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REGENERATION OF STEROID KETONES FROM THEIR SEMICARBAZONES WITH PYRUVIC ACID[1]

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An important step in the separation of ketonic hormone intermediates obtained from the oxidation of sterols is the formation of their sparingly soluble semicarbazones. After suitable purification, the semicarbazones may be "split" by any one of several methods and the steroid ketones are thus recovered. Methods for accomplishing the split include treatment with strong acid, exchange with an excess of some other ketone or aldehyde in non-aqueous solution, and the recently described[2] nitrous acid in acetic acid procedure (1, 2, 3). The first two methods, which are in common use, are drastic and lead to significant amounts of irreversible condensation products or to partial hydrolysis of the acetylated hydroxyl groups which are often present.[3]

Conant and Bartlett (12) made a quantitative study of semicarbazone formation in which they confirmed earlier observations on reaction rates by Grassi (13, 14), and a statement made by Sidgwick (15) that pyruvic acid in aqueous medium is a particularly effective exchange ketone. They found that the hydrolysis constant of pyruvic acid semicarbazone has a low limiting value, resembling that of an aromatic aldehyde semicarbazone. A buffer action is also provided by the presence of carboxyl and amino groups in the same molecule which resists hydrolysis caused by changes in acidity.

In the new method described below, advantage is taken of the favorable properties of pyruvic acid and of its semicarbazone to effect an exchange in acetic acid solution with the semicarbazone moiety of the steroid ketones. Acetic acid is a particularly good solvent for this purpose. It is a good ionizing medium (16) for this reaction, and the steroid semicarbazones are very soluble in the anhydrous solvent and to a considerable extent even in dilute aqueous mixtures. This per-

[1] In a communication to the Editor, Mattox, and Kendall, *J. Am. Chem. Soc.*, **70**, 882 (1948), describe the use of pyruvic acid together with hydrobromic acid in chloroform solution to split methyl 3,11-diketo-12-bromo-Δ^4-cholenate-3-(2,4-dinitrophenylhydrazone). Dr. Kendall first reported this work at the 112th Meeting of the American Chemical Society in New York, September, 1947.

[2] Upon repetition in this laboratory, the yield of crude dehydroisoandrosterone acetate recovered by this method was found to be practically quantitative as reported. However, its quality was poor and the melting point was from 5–10° low, even when a highly purified sample of semicarbazone was employed. In general, the results were not equal to those obtained by the pyruvic acid method.

[3] Examples of the more common hydrolytic methods for splitting semicarbazones are illustrated in the following references; sulfuric acid in alcohol, (4, 5, 6, 7); sulfuric acid in dioxane, (8, 9); hydrochloric and acetic acids, (10). Oxalic acid, which was used by Ruzicka, Goldberg, Meyer, Brüngger, and Eichenberger (11) for the hydrolysis of the semicarbazones from the oxidation of epidihydrocholesterol acetate gave as a product the neutral oxalate ester of two molecules of androsterone.

mits an irreversible shift in the equilibrium, for it is often possible to crystallize the less soluble ketone from the reaction mixture of keto-steroid semicarbazone, pyruvic acid, and acetic acid, by adding water gradually to the refluxing solution. The ketone is thus removed from solution as it is formed, and the yields obtained are nearly quantitative. When the solubility relationships are not favorable, it is sufficient to reflux the solution of the reactants for a short time and then to add water until the saturation point of the ketone is reached. Upon slow cooling, the ketone usually crystallizes from the solution.

The reaction rate is several times greater when using pyruvic acid than with its sodium salt or in the presence of sodium acetate. This advantage is offset by the fact that pyruvic acid in acetic acid solution is a strong acid, approaching oxalic acid in strength, and causes from five to ten per cent hydrolysis of any acetyl protected hydroxyl groups in the molecule. To avoid this hydrolysis, it was found preferable to conduct the reaction in a solution buffered with sodium acetate. In carefully conducted experiments with dehydroisoandrosterone acetate semicarbazone and sodium pyruvate, 97% of the dehydroisoandrosterone acetate could be recovered, whereas the use of pyruvic acid alone resulted in a mixture from which about 92% of ketone acetate was obtained, and dehydroisoandrosterone was found in the mother liquors. When the ketone did not contain acid-sensitive groups it was advantageous to make use of the more rapid hydrolysis obtained with pyruvic acid. However, pyruvic acid semicarbazone is only moderately soluble in dilute acetic acid solution and, for example, in the exchange with cholestenone semicarbazone it sometimes crystallized together with the cholestenone. Under these conditions it was found that the addition of sodium acetate at the end of the reaction formed the more soluble sodium pyruvate semicarbazone and avoided contamination of the steroid ketone.

