[CONTRIBUTION FROM THE FULMER CHEMICAL LABORATORY, STATE COLLEGE OF WASHINGTON]

# Cortical Steroid Analogs. III. Further Synthetic and Structure Studies on Acyclic Dihydroxyacetones Derived from 2,3-Butanedione. 1,3-Dihydroxy-3-phenyl-2-butanone[1]

GARDNER W. STACY, RICHARD A. MIKULEC, C. RICHARD BRESSON, AND LAURENCE D. STARR[2]

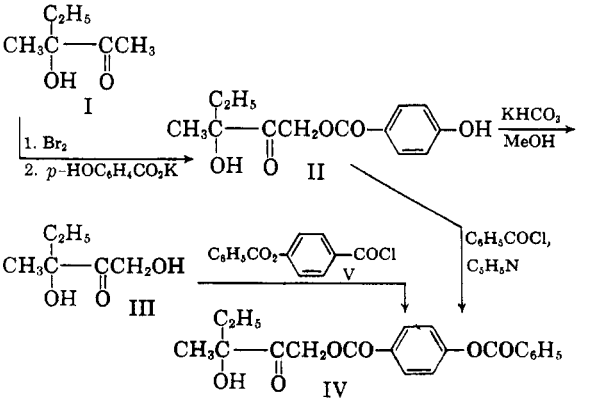
*Received February 9, 1959*

Methods previously employed in the synthesis of 1,3-dihydroxy-3-methyl-2-pentanone (III) failed when applied to 1,3-dihydroxy-3-phenyl-2-butanone (X). Because of this, a study of other ester intermediates which might be employed in this synthesis, was undertaken. 1-(*p*-Hydroxybenzoates) could not be converted readily to dihydroxyacetones; however, hydrolysis of the 1-(*p*-nitrobenzoate) and 1-(*p*-chlorobenzoate) of X led to the synthesis of the desired 1,3-dihydroxy-3-phenyl-2-butanone (X) in good yields (44–66%). Structural relationships between III and its 1-(*p*-hydroxybenzoate) II, as well as the dihydroxyacetone X and its 1-(*p*-nitrobenzoate) IX are described; IX can be cleaved by periodic acid to *O*-*p*-nitrobenzoylglycolic acid (XI) and acetophenone. The alkaline instability of X resulting in the formation of acetophenone has been investigated, and an explanation for this behavior is presented. Compound X showed no significant corticoid, thymolytic, or antiinflammatory activity in assays on adrenalectomized rats.

Although the synthesis of certain types of acyclic dihydroxyacetone derivatives apparently has not been described previously, Diels and Johlin had discussed an attempted synthesis of two such compounds,[3] 1,3-dihydroxy-3-methyl-2-pentanone (III) and 1,3-dihydroxy-3-phenyl-2-butanone (X). Dihydroxyacetones have been of interest in this laboratory as cortisone analogs, and as a part of our program, we recently succeeded in synthesizing compound III starting with 2,3-butanedione.[1] We now report the synthesis of the other dihydroxyacetone, which had been of interest to Diels and Johlin,[3] compound X; experiments confirming the structure of both X and III are also presented.

When the scheme that had been employed for the synthesis of III was applied in the case of X,[1] difficulties were encountered at two stages. If an attempt was made to convert 3-hydroxy-3-phenyl-2-butanone to the corresponding 1-acetate, a reaction mixture resulted, from which a pure product could not be obtained. However, the crude 1-acetate appeared to be at hand, because a corresponding semicarbazone could be isolated from the reaction mixture. Still, it was not possible to produce the desired dihydroxyacetone from the crude 1-acetate, for when this substance was treated in the usual manner with potassium bicarbonate-methanol,[1] an intractable sirup was formed. In an attempt to form a solid derivative from this reaction mixture, it was found that treatment with 2,4-dinitrophenylhydrazine produced a small amount of acetophenone 2,4-dinitrophenylhydrazone. An experiment, to be described subsequently, indicates how this product probably was formed.

