[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Reactions of Hydrazines with γ-Pyrones

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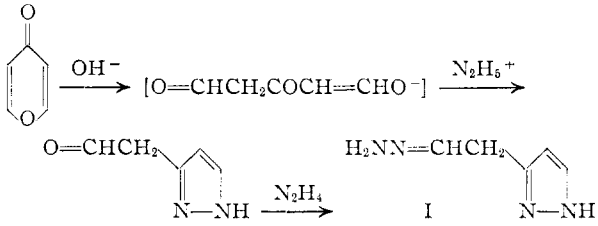
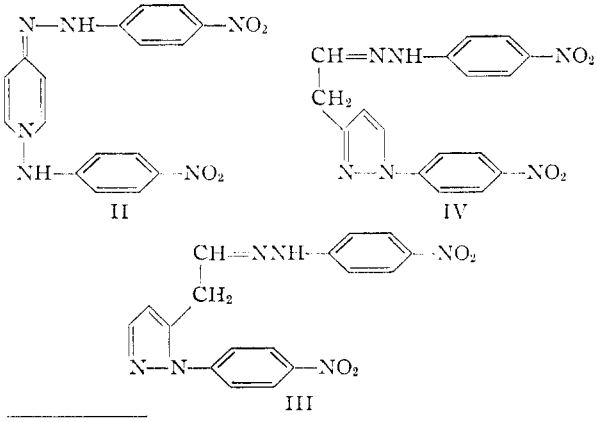
The reactions of a number of hydrazines with γ-pyrones have been investigated and the products obtained have been shown to be pyrazole derivatives. Phenylhydrazine and γ-pyrone gave the 1-phenyl-5-substituted pyrazole and not the isomeric 1,3-disubstituted compound. Hydrazine with 2-hydroxy-6-methyl-4-pyrone gave 3-methyl-5-pyrazoleacetic acid hydrazide rather than the possible pyridone isomer.

The reaction of hydrazine with γ-pyrone to form 3-pyrazoleacetaldehyde hydrazone was reported recently.[1] This probably involves first a ring opening of the pyrone to give the 1,3,5-tricarbonyl derivative, which undergoes condensation with hydrazine in the expected manner to form 3-pyrazoleacetaldehyde. In the presence of excess hydrazine, the hydrazone, I, is obtained.



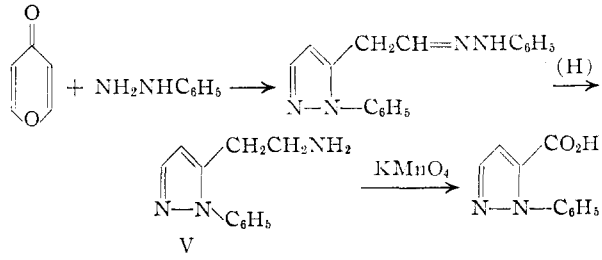
Earlier investigators have described a number of reactions of hydrazines with various γ-pyrone and 1,3,5-tricarbonyl derivatives. The products generally have not been formulated as pyrazoles. Several of these reactions have been examined again in this Laboratory, and together with a number of similar new reactions have all been shown to give pyrazole derivatives.

The condensations of *p*-nitrophenylhydrazine with several γ-pyrones and 1,3,5-tricarbonyl compounds were studied by Deshapande and co-workers.[2,3] The products were said to be γ-pyridone derivatives. Thus, treatment of γ-pyrone with two moles of *p*-nitrophenylhydrazine gave a product which was assigned the structure II.[3] The experiment has been repeated in this Laboratory and the same product obtained ($C_{17}H_{14}N_6O_4$, m.p. 242°); however, the compound cannot have structure II but most likely is the pyrazole III.

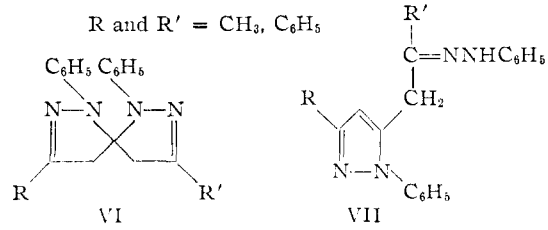


Evidence for structure III was found by subjecting the compound to oxidation with permanganate. A 1-(*p*-nitrophenyl)-pyrazolecarboxylic acid was obtained. This acid sublimed unchanged when heated under reduced pressure, but its silver salt underwent decarboxylation upon heating, to yield 1-*p*-nitrophenylpyrazole,[4] identical in all respects with an authentic sample.

