[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLLEGE OF MEDICINE, UNIVERSITY OF UTAH]

The Reaction of Simple Ketols With 2,4-Dinitrophenylhydrazine[1]

HANS REICH[2] AND BARBARA K. SAMUELS

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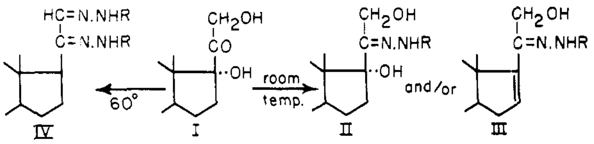
The reaction of acetol, acetol acetate, dihydroxyacetone, *dl*-glyceraldehyde, and benzoyl carbinol with 2,4-dinitrophenylhydrazine has been studied in detail. Depending on the conditions, acetol and its acetate give either the corresponding dinitrophenylhydrazones or pyruvaldehyde dinitrophenylosazone. Likewise dihydroxyacetone yields either the dinitrophenylhydrazone or hydroxypyruvaldehyde dinitrophenylosazone or a mixture of the latter and pyruvaldehyde dinitrophenylosazone. A similar mixture is obtained from *dl*-glyceraldehyde, when the reaction goes beyond the formation of the dinitrophenylhydrazone. Benzoyl carbinol dinitrophenylhydrazone was accompanied by a small amount of phenyl glyoxal dinitrophenylosazone.

It has recently been shown[3,4] that steroid 20,21-ketols which have no hydroxyl group in the 17α-position react normally with dinitrophenylhydrazine in the presence of hydrochloric acid. When the reaction is carried out at room temperature, only traces of osazones are formed, and even at elevated temperature the osazone formation proceeds slowly. Steroid ketols with a 17α-hydroxyl group such as I either give the normal hydrazones (II) or undergo dehydration with the formation of 16,17-unsaturated hydrazones (III). At a reaction temperature of 60°, however, the main product is the osazone IV which can originate from II or III.



$R = -C_6H_3(NO_2)_2$

In connection with these experiments in the steroid series it seemed interesting to investigate the reaction of acetol and dihydroxyacetone with dinitrophenylhydrazine, since these ketols correspond to the two types of steroid ketols mentioned above. For comparison, glyceraldehyde and benzoyl carbinol were included in this investigation.

In contrast to 21-hydroxy- and 21-acetoxypregnenolone, acetol and acetol acetate readily reacted with dinitrophenylhydrazine, when alcoholic solutions of the components were refluxed for several hours. No acid was required, and the dinitrophenylhydrazones were obtained in excellent yields. Acetol acetate furthermore is distinguished from 21-acetoxypregnenolone by its prompt reaction with dinitrophenylhydrazine in chloroform-acetic acid. When attempts were made to prepare acetol acetate dinitrophenylhydrazone in hydrochloric acid solution, it proved necessary to limit the reaction time to less than one hour, since on prolonged standing at room temperature considerable amounts of pyruvaldehyde dinitrophenylosazone were formed, even when only one mole of reagent was used. It is obvious that the first step in this reaction is the hydrolysis of the acetoxy group which proceeds relatively slowly and that the intermediate acetol dinitrophenylhydrazone is rapidly converted to the osazone. Thus the latter precipitates from a solution of acetol dinitrophenylhydrazone in alcoholic hydrochloric acid after short standing at room temperature. It is therefore difficult to prepare pure acetol dinitrophenylhydrazone in hydrochloric acid, although it can be done under controlled conditions, especially when the amount of dinitrophenylhydrazine does not exceed 0.5 mole. These experiments indicate that acetol, acetol acetate, and acetol dinitrophenylhydrazone are much more reactive than is 21-hydroxypregnenolone and its corresponding derivatives.

