**), m.p. 168–170°.

Formazan test. — When compounds **5**, **6**, and **8a** in a mixture of pyridine (1 ml) and methanol (1 ml) were treated with benzenediazonium chloride[8] at 0–5°, a red formazan color developed immediately in the case of compound **8a** only.

Conversion of **5** *and* **6** *into* **8a**. — A suspension of 3-(hydroxymethyl)-5-(2-hydroxy-1-oxoethyl)-1-phenylpyrazole phenylhydrazone (**5** or **6**, 0.1 g) in water (10 ml) was treated with phenylhydrazine (2 ml) and a few drops of acetic acid. The mixture was refluxed for 3 h with small additions of methanol to give a clear solution. The product was extracted with chloroform from the reaction mixture and the extract dried (sodium sulfate) and evaporated to give 3-(hydroxymethyl)-5-(1,2-dioxo-ethyl)-1-phenylpyrazole bis(phenylhydrazone) (**8a**) in quantitative yield.

Oxidation with potassium permanganate. — Compounds **5**, **6**, and **8a** (0.5 g) in water (50 ml) were refluxed with an excess of potassium permanganate for 30 min and the mixtures were filtered. The filtrates were acidified and extracted with ether.

The residue left after evaporation of the ether was crystallized from methanol–water to yield, in each case, 1-phenylpyrazole-3,5-dicarboxylic acid, m.p. 270° (lit.[7] m.p. 270°). (Calc. for $C_{11}H_8N_2O_4$·N, 12.1; Found: N, 12.3%).

Acetylation of the bis(phenylhydrazone) **8a**. — A solution of the bis(hydrazone) **8a** (0.5 g) in dry pyridine (10 ml) was treated with acetic anhydride (5 ml) and kept overnight at room temperature. The mixture was poured onto crushed ice and the acetate was filtered off, washed with water and crystallized from ethanol to give **8b**, m.p. 146–148°.

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REFERENCES

1 W. M. Corbett, *J. Chem. Soc.*, (1959) 3213.
2 A. Marxer and A. F. Thomas, *Chimia*, 14 (1960) 126.
3 I. Ishimoto, T. Washino, H. Ueda, and C. Tatsumi, *J. Agr. Chem. Soc. Jap*, 45 (1971) 36.
4 A. F. Thomas and A. Marxer, *Helv. Chim. Acta*, 41 (1958) 1898.
5 A. R. Katritzky and A. P. Ambler, in A. R. Katritzky (Ed.), *Physical Methods in Heterocyclic Chemistry*, vol. 2, 1963, p. 260.
6 L. Mester, *J. Amer. Chem. Soc.*, 77 (1955) 4301.
7 H. El Khadem and M. M. Mohammed-Aly, *J. Chem. Soc.*, (1963) 4929; M. Claisen and A. Roosen, *Ann.*, 278 (1894) 272, 286.
8 L. Mester, *Methods Carbohyd. Chem.*, 2 (1963) 119.