Assignment of structure III instead of the alternative structure IV is based on analogy with the product of the reaction of γ-pyrone with phenylhydrazine. Contrary to earlier reports,[3] phenylhydrazine reacted readily with γ-pyrone when the two were heated together at 120°. The crude, noncrystalline product was hydrogenated to give 5-(β-aminoethyl)-1-phenylpyrazole (V) and aniline. The structure of V was demonstrated by permanganate oxidation which yielded the known 1-phenyl-5-pyrazolecarboxylic acid.[5]



Reactions of phenylhydrazine with diacetylacetone[6] and with benzoylacetylacetone[7] gave products which have been assigned structures like VI. In the light of the present work the structures are probably better represented by VII.



Hydrazine underwent reactions with the symmetrical 2,6-dimethyl- and 2,6-diphenyl-4-pyrones and gave the expected pyrazole derivatives VIII and IX, respectively. These were hydrogenated to

(1) R. G. Jones and M. J. Mann, THIS JOURNAL, **75**, 4048 (1953).

(2) S. S. Deshapande, V. V. Dingankar and D. N. Kopil, *J. Indian Chem. Soc.*, **11**, 595 (1934) [*C. A.*, **29**, 736 (1935)].

(3) D. N. Bedekar, R. P. Kaushal and S. S. Deshapande, *J. Indian Chem. Soc.*, **12**, 465 (1935) [*C. A.*, **30**, 459 (1936)].

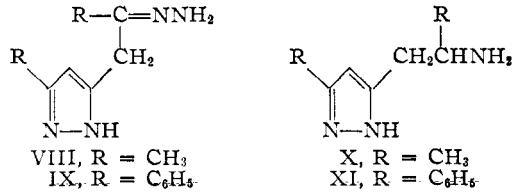
(4) G. S. D'Alcontres, *Gazz. chim. ital.*, **80**, 441 (1950) [*C. A.*, **45**, 3837 (1951)].

(5) L. Claisen, *Ann.*, **278**, 261 (1894).

(6) (a) F. Feist, *ibid.*, **257**, 292 (1890); (b) J. N. Collie and A. A. B. Reilly, *J. Chem. Soc.*, **121**, 1984 (1922).

(7) S. Ruhemann, *ibid.*, **93**, 1281 (1908).

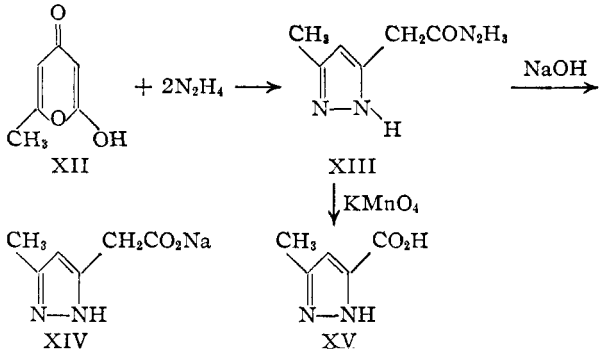
yield the amines X and XI. A condensation of 2,6-dimethyl-4-pyrone with hydrazine had been described previously,[8] but the nature of the product was not clearly defined.



VIII, R = CH_3
IX, R = C_6H_5

X, R = CH_3
XI, R = C_6H_5

The reaction between hydrazine and 2-hydroxy-6-methyl-4-pyrone (XII) was of some interest because of the possibility of several products being formed. Actually only one compound was obtained, and in almost quantitative yield. It was shown to be 3-methyl-5-pyrazoleacetic acid hydrazide (XIII). Hydrolysis by heating in sodium hydroxide solution gave sodium 3-methyl-5-pyrazoleacetate (XIV), and oxidation with permanganate gave the known 3-methyl-5-pyrazolecarboxylic acid (XV).[9]



XII XIII XIV XV

The reaction of XII with phenylhydrazine was reported by Borsche and Blount,[10] who presumed that the product obtained was 5-methyl-1-phenyl-3-pyrazoleacetic acid phenylhydrazide, analogous to compound XIII. The isomeric structure 3-methyl-1-phenyl-5-pyrazoleacetic acid phenylhydrazide would seem more likely in view of compound V obtained from phenylhydrazine and γ-pyrone.

Acknowledgment.—The authors are grateful to W. L. Brown, H. L. Hunter and G. M. Maciak for the microanalyses.