Dihydroxyacetone was found to be very different from Δ^5-pregnene-3β,17α,21-triol-20-one (I) in its behavior towards dinitrophenylhydrazine, but similar to acetol. Its dinitrophenylhydrazone was best obtained in boiling alcohol, while the reaction in hydrochloric acid had to be limited to a short period of time to avoid osazone formation. When three moles of dinitrophenylhydrazine were applied and the time was extended to three days, the only product was hydroxypyruvaldehyde dinitrophenylosazone which was characterized by its crystalline acetate. Upon refluxing for one hour, however, an osazone resulted which melted at a higher temperature and contained more nitrogen than does hydroxypyruvaldehyde dinitrophenylosazone. In spite of its insolubility in all common solvents, a small amount could be separated by chromatography into two osazones. The first was identical with pyruvaldehyde dinitrophenylosazone, the second with hydroxypyruvaldehyde dinitrophenylosazone. Whether only the latter is formed or both osazones are formed simultaneously, certainly depends on the tempera-

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(2) Present address: Biochemistry Department, College of Agriculture, University of Wisconsin, Madison, Wisconsin.

(3) Reich and Samuels, *J. Org. Chem.*, **19**, 1041 (1954).

(4) Reich and Samuels, *J. Org. Chem.*, **21**, 65 (1956).

(5) Katzschmann, *Ber.*, **77**, 579 (1944).

ture, but also on other factors which are not yet fully understood. Thus, mixtures of both osazones were obtained when the reaction was carried out at room temperature either in aqueous hydrochloric acid with only one mole of dinitrophenylhydrazine (reaction time $^{1}/_{2}$ hour) or in alcoholic hydrochloric acid with 3.8 moles of reagent (reaction time three days). With one mole dinitrophenylhydrazine in chloroform-acetic acid-water dihydroxyacetone also gave a mixture of both osazones. The formation of pyruvaldehyde dinitrophenylosazone corresponds to that of the osazone IV from the steroid ketol I. In this case, however, the osazone with the 17α-hydroxyl group had not been isolated.

It is interesting to note that Katzschmann[5] claims to have prepared pure hydroxypyruvaldehyde dinitrophenylosazone from dihydroxyacetone ditrityl ether in boiling hydrochloric acid. If this statement is correct, one has to assume that osazone formation occurs after only one trityl group has been split off and that the second trityl group is eliminated subsequently.

The dinitrophenylhydrazone of *dl*-glyceraldehyde was easily prepared in boiling alcohol and was characterized by its crystalline diacetate. In contrast, the diacetate of dihydroxyacetone dinitrophenylhydrazone was only obtained as an oil. When *dl*-glyceraldehyde was brought into reaction with three moles of dinitrophenylhydrazine in aqueous hydrochloric acid (three days at room temperature), a mixture of the dinitrophenylosazones of pyruvaldehyde and hydroxypyruvaldehyde was obtained. No suitable conditions have been found thus far for the preparation of pure hydroxypyruvaldehyde dinitrophenylosazone from *dl*-glyceraldehyde.

The dinitrophenylhydrazone of benzoyl carbinol was always accompanied by a small amount of phenyl glyoxal dinitrophenylosazone, when the reaction was carried out in alcoholic hydrochloric acid. In chloroform-acetic acid, benzoyl carbinol as well as its acetate reacted with dinitrophenylhydrazine only to a small extent.

EXPERIMENTAL[6]

Acetol acetate dinitrophenylhydrazone. (*a*) To a suspension of 920 mg. of dinitrophenylhydrazine in 30 cc. of chloroform and 5 cc. of glacial acetic acid, 0.6 cc. of acetol acetate (Eastman, practical; 20% excess) was added. After standing for $3^{1}/_{2}$ hours with occasional shaking a clear solution resulted which was diluted with 120 cc. of hexane. Yellow needles precipitated which melted at 114–115°; a chromatographed sample (eluted with benzene) melted at 115–115.5°; maxima at 255 mμ and 354 mμ (ϵ 10,893 and 20,360), minimum at 293 mμ (ϵ 2,443); infrared band at 5.72 μ.

Anal. Calc'd for $C_{11}H_{12}N_4O_6$: C, 44.59; H, 4.08; N, 18.92. Found: C, 44.73; H, 4.07; N, 19.00.