Because of these difficulties, it appeared strategic to synthesize crystalline ester intermediates by derivation from aromatic acids. Also, it seemed wise to conduct initial experiments of this nature in the series relating to the dihydroxyacetone III which had been previously prepared.[1] Accordingly, the intermediate bromo ketone, prepared from 3-hydroxy-3-methyl-2-pentanone (I), was heated under reflux in acetone with potassium *p*-hydroxybenzoate, and, indeed, a nicely crystalline 1-(*p*-hydroxybenzoate) intermediate II was formed. The structure of II and the previously prepared dihydroxyacetone III were compared by the following interrelationships. The 1-(*p*-hydroxybenzoate) II readily underwent benzoylation to give the derivative IV, identical with the substance formed by the reaction of the acid chloride V with a sample of III. The acid chloride V had not been previously reported but was readily prepared from the corresponding known acid.[4] Methanolysis of the




(1) Presented in part before the Division of Organic Chemistry at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 20, 1956, and in part before the Northwest Scientific Association, Spokane, Wash., Dec. 27, 1957. Paper II, G. W. Stacy, R. A. Mikulec, S. L. Razniak, and L. D. Starr, *J. Am. Chem. Soc.*, **79**, 3587 (1957).

(2) In part abstracted from theses submitted by Richard A. Mikulec and Laurence D. Starr, respectively, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, State College of Washington, June, 1956, 1955.

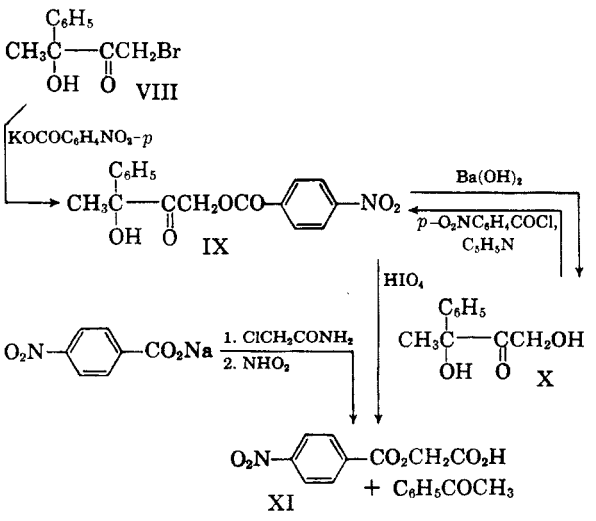
(3) O. Diels and J. M. Johlin, *Ber.*, **44**, 403 (1911).

(4) K. V. Rao and T. R. Seshadri, *J. Chem. Soc.*, 122 (1947).

1-(*p*-hydroxybenzoate) II gave a low yield of III along with a substantial recovery of unreacted II.

When these procedures were extended to the phenyl series, discrepancies were revealed. Attempted formation of a 1-(*p*-hydroxybenzoate) gave what appeared to be 1,3-dihydroxy-3-phenyl-2-butanone 1-(*p*-hydroxybenzoate) (VI); however, repeated endeavor to obtain this substance in a state of analytical purity met with failure. Various samples, obtained by chromatography or recrystallization, gave results about 1% high on the carbon analysis. This analytically impure product could be accounted for by occlusion of benzene, and it did give a pure benzoyl derivative VII in a 74% yield. Attempts to convert the impure 1-(*p*-hydroxybenzoate) VI by methanolysis to the desired dihydroxyacetone failed, giving recovered starting material and a small amount of acetophenone.

A continuation of our search for appropriate ester intermediates led to the 1-(*p*-nitrobenzoate) (IX), the basis of selection being the relative ease of hydrolysis of ethyl *p*-nitrobenzoate.[5] It was found that 1,3-dihydroxy-3-phenyl-2-butanone 1-(*p*-nitrobenzoate) (IX) was obtained in significantly better yields than had been the case with the corresponding 1-(*p*-hydroxybenzoate). In an attempt to obtain X, methanolysis of IX gave a reddish-brown oil; however, it appeared certain that removal of the *p*-nitrobenzoate group had taken place, as methyl *p*-nitrobenzoate was isolated. This suggested that the failure to obtain the dihydroxyacetone X might well be due to its sensitivity to the prolonged alkaline conditions of the reaction. It seemed possible that a procedure involving a shorter exposure, as well as mild alkaline conditions, might be more suitable. Such a procedure had been employed by Smith and Anderson for the hydrolysis of phenyldihydroxyacetone diacetate by barium hydroxide solution at 0°.[6] Extension of precisely the same procedure failed, as had the methanolysis; however, a modification, employing considerably less barium hydroxide, resulted finally in the synthesis of 1,3-dihydroxy-3-phenyl-2-butanone (X) in yields as high as 66%.