Experimental[11]

1-(*p*-Nitrophenyl)-5-pyrazoleacetaldehyde *p*-Nitrophenylhydrazone (III).—A mixture of 9.6 g. (0.1 mole) of γ-pyrone,[1] 30.6 g. (0.2 mole) of *p*-nitrophenylhydrazine and 200 ml. of glacial acetic acid was heated on the steam-bath for one hour. The solution was cooled, and the solid product was collected on a filter and recrystallized from 250 ml. of pyridine. A yield of 8 g. (22%) of yellow crystalline solid was obtained; m.p. 242–243° dec. (lit.[3] m.p. 242°).

Anal. Calcd. for $C_{17}H_{14}N_6O_4$: N, 22.94. Found: N, 22.97.

A mixture of 3.7 g. (0.01 mole) of 1-(*p*-nitrophenyl)-5-pyrazoleacetaldehyde *p*-nitrophenylhydrazone, 8 g. (0.05 mole) of potassium permanganate and 200 ml. of water was heated on the steam-bath for two hours. The manganese dioxide was removed by filtration, and the filtrate was evaporated to a volume of 50 ml. When this solution was acidified with hydrochloric acid a solid was precipitated. This was recrystallized from ethanol–water and obtained as prisms; m.p. 240° dec., yield 0.5 g. (22%). A sample was sublimed by heating at 220° under 0.5 mm. pressure. 1-*p*-Nitrophenyl-5-pyrazolecarboxylic acid was obtained as yellow prisms, m.p. 245° dec.

Anal. Calcd. for $C_{10}H_7N_3O_4$: C, 51.51; H, 3.03; N, 18.02. Found: C, 51.71; H, 3.02; N, 18.25.

The silver salt of the above carboxylic acid was precipitated by treating the acid in ethanol–water solution with silver nitrate. It decomposed when heated to 200° under 0.5 mm. pressure and a sublimate was obtained that melted at 170–171°. This product was identified as 1-*p*-nitrophenylpyrazole (see below) by its melting point, mixed melting point and infrared spectrum.

1-*p*-Nitrophenylpyrazole.—A mixture of 22 g. (0.1 mole) of 1,1,3,3-tetraethoxypropane,[12] 15.3 g. (0.1 mole) of *p*-nitrophenylhydrazine, 75 ml. of ethanol and 75 ml. of 0.8 *N* hydrochloric acid was heated on the steam-bath for one hour. The solvent was removed by evaporation under reduced pressure, and the residue was washed with dilute sodium hydroxide solution. The dark solid was recrystallized from 500 ml. of 95% ethanol, using decolorizing carbon, to give 12 g. (64% yield) of pale yellow needles; m.p. 168–169° (lit.[4] m.p. 168.5–169°). A sample was sublimed by heating under reduced pressure, m.p. 171–172°.

Anal. Calcd. for $C_9H_7N_3O_2$: C, 57.14; H, 3.73. Found: C, 57.25; H, 3.61.

1-Phenyl-5-β-aminoethylpyrazole (V).—A mixture of 14.4 g. (0.15 mole) of γ-pyrone[1] and 32 g. (0.3 mole) of phenylhydrazine was heated up to 120°. An exothermic reaction took place, and the temperature rapidly went up to 150°. The temperature of the mixture was kept at about 120° for one hour. The crude sirupy reaction product was dissolved in 100 ml. of methanol containing 10 g. of ammonia and hydrogenated with Raney nickel catalyst at 90° and 1000 lb. pressure. After removal of the catalyst by filtration and the solvent by evaporation, the residual liquid was distilled under reduced pressure. A forerun of aniline was obtained and then 16 g. (57% yield) of 1-phenyl-5-β-aminoethylpyrazole came over as a colorless liquid at 133° (0.5 mm.).

Anal. Calcd. for $C_{11}H_{13}N_3$: N, 22.44. Found: N, 22.01.

The hydrochloride was prepared in the usual way and was recrystallized from methanol by adding dry ether. It was obtained as colorless platelets, m.p. 143–145°.

Anal. Calcd. for $C_{11}H_{13}N_3 \cdot HCl$: N, 18.79. Found: N, 18.46.