Oximes as a class are less useful in the separation of steroid ketones than the semicarbazones, for they are sometimes more soluble and often differ but slightly in properties from their parent ketones. Furthermore they are less readily hydrolyzed than semicarbazones and require longer treatment with pyruvic acid or its salt. A few examples have been included, however, for a general procedure is lacking.

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EXPERIMENTAL PART[4, 5]

The experimental procedure developed for dehydroisoandrosterone acetate semicarbazone is typical and is reported in detail. The other ketones and diketones were selected to represent different types that might establish the generality of the method as completely as possible. Of necessity, the procedure must be adapted to the peculiarities of the ketone being recovered, its solubility, its ease of crystallization, and its initial purity.

A 50% molar excess of pyruvic acid over the semicarbazone was employed. At first, anhydrous pyruvic acid was used but it was later found that the small amount of water introduced by a 50% solution had a negligible effect. Technical pyruvic acid was carefully

[4] All melting points are corrected.

[5] Microanalyses were performed by Mr. Edwin Conner of this laboratory.

distilled in a vacuum without fractionation at a pressure of 5–10 mm. The aqueous product, adjusted by titration to a 50% by weight concentration, was stable, and did not discolor upon standing.

Dehydroisoandrosterone acetate. (a) *Semicarbazone exchange with sodium pyruvate.* To a solution of 10.00 g. of dehydroisoandrosterone acetate semicarbazone (m.p. 279.5–280.5° dec., inserted at 255°; prepared from dehydroisoandrosterone acetate m.p. 170.2–170.7°) in 30 cc. of glacial acetic acid warmed to 110° was added a solution consisting of 3.2 g. of anhydrous sodium acetate and 7.0 g. of a 50% by weight solution of aqueous pyruvic acid in 15 cc. of hot acetic acid. The second flask was rinsed with an additional 5-cc. portion of acetic acid which was added to the reaction flask. A small amount of semicarbazone separated at this point but redissolved after shaking for a few minutes. After 10 minutes at 105–110°, water was added dropwise until 15 cc. had been added and the solution was sufficiently dilute so that refluxing occurred below 110°. The dropwise addition of water was continued at a rate such that after about 25 minutes a total of 46 cc. of water had been added and the solution was saturated, so that upon seeding and slight cooling the ketone acetate began to crystallize. The boiling point of the solution was now 105° and the addition of water was continued at an increased rate while under reflux so that after an elapsed time of about 35 minutes from the beginning of the addition a total of 100 cc. of water had been added, and the solution was thick with crystalline product. Then the flask was set aside to cool slowly so that thick needles formed which permitted easy filtering and washing. After standing from one to two hours at room temperature, the flask was placed in the refrigerator to cool to 0° and the product was collected with suction, washed with ice-cold dilute acetic acid (25% by volume), and with water. Upon drying at 110° under vacuum for from 1–2 hours there was obtained 8.28 g. of dehydroisoandrosterone acetate (97.2%), m.p. 170.2–170.9°.

(b) *Semicarbazone exchange with pyruvic acid.* The following experiment illustrates the behavior of a typical acetate under the mildly hydrolytic conditions which exist when using pyruvic acid.

A suspension of finely divided solid was prepared by diluting a solution of 10.00 g. of dehydroisoandrosterone acetate semicarbazone in 50 cc. of hot acetic acid with 25 cc. of hot water. Thereupon 7.0 g. of 50% pyruvic acid was added and the suspension was heated to reflux. After 5 to 7 minutes the semicarbazone dissolved and the solution was refluxed for 10 minutes longer. Then 75 cc. of water was added over a 10-min. period and the flask was put in the refrigerator to cool slowly. After 2 hours the contents were at about 5° and the crystalline product was collected, washed twice with ice-cold dilute (25%) acetic acid and finally with water. After drying as before, there was obtained 7.90 g. (92.5%), m.p. 170.0–170.7°.