The dihydroxyacetone X, like its predecessor III, was characterized by its ability to reduce Benedict's solution and Tollen's reagent, an infrared absorption spectrum consistent with the structure assigned, and the formation of a 2,4-dinitrophenylhydrazone. It was also possible to reconvert X to the 1-(*p*-nitrobenzoate) IX by reaction with *p*-nitrobenzoyl chloride in pyridine. Further evidence for the structure of IX, and hence X, was obtained by the periodic acid oxidation of IX to yield *O*-*p*-nitrobenzoylglycolic acid (XI) and acetophenone. For comparison, it was necessary to prepare an authentic sample of XI (previously unreported) from the corresponding amide, which had been synthesized by Einhorn and Seuffert by the reaction of sodium *p*-nitrobenzoate and chloroacetamide.[7] The amide was converted to the desired acid by treatment with nitrous acid under conditions critical to success of the reaction.

Of further interest was the preparation of a 1-(*p*-chlorobenzoate) intermediate XII in a yield approximating that which had been obtained for IX. The 1-(*p*-chlorobenzoate) XII also was hydrolyzed by the barium hydroxide procedure to give X in good yield.

Now that the dihydroxyacetone X was at hand, it was of interest to explain the formation of acetophenone in the experiments on the methanolysis of the crude 1-acetate and 1-(*p*-hydroxybenzoate), which were discussed earlier. Therefore, the pure dihydroxyacetone X was submitted to similar conditions by stirring in methanolic potassium bicarbonate for 2 days, and X, indeed, proved to be unstable forming a small amount of acetophenone. In parallel with our observations, the the alkaline instability of the dihydroxyacetone side chain in steroids has been noted in at least two instances.[8,9] Wendler and Graber[9] have observed that 3$\alpha$, 17$\alpha$, 21-trihydroxypregnane-11,20-dione is converted by dilute methanolic potassium hydroxide under nitrogen to 3$\alpha$-hydroxyetiocholane-11,17-dione. These authors suggest that the course of the reaction involves a retroaldolization of the equilibrium aldotriose. A similar explanation may be advanced in the present instance for the formation of acetophenone from X.

*Biological evaluation.* 1,3-Dihydroxy-3-phenyl-2-butanone (X) and its 1-(*p*-nitrobenzoate) IX

(5) L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill Book Co., New York, N. Y., 1940, p. 121.

(6) L. I. Smith and R. H. Anderson, *J. Org. Chem.*, **16**, 963 (1951).

(7) A. Einhorn and R. Seuffert, *Ber.*, **43**, 2995 (1910).

(8) H. L. Mason, *Proc. Mayo Clin.*, **13**, 235 (1938).

(9) N. L. Wendler and R. P. Graber, *Chem. and Ind. (London)*, 549 (1956).

were investigated for possible corticoid activity by the glycogen deposition assay in adrenalectomized rats. No significant activity was observed in either case, and even when a massive dose of lg./kg. of X was administered, no response was noted. Further, no response was observed in either case for thymolytic and anti-inflammatory assays in adrenalectomized rats.

## EXPERIMENTAL[10]

*1,3-Dihydroxy-3-phenyl-2-butanone 1-acetate semicarbazone.* The intermediate bromo ketone VIII was obtained by the addition of 14.1 g. (0.086 mole) of bromine in 200 ml. of chloroform over a period of 2 hr. to 14.0 g. (0.085 mole) of 3-hydroxy-3-phenyl-2-butanone[11] in 400 ml. of chloroform. Throughout, the reaction mixture was stirred, and after addition of bromine had been completed, stirring was continued for 1 hr., at the end of which time only a small amount of free bromine remained. The reaction mixture was washed with dilute sodium bisulfite solution (9 × 100 ml.) and once with water (100 ml.). The chloroform solution was dried over 100 g. of anhydrous sodium sulfate, containing 0.1 g. of magnesium oxide.[12] After the solvent had been removed, the residual, crude bromo ketone was employed immediately in the next step.

A mixture of 42.7 g. of potassium bicarbonate, 230 ml. of acetone, and 26.9 g. of glacial acetic acid was heated under reflux for 1 hr. with stirring and then was cooled in an ice bath. The crude bromo ketone VIII from above, in 50 ml. of acetone, was added dropwise with stirring to the acetone–potassium acetate mixture over a period of 1 hr. The resulting mixture was heated under reflux for 16 hr. Dissolved potassium acetate was precipitated by stirring and cooling the reaction mixture in an ice bath for 1 hr. Removal of the acetone by distillation through a 30-cm. Vigreux column gave 7.15 g. (38% yield) of a brown residue. Attempts to crystallize or distill this material either by conventional methods or by a molecular distillation failed.