(*b*) A suspension of 400 mg. of dinitrophenylhydrazine in 10 cc. of ethanol was refluxed for 3 hours after addition of 0.3 cc. of acetol acetate. The mixture was diluted with water, extracted with chloroform, and the chloroform solution was dried and evaporated. One recrystallization from alcohol gave yellow needles, m.p. 115°.

(*c*) To a solution of 92.5 mg. of dinitrophenylhydrazine in 23 cc. of 2 *N* hydrochloric acid 0.05 cc. of acetol acetate was added. The dinitrophenylhydrazone was filtered after 5 minutes, washed with hydrochloric acid and water, dried, and recrystallized from alcohol. Yellow needles, m.p. 115°.

Acetol acetate dinitrophenylhydrazone was previously prepared from chloroacetone dinitrophenylhydrazone and sodium acetate [reported: m.p. 106–107°].[7] A sample synthesized according to this method and purified by chromatography had m.p. 115°; maximum at 353 mμ.

Acetol dinitrophenylhydrazone. (*a*) *From acetol acetate.* A solution of 0.5 cc. of acetol acetate in 5 cc. of 2 *N* hydrochloric acid was allowed to stand overnight. Then a cold solution of 500 mg. of dinitrophenylhydrazine in 50 cc. of 2 *N* hydrochloric acid was added. The crystals which precipitated were filtered after one hour, washed with water, dried, and extracted with hot ethanol. This solution, upon concentration, gave small yellow needles which were recrystallized from chloroform-hexane and ethanol; m.p. 132–134°. A chromatographed sample (eluted with benzene-chloroform 1:1) had m.p. 134.5–136.5° [reported: m.p. 127.5–129.5°];[8] maxima at 255 mμ and 354 mμ (ϵ 10,847 and 22,676), minimum at 292 mμ (ϵ 2,268).

Anal. Calc'd for $C_9H_{10}N_4O_5$: N, 22.04. Found: N, 22.08.

In a second experiment the mixture of 1 cc. of acetol acetate, 10 cc. of ethanol and 1 cc. of conc'd hydrochloric acid was allowed to stand overnight. After addition of excess silver carbonate and filtration, 1.2 cc. of the filtrate was refluxed for three hours with 185 mg. of dinitrophenylhydrazine and 5 cc. of alcohol. The crystals which precipitated on cooling were recrystallized from alcohol; m.p. 135°.

(*b*) *From chloroacetone.* A mixture of 1 cc. of chloroacetone, 1.58 g. of potassium formate (1.5 moles) and 7 cc. of abs. methanol was refluxed for 19 hours. After cooling, 1 cc. of the supernatant was mixed with 0.16 cc. of glacial acetic acid and 125 mg. of dinitrophenylhydrazine. The latter dissolved rapidly and yellow needles precipitated. They were filtered after one hour and were recrystallized from abs. ethanol; m.p. 134.5–136.5°.

Pyruvaldehyde dinitrophenylosazone. (*a*) A mixture of 0.1 cc. of acetol acetate and 0.5 cc. of methanol was added to a solution of 185 mg. of dinitrophenylhydrazine in 22 cc. of methanol and 2.2 cc. of conc'd hydrochloric acid. Crystallization started after 80 minutes. After standing overnight, the crystals were filtered and extracted twice with boiling chloroform; m.p. 308–309° [reported: m.p. 300°;[7] m.p. 313°];[9] maxima at 393 mμ and 436 mμ.

(*b*) A mixture of 400 mg. of dinitrophenylhydrazine, 0.05 cc. of acetol acetate, and 100 cc. of 2 *N* hydrochloric acid was refluxed for one hour. The crystals were filtered, washed with hydrochloric acid and water, dried, and recrystallized from nitrobenzene; m.p. 310°.

(*c*) A sample of acetol dinitrophenylhydrazone was dissolved in 3 cc. of hot abs. ethanol, and 12 drops of conc'd hydrochloric acid were added. The brown crystals which appeared after 45 minutes were washed with ethanol; m.p. 303–305°.