A mixture of 1 g. of the free amine (V), 1 g. of potassium permanganate and 20 ml. of water was heated on the steam-bath for one hour. The resulting mixture was filtered and the filtrate strongly acidified with hydrochloric acid. The resulting white precipitate was collected and recrystallized from water. It was obtained as needles; m.p. 185–186°. The melting point was not depressed when the compound was mixed with an authentic sample of 1-phenyl-5-pyrazolecarboxylic acid.[5,13]

3-Methyl-5-β-aminopropylpyrazole (X).—The reaction between one mole of 2,6-dimethyl-4-pyrone[14] and two moles of hydrazine in methanol solution took place exothermically. The product, after evaporation of the methanol, was a pale yellow, viscous sirup as described by Kizhner.[8] The methanol solution containing ammonia was hydrogenated and worked up as described for the preparation of 3-β-aminoethylpyrazole from hydrazine and γ-pyrone.[1] The 3-methyl-5-β-aminopropylpyrazole was obtained in 60% yield as a colorless oil, b.p. 128° (0.1 mm.). After long standing it crystallized, m.p. 38°.

Anal. Calcd. for $C_7H_{13}N_3$: C, 60.40; H, 9.41. Found: C, 60.09; H, 9.26.

The dihydrochloride was prepared in the usual way and recrystallized from ethanol solution by dilution with dry ether. It melted at 185°.

(8) N. Kizhner, *J. Russ. Phys. Chem. Soc.*, **55**, 539 (1924) [*C. A.*, **19**, 2822 (1925)].
(9) R. Rothenburg, *Ber.*, **27**, 1097 (1894).
(10) W. Borsche and B. K. Blount, *ibid.*, **65**, 820 (1932).
(11) Melting points were determined on a Fisher-Johns block.
(12) Obtained from Carbide and Carbon Chemicals Corporation.
(13) The authentic sample was prepared by Mrs. M. J. Mann.
(14) F. Arndt, B. Eistert, H. Scholz and E. Aron, *Ber.*, **69**, 2373 (1936).

Anal. Calcd. for $C_7H_{13}N_3 \cdot 2HCl$: Cl, 33.43; N, 19.81. Found: Cl, 33.16; N, 19.50.

The dipicrate was prepared and recrystallized from methanol, m.p. 210°.

Anal. Calcd. for $C_{19}H_{19}N_9O_{14}$: N, 21.10. Found: N, 21.11.

5-(3-Phenylpyrazolyl)-methyl Phenyl Ketone Hydrazone (IX).—To a solution of 5 g. (0.02 mole) of 2,6-diphenyl-4-pyrone[15] in 25 ml. of methanol was added 5 ml. (0.1 mole) of hydrazine hydrate. A mild exothermic reaction took place. The solution was heated under reflux for one hour and then cooled in an ice-bath. The white crystalline solid that separated was collected and recrystallized from methanol. The yield was 2.8 g. (50%), m.p. 175–176°.

Anal. Calcd. for $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.28. Found: C, 73.59; H, 6.13; N, 20.17.

3-Phenyl-5-(β-phenyl-β-amino)-ethylpyrazole (XI).—The above hydrazone was hydrogenated under high pressure in ammoniacal methanol solution using Raney nickel catalyst. After removal of the catalyst and solvent, the residue was dissolved in 50 ml. of ethanol and treated with 4.6 g. of picric acid in 100 ml. of ethanol. The resulting picrate, 5.0 g. (66% yield), was recrystallized from ethanol, m.p. 204°.

Anal. Calcd. for $C_{29}H_{23}N_9O_{14}$: C, 48.27; H, 3.21; N, 17.47. Found: C, 48.51; H, 3.34; N, 17.31.

3-Methyl-5-pyrazoleacetic Acid Hydrazide (XIII).—A solution of 12.6 g. (0.1 mole) of 2-hydroxy-6-methyl-4-pyrone[16] in 50 ml. of methanol was treated with 12 ml. (0.22 mole) of hydrazine hydrate. An exothermic reaction took place. After the initial reaction, the solution was heated for a short time and then evaporated to dryness under reduced pressure leaving a crystalline solid. This was washed with ether and air dried. The yield was quantitative. A sample was recrystallized from dioxane and obtained as white needles, m.p. 145°.

Anal. Calcd. for $C_6H_{10}N_4O$: C, 46.74; H, 6.54; N, 36.34. Found: C, 46.80; H, 6.86; N, 36.01.