A semicarbazone was prepared from 1.00 g. (4.5 mmoles) of the above crude product and 1.00 g. (9.0 mmoles) of semicarbazide hydrochloride in ethanol-water. The crude product (820 mg.) was recrystallized from ethanol-water to give 620 mg. (49% yield) of colorless needles, m.p. 185.5–186°. A second recrystallization raised the melting point to 187.4–187.5°.

*Anal.* Calcd. for $C_{13}H_{17}N_3O_4$: C, 55.90; H, 6.14; N, 15.04. Found: C, 55.91; H, 6.31; N, 15.13.

*Attempted conversion of the crude 1-acetate to 1,3-dihydroxy-3-phenyl-2-butanone* (X). A mixture of crude 1-acetate (obtained from 14.0 g. of 3-hydroxy-2-butanone, as described above), 64.0 g. of potassium bicarbonate, and 320 ml. of methanol was stirred under nitrogen for 2 days. After the reaction mixture had been cooled in an ice bath, it was filtered and concentrated. The resulting cake was triturated with cold acetone, and the mixture was filtered. Removal of the acetone under reduced pressure produced 19.1 g. of a dark brown, tar-like product.

Attempted isolation of product as a derivative, when 2.00 g. of the crude reaction mixture was treated with 2,4-dinitrophenylhydrazine, gave, after two recrystallizations from 1,4-dioxane-ethanol, 220 mg. of red-orange needles; m.p. 246°. This substance gave an analysis consistent with the composition of acetophenone 2,4-dinitrophenylhydrazone, lit.[13] m.p. 240–241°; a mixed melting point determination with an authentic sample showed no depression.

*1,3-Dihydroxy-3-methyl-2-pentanone 1-(p-hydroxybenzoate)* (II). To form potassium *p*-hydroxybenzoate, a mixture of 20.0 g. (0.20 mole) of potassium bicarbonate and 29.1 g. (0.21 mole) of *p*-hydroxybenzoic acid in 240 ml. of acetone was heated under reflux for 0.5 hr. The crude bromo ketone intermediate, obtained from 4.65 g. (0.040 mole) of I and 6.47 g. (0.0405 mole) of bromine,[1] was dissolved in 30 ml. of acetone and was added dropwise with stirring to the refluxing acetone-potassium *p*-hydroxybenzoate suspension. The resulting mixture was heated under reflux for 20 hr., cooled, and filtered. The filtrate was evaporated under reduced pressure, and the residue was suspended in 150 ml. of 5% sodium bicarbonate solution. The suspension was extracted with chloroform (4 × 100 ml.), and the extracts were combined, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give 5.86 g. (58% yield) of crude product, m.p. 103–114°. One recrystallization from chloroform gave 3.22 g. (32% yield) of colorless plates, m.p. 114.5–115.5°. An analytical sample was prepared by three additional recrystallizations from chloroform, m.p. 116–117°.

*Anal.* Calcd. for $C_{13}H_{16}O_5$: C, 61.89; H, 6.39. Found: C, 61.67; H, 6.37.

*1,3-Dihydroxy-3-methyl-2-pentanone 1-(p-benzoyloxybenzoate)* (IV) *from* II. A solution of 0.50 g. (2.0 mmoles) of II and 0.28 g. (2.0 mmoles) of benzoyl chloride in 3.0 ml. of pyridine was heated on a steam bath for 1 min. and then poured into 10 ml. of water with vigorous stirring. The resulting precipitate was washed with 5% sodium carbonate solution and water to give 0.63 g. (89% yield) of colorless needles, m.p. 101.5–102.5°. Recrystallization from aqueous acetone or carbon tetrachloride gave sharp-melting products (m.p. 102.5–103°) which, however, did not give analytical data in close agreement with the calculated values. Finally, an analytical sample was obtained by suspending the compound on the alumina column and eluting with benzene-ether (1:1), m.p. 103–103.5°.

*Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.82; H, 5.43.

*p-Benzoyloxybenzoyl chloride* (V). To a suspension of 4.84 g. (0.020 mole) of *p*-benzoyloxybenzoic acid[4] in 17 ml. of carbon tetrachloride was added 4.60 g. (0.022 mole) of phosphorus pentachloride with warming on a steam bath until a clear solution resulted. This solution was evaporated to dryness under reduced pressure, and the residue was recrystallized twice from carbon tetrachloride to give 4.46 g. (86% yield) of large, hard clumps of colorless burrs, m.p. 131–132°.

*Anal.* Calcd. for $C_{14}H_9ClO_3$: C, 64.50; H, 3.48; Cl, 13.60. Found: C, 64.36; H, 3.51; Cl, 13.45.