Dihydroxyacetone dinitrophenylhydrazone. A mixture of 90 mg. of dihydroxyacetone and 200 mg. of dinitrophenyl-

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[6] All melting points were taken on a Kofler micro hot stage and are corrected. The ultraviolet spectra were taken in chloroform in a Beckman spectrophotometer Model DU, the infrared spectra in chloroform in a Perkin-Elmer spectrophotometer Model 21. The microanalyses were carried out by Huffman Microanalytical Laboratories, Wheatridge, Colorado. If not mentioned otherwise, the aluminum oxide used was acid-washed and kindly supplied by Dr. H. B. MacPhillamy, Ciba Pharmaceutical Products, Summit, New Jersey.

(7) Bülow and Seidel, *Ann.*, **439**, 48 (1924).

(8) Strain, *J. Am. Chem. Soc.*, **57**, 758 (1935).

(9) Neuberg and Strauss, *Arch. Biochem.*, **7**, 211 (1945)

hydrazine in 5 cc. of abs. ethanol was refluxed for 24 hours. On cooling crystals precipitated which, after washing with cold ethanol, melted at 168–169°. The m.p. did not change after recrystallization from ethanol [reported: m.p. 163–164°;[10] m.p. 167°];[11,12] maximum at 359 mμ (ε 22,174), minimum at 293 mμ (ε 1,907).

Anal. Calc'd for $C_9H_{10}N_4O_6$: N, 20.74. Found: N, 20.84.

Diacetate. The mother liquors of the preceding dinitrophenylhydrazone were dried, dissolved in pyridine-acetic anhydride 2:1, and allowed to stand overnight. After addition of ice, the suspension was extracted with chloroform. The chloroform solutions were washed repeatedly with hydrochloric acid and water, dried, evaporated, and the residue was chromatographed. Benzene eluted a yellow oil which did not crystallize; maxima at 255 mμ and 351 mμ (ε 9,282 and 17,356), minimum at 292 mμ (ε 2,466).

Anal. Calc'd for $C_{13}H_{14}N_4O_8$: N, 15.82. Found: N, 15.60.

Hydroxypyruvaldehyde dinitrophenylosazone. Dihydroxyacetone (30 mg.) was added to a solution of 200 mg. of dinitrophenylhydrazine in 50 cc. of 2 *N* hydrochloric acid. The mixture was allowed to stand at room temperature for 3 days. The precipitate was filtered, washed with hydrochloric acid and water, extracted with chloroform, and recrystallized from pyridine-acetic acid-water; m.p. 278° [reported: m.p. 275–277°].[11,12]

Anal. Calc'd for $C_{15}H_{12}N_8O_9$: N, 25.00. Found: N, 25.01.

Acetate. The preceding dinitrophenylhydrazone was dissolved in hot pyridine-acetic acid, and the solution was filtered and mixed with acetic anhydride. Crystals precipitated on standing overnight. They were filtered, washed with acetic acid and water, and dried; orange needles, m.p. 254°.

Anal. Calc'd for $C_{17}H_{14}N_8O_{10}$: N, 22.86. Found: N, 22.91.

Pyruvaldehyde and hydroxypyruvaldehyde dinitrophenylosazones from dihydroxyacetone. (*a*) A solution of 268 mg. of dinitrophenylhydrazine (equivalent to 4 moles) in 67 cc. of 2 *N* hydrochloric acid was mixed with a solution of 30 mg. of dihydroxyacetone in 2 *N* hydrochloric acid and refluxed for one hour. The precipitate was filtered, washed with hydrochloric acid and water, dried, and extracted with hot methanol; m.p. 292°. After recrystallization from pyridine-acetic acid-water, the red-orange crystals melted at 294°. A sample was extracted three times with hot benzene-chloroform 4:1 (the insoluble material had m.p. 295°). These solutions were chromatographed on neutral aluminum oxide Woelm. The filtrate and the benzene-chloroform 1:1 eluates were combined and concentrated. The precipitate, after washing with benzene, melted at 310° and was identical with pyruvaldehyde dinitrophenylosazone. The fraction eluted with chloroform gave a red precipitate on concentration which was washed with benzene. It melted at 278° and was identical with hydroxypyruvaldehyde dinitrophenylosazone.