Dilution of the acetic acid mother liquors with water to a volume of about 900 cc. gave 0.42 g. of solid, m.p. 124–129°. This material which in other experiments was purified to give dehydroisoandrosterone m.p. 147.5–149.5°, (the m.p. of a mixture with authentic material was the same) was acetylated by boiling for 5 minutes with a few cc. of acetic anhydride and then crystallized directly by adding water dropwise to the boiling solution until saturation had been reached. There was obtained upon cooling 0.38 g. of acetate (4.5%), m.p. 168–170°.

Isoandrosterone. A suspension of 1.00 g. of the semicarbazone (m.p. 286–288° dec., prepared from isoandrosterone m.p. 170.5–172.5°) in 15 cc. of acetic acid, 15 cc. of water, 0.78 g. of 50% pyruvic acid, and 0.37 g. of sodium acetate was refluxed for 1 hr., when solution was complete. Then 20 cc. of water was added and upon slow cooling 0.65 g. of isoandrosterone m.p. 170–172.5° was obtained. Dilution of the mother liquor with several volumes of water gave an additional 0.11 g. with m.p. 169–172° (total 91%). Though the reaction was slow, better results were obtained in dilute acetic acid solution, possibly due to the easy acetylation of isoandrosterone in strong acetic acid with resulting contamination of the product by the lower-melting acetate.

Dihydrotestosterone. [Androstan-17(α)-ol-3-one. According to evidence recently

presented by Goldberg *et al.* (17) the actual configuration is (β)]. A solution of 0.75 g. of the semicarbazone [m.p. 261–262° dec., inserted at 255°, prepared from ketone m.p. 180.5–181.5°; Butenandt, Tscherning, and Hanish (18) report this semicarbazone with the m.p. 237–243° uncorr.] was dissolved in 5 cc. of acetic acid, 0.59 g. of 50% pyruvic acid and 0.28 g. of sodium acetate, and refluxed for one hour. After diluting with water to saturation and slow cooling and seeding, there was obtained 0.59 g. of product m.p. 175–179° (94%). Recrystallization from acetone-ligroin gave 0.52 g. (83%) of dihydrotestosterone, m.p. 180–181.5°.

Δ^5-*Pregnen-3-ol-20-one acetate semicarbazone* (2.00 g., m.p. 265–266° dec., inserted at 257°; from pregnenolone acetate m.p. 142–148°) was warmed to boiling with 1.4 g. of 50% pyruvic acid, 0.64 g. of sodium acetate and 10 cc. of acetic acid. Over a one-half hour period 20 cc. of water was added dropwise, which resulted in saturation of the solution. Upon cooling and seeding there was obtained 1.70 g. (100%) of crystalline material, m.p. 145.5–148.5°. This was recrystallized from methanol to give 1.49 g. (88%) of glistening white leaflets, m.p. 147–149°.

Δ^5-*Norcholesten-3-ol-25-one acetate semicarbazone* (2.00 g., m.p. 234–235° dec.) was refluxed for 10 minutes with a solution of 12 cc. of acetic acid, 3 cc. of water, 1.14 g. of pyruvic acid and 0.52 g. of sodium acetate. The solution was cooled to about 90°, whereupon glistening leaflets began to separate. More water was added dropwise and the solution was brought to a boil and refluxed. After 30 minutes a total of 17 cc. of water had been added, and upon cooling the suspension to 0°, collecting and drying, there was obtained 1.76 g. of ketone m.p. 138.5–141° (99%). Recrystallization from dilute acetic acid gave 1.70 g. (96%) of glistening white leaflets, m.p. 139.5–141°.

Δ^4-*Cholesten-3-one semicarbazone* (5.00 g., m.p. 237–239° dec., inserted at 230°; prepared from cholestenone m.p. 79–80°) was suspended in a solution of 25 cc. of acetic acid, 3.1 g. of 50% pyruvic acid and 5 cc. of water and heated to reflux. After 10 minutes 25 cc. of water was added dropwise and the flask was then cooled in an ice-bath. The milky dilute acid layer was decanted from the viscous oil, which was treated with 50 cc. more of water and brought to a boil. After cooling, the oil slowly crystallized and the solid was collected, washed with water, and dried; 4.25 g. (97%), m.p. 80–86°. Recrystallization of 4.18 g. from acetone-methanol gave 3.81 g. (89%) of cream-colored prisms, m.p. 79–80°.