*1,3-Dihydroxy-3-methyl-2-pentanone 1-(p-benzoyloxybenzoate)* (IV) (*from the dihydroxyacetone* III). A mixture of 264 mg. (2.0 mmoles) of III[1] and 521 mg. (2.0 mmoles) of V in 3.0 ml. of pyridine was warmed on a steam bath for 15 min. and poured into 20 ml. of water. The resulting precipitate was removed by filtration and washed with 5% sodium carbonate solution (3 × 2 ml.) and water, and 501 mg. (70% yield) of a colorless solid, m.p. 96–99°, was obtained. This material was suspended on a column of 40 g. of alumina, which was eluted with 150 ml. of benzene-ether (1:1) to yield 368 mg. (52%) of colorless needles, m.p. 102.5–103.5°. This substance was shown by a mixed melting point determination (m.p. 102–103.5°) and by similarity of

(10) All melting points are corrected, and boiling points are uncorrected. The microanalytical work was performed by Weiler and Straus Laboratories, Oxford, England, and Galbraith Laboratories, Knoxville, Tenn. The infrared absorption spectra were determined by a Perkin-Elmer Double Beam Infrared Spectrometer, Model 21; the solid samples were dispersed in potassium bromide disks.

(11) Prepared in a 48% yield by the method of J. Wegmann and H. Dahn, *Helv. Chim. Acta*, **29**, 101 (1946).

(12) Magnesium oxide is said to stabilize α-bromo ketones; J. R. Catch, D. F. Elliott, D. H. Hey, and E. R. H. Jones, *J. Chem. Soc.*, 272 (1948).

(13) H. E. Zimmerman and J. English, *J. Am. Chem. Soc.*, **76**, 2294 (1954).

infrared spectra to be identical with the 1-(*p*-benzoyloxybenzoate) formed from II, which was described above.

*1,3-Dihydroxy-3-phenyl-2-butanone 1-(p-hydroxybenzoate)* (VI). The crude bromo ketone VIII, obtained from the reaction of 6.57 g. (0.040 mole) of 3-hydroxy-3-phenyl-2-butanone and 6.47 g. (0.0405 mole) of bromine, in 30 ml. of acetone was added to an acetone–potassium *p*-hydroxybenzoate mixture in a manner similar to that used for the preparation of II above. After the mixture had been worked up similarly, there was obtained 4.11 g. (34% yield) of a solid, m.p. 93–97°. Six recrystallizations from benzene gave a product of constant melting point but consistently lower than the original crude material, m.p. A 88.5–92.5°. A sample of the substance was also chromatographed on an alumina column by eluting with benzene-ether (1:1), m.p. B 90–92°.

*Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.87; $C_{17}H_{16}O_5 \cdot {}^1/_6C_6H_6$: C, 68.90; H, 5.47. Found: C, A 68.85, B 68.90; H, A 5.44, B 5.58.

*1,3-Dihydroxy-3-phenyl-2-butanone 1-(p-benzoyloxybenzoate)* (VII). By a procedure similar to that employed in the preparation of IV from II, as described above, 0.60 g. (2.0 mmoles) of VI and 0.28 g. (2.0 mmoles) of benzoyl chloride in 3.0 ml. of pyridine yielded 0.73 g. (90%) of colorless needles, m.p. 112–113°. Recrystallization from carbon tetrachloride raised the melting point to 112.5–113.5° (0.60 g.; yield, 74%).

*Anal.* Calcd. for $C_{24}H_{20}O_6$: C, 71.28; H, 4.98. Found: C, 71.09; H, 5.03.

*Methanolysis experiments on 1-(p-hydroxybenzoates). A. Compound* II. A mixture of 5.05 g. (0.020 mole) of II and 14.5 g. of potassium bicarbonate in 75 ml. of methanol was stirred at room temperature under nitrogen for 2 days and then was allowed to stand for an additional day. The mixture was filtered, and the solid was washed throughly with methanol. The filtrate was concentrated under reduced pressure and distilled through a 15-cm. Vigreux column. There was obtained 270 mg. (10% yield) of the dihydroxyacetone VI; b.p. 51° (0.15 mm.), $n_D^{25}$ 1.4545, lit.[1] b.p. 51–58.5° (0.1–0.25 mm.), $n_D^{25}$ 1.4548.