(*b*) A solution of 30 mg. of dihydroxyacetone in 3 cc. of ethanol was added to a solution of 250 mg. of dinitrophenylhydrazine (3.8 moles) in 30 cc. of ethanol and 3 cc. of conc'd hydrochloric acid. Crystallization started after 15 minutes. The precipitate was filtered after three days, washed with alcoholic hydrochloric acid and alcohol, and dried. It weighed 144.8 mg. and melted at 295°.

Anal. Calc'd for $C_{15}H_{12}N_8O_8$: N, 25.92. Calc'd for $C_{15}H_{12}N_8O_9$: N, 25.00. Found: N, 25.42.

The mother liquor and washes were combined and treated with 8 g. of potassium acetate in 40 cc. of water and 3 drops of pyruvic acid. After three hours, the mixture was extracted with chloroform and the pyruvic acid dinitrophenylhydrazone was isolated in the usual manner.[13] From its weight it was calculated that 2.3 moles of dinitrophenylhydrazine had been utilized. This would correspond to a formation of 30% hydroxypyruvaldehyde dinitrophenylosazone and 70% pyruvaldehyde dinitrophenylosazone. The neutral chloroform solution which resulted after removal of pyruvic acid dinitrophenylhydrazone was evaporated and the residue was chromatographed on neutral aluminum oxide Woelm. The fractions eluted with benzene weighed 16.3 mg. (27%) and showed a u.v. maximum at 327 mμ. Crystallization from dil. ethanol gave yellow prisms of m.p. 178°, identical with dinitroaniline.

(*c*) A solution of 90 mg. of dihydroxyacetone in 9 cc. of water was added to a solution of 200 mg. of dinitrophenylhydrazine (1 mole) in 50 cc. of 2 *N* hydrochloric acid. After 1/2 hour the precipitate was filtered, washed with hydrochloric acid and water, and extracted with chloroform. This solution, upon concentration, gave a red-orange precipitate which melted at 297°. Dihydroxyacetone dinitrophenylhydrazone could not be isolated.

(*d*) Dihydroxyacetone (30 mg.) and 66 mg. of dinitrophenylhydrazine was shaken overnight in a mixture of 36 cc. of chloroform, 6 cc. of glacial acetic acid and 40 cc. of water. The aqueous layer was extracted repeatedly with chloroform. The chloroform solutions were combined, washed with sodium carbonate solution and water, dried, and concentrated. After addition of 4 drops of pyruvic acid and standing for several hours, the solution was washed again with sodium carbonate solution and water, dried, and filtered through 5 g. of neutral aluminum oxide Woelm. The fractions eluted with chloroform were recrystallized from pyridine-acetic acid-water and gave orange crystals, m.p. 300° (pyruvaldehyde dinitrophenylosazone). Fresh chloroform (contg. 0.75% of alcohol) eluted the red hydroxypyruvaldehyde dinitrophenylosazone which, after recrystallization from pyridine-acetic acid-water, melted at 275°.

(*e*) A sample of dihydroxyacetone dinitrophenylhydrazone was dissolved in 3 cc. of hot abs. ethanol. On addition of 12 drops of conc'd hydrochloric acid a precipitate appeared immediately which was filtered after standing overnight and washed with ethanol; m.p. 297–300°.

dl-Glyceraldehyde dinitrophenylhydrazone. dl-Glyceraldehyde (90 mg.) and 200 mg. of dinitrophenylhydrazine in 5 cc. of ethanol were refluxed for three hours. After cooling and addition of 4 cc. of water, the yellow crystals were filtered, washed with dil. ethanol, and recrystallized from ethanol; m.p. 170° [reported: m.p. 167°].[10,12]

Diacetate. A sample of the preceding dinitrophenylhydrazone was dissolved in 1 cc. of pyridine and 0.5 cc. of acetic anhydride. After standing overnight, ice was added, and the precipitate was filtered, washed with water, and recrystallized from ethanol. The yellow prisms melted at 125°.