The dilute acetic acid mother liquors deposited pyruvic acid semicarbazone upon standing overnight, m.p. 121–122° dec.

Δ^4-*Androstene-3,17-dione disemicarbazone and its exchange.* A 2.50-g. portion of androstenedione, m.p. 171.5–173°, dissolved in 15 cc. of boiling acetic acid was treated with a solution of 5.9 g. of semicarbazide hydrochloride and 5.1 g. of sodium acetate in 14 cc. of water. The disemicarbazone soon formed in a microcrystalline condition and the reaction was completed by warming the mixture for one hour on the steam-bath. In three successive experiments there was obtained 3.65 g. (91%), 3.81 g. (95%), and 3.88 g. (96.5%) of material with no definite melting point.

The product crystallizes with one molecule of acetic acid of crystallization as indicated by the following analysis:

Anal. Calc'd for $C_{21}H_{32}N_6O_2 \cdot CH_3COOH$: N, 18.25. Found: N, 17.99.

Androstenedione dioxime. A solution of 7.3 g. of sodium acetate and 4.9 g. of hydroxylamine hydrochloride dissolved in 30 cc. of water was added to a hot solution of 2.50 g. of androstenedione in 75 cc. of ethyl alcohol. The resulting clear solution was refluxed for 2.5 hours and then the concentration was adjusted by the addition of 75 cc. of water so that a crystalline precipitate was obtained upon cooling. After collecting, washing with 50% methanol, and drying there was obtained 2.64 g. (95%) of dioxime, m.p. 195–210°. The product is a mixture of isomers from which one could be separated after three successive recrystallizations from 50% ethyl alcohol and three more from methanol. It formed colorless rectangular prisms, m.p. 229–230° which became opaque upon drying at 100° for 3 hrs. *in vacuo*. Butenandt and Kudzus (19) report this compound with the melting point 143° (uncorr.).

Anal. Calc'd for $C_{19}H_{28}N_2O_2$: N, 8.86. Found: N, 9.00.

The exchange of 1.00 g. of dioxime, m.p. 195–210° in a refluxing solution of 5 cc. of acetic acid, and 10 cc. of water with 1.7 g. of pyruvic acid was complete in 50 min. Upon cooling, two crops of needle-shaped crystals were obtained: 0.60 g., m.p. 168–171°, and 0.11 g., m.p. 165–167° (total, 78%). Recrystallization of the combined material gave 0.61 g., m.p. 170–172°.

Progesterone dioxime. The dioxime was made in the usual way (yield, 94%) and formed a micro-crystalline powder which upon recrystallization from alcohol melted at 247–256° (20). Though it had the proper nitrogen content, it evidently was a mixture of isomers.

Anal. Calc'd for $C_{21}H_{32}N_2O_2$: N, 8.13. Found: N, 8.20.

The exchange of 0.30 g. of dioxime with 0.48 g. of pyruvic acid was accomplished by refluxing the components in 10 cc. of 50% acetic acid for 3 hours. After adding 0.22 g. of sodium acetate and diluting with water to saturation at about 60°, there was obtained upon cooling 0.21 g. (77%) of material, m.p. 126–128.5°.

Dehydroisoandrosterone acetate oxime. The oxime prepared in the usual way in dilute alcohol (2:1) in 92% yield, was microcrystalline, m.p. 162–163° (from dilute methanol or from benzene-ligroin).

Anal. Calc'd for $C_{21}H_{31}NO_3$: N, 4.05. Found: N, 4.20.

Dehydroisoandrosterone acetate was recovered in 75% yield upon refluxing 1.00 g. of the oxime for 4 hours with a solution of 0.78 g. of pyruvic acid and 0.38 g. of sodium acetate in a mixture of 5 cc. of acetic acid in 2 cc. of water. The solution became brown due to side reactions of the sodium pyruvate.

SUMMARY

A general procedure using pyruvic acid has been devised for the splitting of both keto-steroid semicarbazones and oximes, and recovery of the ketones. Its application to a number of mono and diketones is illustrated.

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