A solution of 3 g. of the above hydrazide and 19 g. of potassium permanganate in 150 ml. of water was heated on the steam-bath for two hours. The manganese dioxide was removed by filtration and the filtrate was acidified with hydrochloric acid. It was evaporated to dryness under reduced pressure. The residue was extracted with a little warm ethanol, and the filtered extract was evaporated to dryness. The residual solid was recrystallized from a small volume of water. It was identified as 3-methyl-5-pyrazolecarboxylic acid, m.p. 241–242° (lit.[9] m.p. 236–238° dec.).

Anal. Calcd. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.57; H, 4.91; N, 21.97.

Sodium 3-Methyl-5-pyrazoleacetate (XIV).—A solution of 1.5 g. of 3-methyl-5-pyrazoleacetic acid hydrazide in 20 ml. of 1 *N* sodium hydroxide solution was heated under reflux overnight. The solution was evaporated to dryness, and the residue was extracted with ethanol. When the alcohol solution was diluted with ether, sodium 3-methyl-5-pyrazoleacetate separated as a white crystalline solid, m.p. 196–197°.

Anal. Calcd. for $C_6H_7N_2NaO_2$: N, 17.28. Found: N, 17.37.

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(15) J. Kalff, *Rec. trav. chim.*, **46**, 594 (1927) [*C. A.*, **22**, 240 (1928)].
(16) J. N. Collie, *J. Chem. Soc.*, **59**, 607 (1891).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Microbiological Transformations of Steroids.[1] X. The Oxygenation of Androgens by *Rhizopus*[2]

BY S. H. EPPSTEIN, P. D. MEISTER, H. MARIAN LEIGH, D. H. PETERSON, H. C. MURRAY, L. M. REINEKE AND A. WEINTRAUB

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The three androgens, 4-androstene-3,17-dione (I), testosterone (VIII) and 17α-methyltestosterone (XV), were subjected to enzymatic transformation by various species of *Rhizopus*. In each case the corresponding 11α-hydroxylated derivative was isolated as the major product and the 6β-hydroxylated derivative as a minor product. In the case of testosterone, 17β-hydroxyandrostane-3,6-dione was also isolated from the fermentation.

Discussion

In the continuation of our survey of steroid oxygenation by fungi, the androgens, 4-androstene-3,17-dione (I), testosterone (VIII) and 17α-methyltestosterone (XV), were added to various species of *Rhizopus*, notably *Rhizopus nigricans*, *Rhizopus arrhizus* and *Rhizopus reflexus*, and the transformation products isolated and characterized. No essential difference was observed among the three species of fungus used so that the conversion of each androgen will be illustrated with a different species of *Rhizopus*. The methods used for the bioconversion and extraction of steroids from the fermentation liquor, as well as the procedures for paper and alumina column chromatography used in the isolation of the steroids, have been described in our earlier communications.[3] Paper chromatography of the methylene dichloride extractives of the androgen fermentations indicated that the steroid substrates were practically completely converted into more polar substances. In each case two new compounds of different polarity were the major conversion products. The more polar compound in each instance was the 11α-hydroxylated steroid, present in greatest amount, and the less polar, the 6β-hydroxylated steroid.

From fermentations with 4-androstene-3,17-dione (I), 11α-hydroxy-4-androstene-3,17-dione (II) and 6β-hydroxy-4-androstene-3,17-dione (III) were isolated. The configuration of II was established by oxidation to adrenosterone (IV).[4] The melting point of II (225–227°) differed greatly from that of 11β-hydroxy-4-androstene-3,17-dione (189–191°) reported by Reichstein.[5] The ease of acetylation of the hydroxyl on carbon number 11 of compound II and its contribution to the molecular rotation established its α orientation.[6]

(1) Paper IX of this series, THIS JOURNAL, **75**, 5768 (1953).
(2) Presented in part before the Division of Biological Chemistry at the 123rd Meeting of the American Chemical Society, Los Angeles, California, March 15–19, 1953 (Division of Biological Chemistry, Abstract 5C).
(3) Paper I of this series by D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, THIS JOURNAL, **74**, 5933 (1952).
(4) T. Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937).
(5) T. Reichstein, *ibid.*, **20**, 978 (1937).
(6) Since the completion of this work a fuller characterization of 11β-hydroxy-4-androstene-3,17-dione has been reported by R. W. Jeanloz, *et al.* (*J. Biol. Chem.*, **203**, 453 (1953)). Their compound, m.p. 197–199.5°, $[\alpha]_D$ +207.6° (chloroform), did not acetylate with acetic anhydride in pyridine.