Anal. Calc'd for $C_{13}H_{14}N_4O_8$: N, 15.82. Found: N, 15.55.

Mixture of dinitrophenylosazones from dl-glyceraldehyde. dl-Glyceraldehyde (30 mg.) was added to a solution of 200 mg. of dinitrophenylhydrazine in 50 cc. of 2 *N* hydrochloric acid. After standing for three days at room temperature, the precipitate was filtered, washed with hydrochloric acid, and extracted with chloroform. It melted at 294°. The chloroform solution, upon concentration, gave a precipitate, m.p. 302–305°, identical with pyruvaldehyde dinitrophenylosazone. The first precipitate was acetylated as described for hydroxypyruvaldehyde dinitrophenylosazone. The acetylation mixture stayed clear. After addition of chloroform, the solution was washed with hydrochloric acid and sodium carbonate solution, dried, concentrated, mixed with an equal volume of benzene, and chromatographed on neutral aluminum oxide Woelm. The fractions eluted with benzene-chloroform 1:1 were concentrated and yielded an orange precipitate, m.p. 310° (pyruvaldehyde dinitrophenylosazone). The mother liquors gave crystals of lower m.p. which probably contained acetoxypyruvaldehyde dinitrophenylosazone (see above). It could not be isolated in pure form.

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(10) Neuberg and Collatz, *Biochem. Z.*, **223**, 494 (1930).

(11) Collatz and Neuberg, *Biochem. Z.*, **255**, 27 (1932).

(12) This compound is not listed in Chemical Abstracts, neither in the subject nor in the formula index.

(13) Reich, Crane, and Sanfilippo, *J. Org. Chem.*, **18**, 822 (1953).

Benzoyl carbinol dinitrophenylhydrazone. A solution of 10.5 mg. of benzoyl carbinol and 32.7 mg. of dinitrophenylhydrazine in 6 cc. of abs. ethanol and 8 drops of conc'd hydrochloric acid was allowed to stand overnight. The reagent utilized amounted to 1.20 moles. The crude reaction product was chromatographed and gave a small amount of red prisms (eluted with benzene-chloroform 4:1) which after recrystallization from chloroform-ethanol melted at 295–297.5° [reported for phenyl glyoxal dinitrophenylosazone: m.p. 280.5°];[9] maxima at 398 mμ and 446 mμ. Chloroform and chloroform-ethanol 4:1 eluted the benzoyl carbinol dinitrophenylhydrazone which was recrystallized from chloroform-ethanol; red prisms, m.p. 233–236°; maximum at 385 mμ (ε 28,635), minimum at 315 mμ (ε 3,312).

Anal. Calc'd for $C_{14}H_{12}N_4O_5$: N, 17.72. Found: N, 17.97.

The *acetate* was prepared in pyridine-acetic anhydride 2:1 by standing overnight at room temperature and was precipitated by addition of ice. Recrystallization from ethanol gave yellow needles, m.p. 192–194°; maximum at 376 mμ (ε 27,432), minimum at 312 mμ (ε 3,695).

Anal. Calc'd for $C_{16}H_{14}N_4O_6$: C, 53.63; H, 3.94. Found: C, 53.50; H, 3.99.

The acetate was also prepared from benzoyl carbinol acetate in ethanol-hydrochloric acid. After standing overnight the amount of reagent utilized amounted to 0.99 mole. Hydrolysis of the acetoxy group had occurred only to a slight extent.

In chloroform-acetic acid solution benzoyl carbinol and its acetate reacted with only 0.36 and 0.16 mole of dinitrophenylhydrazine respectively.

A sample of benzoyl carbinol acetate dinitrophenylhydrazone was suspended in 3 cc. of abs. ethanol and 12 drops of conc'd hydrochloric acid were added. The red prisms isolated after 11 days melted at 286–290° and were identical with phenyl glyoxal dinitrophenylosazone.

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SALT LAKE CITY, UTAH