The residue from the distillation was crystallized from chloroform to yield 2.82 g. (54% recovery) of unreacted II, m.p. 112.5–116°, mixed m.p. 114–116.5°. From the solid which had been filtered off from the reaction mixture, there was obtained, upon dissolving it in water and acidifying the solution, 413 mg. (15% yield) of *p*-hydroxybenzoic acid, m.p. 213.5–214.5°, lit.[14] m.p. 214–215°, mixed m.p. 213.5–214.5°.

*B. Compound* VI. A mixture of 1.17 g. (3.9 mmoles) of VI and 2.84 g. of potassium bicarbonate in 15 ml. of methanol was treated as above. The mixture was filtered, the solid was washed with methanol and the filtrate was concentrated under reduced pressure. The residue was taken up in 25 ml. of benzene and washed with water (2 × 5 ml.). The benzene solution was dried over anhydrous sodium sulfate and evaporated to yield 0.34 g. of yellow crystals; recrystallization of this crude substance from ether-ligroin (b.p. 35–60°) gave 0.23 g. (19% recovery) of VI, m.p. 81–93°, mixed m.p. 88–95°. None of the desired dihydroxyacetone X was isolated.

Since the aqueous washings from above possessed an odor of acetophenone, they were added to 100 ml. of 2*N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. From this mixture there was obtained a gummy solid, which was recrystallized from ethyl acetate–ethanol to yield 8 mg. of impure acetophenone 2,4-dinitrophenylhydrazone; m.p. 230–235°, mixed m.p. 238–240°.

*1,3-Dihydroxy-3-phenyl-2-butanone 1-(p-nitrobenzoate)* (IX). As a result of a number of experiments, the most convenient procedure for preparing the intermediate bromo ketone VIII was found to involve the addition of 1 ml. of a solution of 16.8 g. (0.105 mole) of bromine in 225 ml. of chloroform (reagent) with stirring to 16.4 g. (0.10 mole) of 3-hydroxy-3-phenyl-2-butanone[11] in 475 ml. of chloroform to which a few drops of acetic acid had been added; the mixture was warmed until the color of bromine disappeared. The bromine solution was then added as rapidly as possible (maintenance of a straw-colored solution); a period of 20 min. was required. The solution was stirred for an additional 15 min. while being cooled in an ice bath. After the solution had been washed with cold 5% sodium hydroxide solution (115 ml.) and saturated sodium chloride solution (2 × 65 ml.), it was dried over anhydrous sodium sulfate to which 0.3 g. of magnesium oxide had been added.[12] The chloroform was removed by distillation *in vacuo*, and the residual bromo ketone was taken up immediately in 40 ml. of acetone in preparation for the next step.

A mixture of 50.1 g. (0.50 mole) of potassium bicarbonate and 87.7 g. (0.52 mole) of *p*-nitrobenzoic acid in 1750 ml. of acetone was heated under reflux for 0.5 hr. to form potassium *p*-nitrobenzoate. To this was added dropwise with stirring the acetone solution of bromo ketone (above). The resulting mixture was heated under reflux for 20 hr. with stirring and then allowed to stand for an additional 20 hr. at room temperature without stirring. The mixture was filtered, and the solid was washed with acetone. The filtrate was concentrated under reduced pressure until bumping commenced. To the residue was added 375 ml. of 5% sodium bicarbonate solution with shaking, which caused crystallization of the residual oil. The large lumps were broken up, filtered, and washed with water to give 22.4 g. (68% yield) of a light brown solid, m.p. 105.5–107.5°. Recrystallization from carbon tetrachloride gave 19.0 g. (58% yield) of small, light tan needles, m.p. 108–108.5°.

*Anal.* Calcd. for $C_{17}H_{15}NO_6$: C, 62.00; H, 4.59; N, 4.25. Found: C, 61.85; H, 4.52; N, 3.97.

The infrared absorption spectrum showed bands that were assignable to OH stretching frequency (3425 cm.$^{-1}$, m), *t*-OH (1350 cm.$^{-1}$, s; 1130 cm.$^{-1}$, m), C=O (1725 cm.$^{-1}$, s), and $NO_2$ (1530 cm.$^{-1}$, s; 1350 cm.$^{-1}$, s).

*1,3-Dihydroxy-3-phenyl-2-butanone 1-(p-chlorobenzoate)* (XII). The procedure was identical with that above for IX. From identical quantities of reagents and solvents and 82.2 g. (0.525 mole) of *p*-chlorobenzoic acid was obtained 23.0 g. (72% yield) of a tan product, m.p. 99–105°. Two recrystallizations from carbon tetrachloride gave 16.6 g. (52%) of light tan crystals, m.p. 107–108°. An analytical sample was prepared by further recrystallization from carbon tetrachloride, m.p. 108–108.5°.

*Anal.* Calcd. for $C_{17}H_{15}ClO_4$: C, 64.05; H, 4.74; Cl, 11.12. Found: C, 63.84; H, 4.46; Cl, 11.05.

*Attempted methanolysis of* IX *to* X. The 1-(*p*-nitrobenzoate) IX (6.60 g., 0.020 mole) was treated as in the methanolysis experiment A above. A part (417 mg.) of the methanol-washed solid was water insoluble and was identified as methyl *p*-nitrobenzoate; m.p. 96–97°, lit.[15] m.p. 96–96.5°, mixed m.p. 95.5–96.5°. The methanol solution was evaporated under reduced pressure to yield a residue, which was extracted with ether and then with acetone. When it was dissolved in water and the solution acidified, the solid insoluble in the solvents yielded 58 mg. of *p*-nitrobenzoic acid; m.p. 217–225°, lit.[16] m.p. 238°. Recrystallization from water raised the melting point to 238–239°, mixed m.p. 239–240°. The ether and acetone extracts from above were evaporated *in vacuo*, and the residue was crystallized from aqueous methanol to give 2.60 g. of methyl *p*-nitrobenzoate (total yield 71%), m.p. 90–93.5°; recrystallization from ligroin (b.p. 66–75°) raised the melting point to 94.5–95.5°. The mother liquors were concentrated to yield 2.60 g. of a reddish-brown oil which could not be induced to crystallize from any of the common solvents, nor distilled without decomposition.

(14) R. Willstätter and W. Mieg, *Ann.*, **408**, 61 (1915).

(15) W. E. Caldwell and K. R. Mac Lean, *J. Am. Chem. Soc.*, **55**, 3458 (1933).

(16) E. Widmann, *Ann.*, **193**, 202 (1878).

*1,3-Dihydroxy-3-phenyl-2-butanone* (X). A. *From the 1-(p-nitrobenzoate)* IX. A solution of 3.29 g. (0.010 mole) of IX in 200 ml. of 95% ethanol was cooled to 0°. To this was added an ice-cold solution of 1.75 g. (1.1 equiv.) of barium hydroxide octahydrate in 85 ml. of boiled water. The addition was carried out over a period of 1 hr., and then the weakly basic (*p*H 8) mixture was stirred at 0° for 2 hr. The resulting solution was concentrated under reduced pressure to a small volume and was extracted with ether (1 × 75 ml., 5 × 15 ml.). The ether extracts were combined, washed with 20 ml. of 5% sodium bicarbonate solution, and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave 1.56 g. (87% yield) of crude X, m.p. 60.5–66°. Recrystallization from carbon tetrachloride afforded 1.20 g. (66% yield, yields in several other runs ranged from 44–63%) of colorless needles, m.p. 69.5–70°. Two additional recrystallizations from carbon tetrachloride furnished an analytical sample, m.p. 70–70.5°.

*Anal.* Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.68; H, 6.89.

The above material reduced Benedict's solution and Tollen's reagent and gave an infrared absorption spectrum, which showed bands that were assignable to OH stretching frequency (3410 cm.$^{-1}$, s), *t*-OH (1365 cm.$^{-1}$, w), and C=O (1715 cm.$^{-1}$, s).

A *2,4-dinitrophenylhydrazone* was prepared in a manner similar to that employed for that of III.[1] Small clumps of yellow needles were obtained from benzene-methanol in a 28% yield, m.p. 173.5–174°.

*Anal.* Calcd. for $C_{16}H_{16}N_4O_6$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53.26; H, 4.70; N, 15.74.

*B. From the 1-(p-chlorobenzoate)* XII. Hydrolysis of 3.19 g. (0.01 mole) of XII was carried out by essentially the same procedure and involving the same quantities of reagents as in the case of IX above. Crude X was obtained in a yield of 1.28 g. (71%), m.p. 61–66°. Recrystallization from carbon tetrachloride gave 928 mg. (52%) of relatively pure X, m.p. 68–69°.

*Formation of the 1-(p-nitrobenzoate)* IX *from* X. A mixture of 180 mg. (1.0 mmole) of the dihydroxyacetone X and 186 mg. (1.0 mmole) of recrystallized *p*-nitrobenzoyl chloride in 1.5 ml. of pyridine was warmed on a steam bath for 15 min. and poured into 10 ml. of water. It was necessary to cool the mixture overnight before the resulting oil solidified. The solid was filtered off and washed with 5% sodium carbonate solution (3 × 1 ml.) and with water. There was obtained 172 mg. (52% yield) of IX, m.p. 106.5–107.5°. Recrystallization from chloroform gave 151 mg. (46% yield), m.p. 107.5–108°. A mixture of this substance with IX formed from the bromo ketone VIII resulted in no depression in melting point (108–108.5°), and the infrared spectra of these two substances were identical.

*Authentic O-p-nitrobenzoylglycolic acid* (XI). A solution of 897 mg. (4.0 mmoles) of *O-p*-nitrobenzoylglycolamide[7] in 10 ml. of concentrated sulfuric acid was cooled in an ice bath. An ice cold solution of 1.64 g. of sodium nitrite in 3 ml. of water was added dropwise with stirring over a period of 0.5 hr. The mixture was allowed to warm to room temperature and diluted with 50 ml. of water. The resulting precipitate was removed by filtration, washed with water, and dried to give 748 mg. (83% yield) of a light yellow solid, m.p. 144–145.5°. Recrystallization from benzene afforded 631 mg. (70% yield) of light yellow plates, m.p. 146–147°.

*Anal.* Calcd. for $C_9H_7NO_6$: C, 48.01; H, 3.13; N, 6.22. Found: C, 47.87; H, 3.07; N, 6.25.

*Periodic acid oxidation of the 1-(p-nitrobenzoate)* IX. A solution of 165 mg. (0.5 mmole) of IX in 10 ml. of methanol was treated with 1.85 ml. of 0.54 *M* periodic acid solution, and the mixture was allowed to stand at room temperature for 1 hr. with occasional swirling. The reaction mixture was distilled to dryness under reduced pressure, the distillate being collected in a Dry Ice-cooled receiver. To the distillate was added 50 ml. of 2*N* hydrochloric acid saturated (at 5°) with 2,4-dinitrophenylhydrazine.[17] After dilution with 10 ml. of 2*N* hydrochloric acid, the mixture was allowed to remain at 5° for 2 hr. The precipitate was collected in a coarse fritted-glass filter and washed with 25 ml. of 2*N* hydrochloric acid and with distilled water until the washings gave a negative test for chloride ion with silver nitrate solution. After the precipitate had been dried to constant weight, there was obtained 137 mg. (91% yield) of acetophenone 2,4-dinitrophenylhydrazone, m.p. 239–240.5°, mixed m.p. 241–242°. The residue from the distillation was suspended in a small volume of water, filtered, washed with water, and dried to give 101 mg. (89% yield) of *O-p*-nitrobenzoylglycolic acid (XI), m.p. 146–147°, mixed m.p. 146–147°.

*Alkaline decomposition of the dihydroxyacetone* X. A mixture of 329 mg. (1.0 mmole) of X and 0.72 g. of potassium bicarbonate in 5 ml. of methanol was stirred under nitrogen for 2 days; the reaction mixture was then allowed to stand for an additional day. The mixture was filtered, and the filter cake was washed well with methanol. The filtrate and washings were evaporated (without heating) under reduced pressure, the distillate being collected in a receiver immersed in a Dry Ice bath. To the distillate was added 100 ml. of 2*N* hydrochloric acid saturated (at 5°) with 2,4-dinitrophenylhydrazine; the mixture was maintained at 5° for 2 hr. By a procedure similar to that described above, 34 mg. (11% yield) of acetophenone 2,4-dinitrophenylhydrazone was isolated, m.p. 237–239°, mixed m.p. 241–242°.

*Acknowledgment.* This investigation was supported in part by a research grant (A-253, C2-C4) and in part by a predoctoral fellowship to R.A.M. (AF-5336) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service. For biological evaluations, we wish to extend our thanks to Mr. S. C. Lyster and Mr. L. E. Barnes, Department of Endocrinology, The Upjohn Co., Kalamazoo, Mich., and to a group under the direction of Dr. Erwin P. Vollmer, Consultant in Endocrinology, National Service Center, Public Health Service, Bethesda, Md. For determination of infrared absorption spectra, we wish to thank Mr. George D. Wagner and Mr. David L. Frasco of the Division of Industrial Research and Mr. Roland W. Lovejoy of the Department of Chemistry, State College of Washington.

PULLMAN, WASH.

(17) Determination of ketones as 2,4-dinitrophenylhydrazones is based on the procedure of H. A. Iddles, A. W. Low, B. D. Rosen, and R. T. Hart, *Ind. Eng. Chem., Anal. Ed.*, 11, 102 (1939).