JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

(Registered in U. S. Patent Office) (Copyright, 1952, by the American Chemical Society)

VOLUME 74 DECEMBER 12, 1952 NUMBER 23

[CONTRIBUTION FROM THE MAYO FOUNDATION, UNIVERSITY OF MINNESOTA AND MAYO CLINIC]

Steroids Derived from Bile Acids. XVIII. Introduction of the 4,5-Double Bond of Cortisone[1,2]

BY WARREN F. MCGUCKIN AND EDWARD C. KENDALL

RECEIVED May 26, 1952

Formation of the 3-semicarbazone of 4-bromo-4,5β-dihydrocortisone acetate afforded cortisone acetate 3-semicarbazone in a yield of about 93% when carried out in a solvent (a) relatively non-polar (tetrahydrofuran, or dioxane) or, (b) inert because of steric hindrance (*t*-butyl alcohol). In aqueous acetic acid at room temperature cortisone acetate semicarbazone was hydrolyzed to cortisone acetate in a yield of 85% in the absence of an acceptor for the semicarbazide. The addition of 2 equivalents of pyruvic acid increased the yield to 99%. The probable mechanism of the dehydrobromination is discussed.

Through the use of 2,4-dinitrophenylhydrazine, cortisone acetate can be prepared from 4-bromo-4,5β-dihydrocortisone acetate in a yield of 65 to 70%.[3] Introduction of the double bond involves first, formation of the Δ^4-hydrazone and, second, restoration of the ketone group. Each of these steps affords a yield of 80 to 85%. In the preparation of cortisone acetate from desoxycholic acid an increase in the yields of these two steps would augment the yield of cortisone acetate more than could accrue from an equal improvement at any other step, since introduction of the double bond is the last operation.

For this reason introduction of the double bond has been reinvestigated. It has been found that under certain conditions semicarbazide can be used advantageously in place of 2,4-dinitrophenylhydrazine. Semicarbazide has been used by several investigators[4-8] as an agent for dehydrobromination, but the conditions for the optimal yield of unsaturated ketone have not been established.

(1) This work was supported in part by a grant from E. R. Squibb & Sons, New York.

(2) Abridgment of portion of thesis submitted by Mr. McGuckin to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).

(4) Carl Djerassi, THIS JOURNAL, **71**, 1003 (1949).

(5) B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, *J. Biol. Chem.*, **184**, 393 (1950).

(6) V. R. Mattox and E. C. Kendall, THIS JOURNAL, **72**, 2290 (1950).

(7) N. L. Wendler, Huang-Minlon and M. Tishler, *ibid.*, **73**, 3818 (1951).

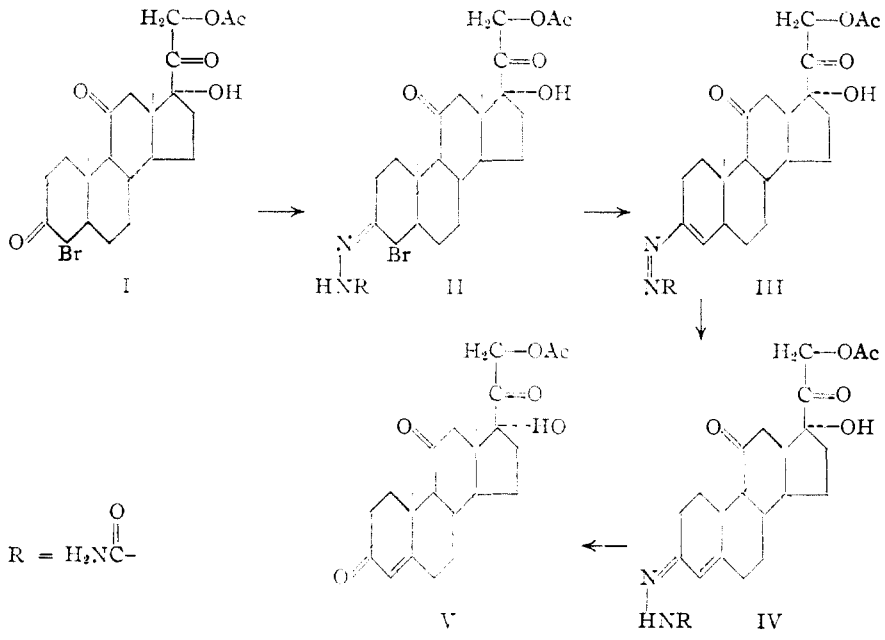
(8) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

With semicarbazide and 4-bromo-4,5β-dihydrocortisone acetate (I) in acetic acid there is a tendency for formation of the 4-acetoxy derivative of the steroid, and it became obvious that semicarbazide could be used to the best advantage only in a medium which prevented substitution at C-4. Accordingly, solvents which could not furnish an easily substitutable anion were explored. Dioxane, tetrahydrofuran, ethylene glycol dimethyl ether and *t*-butyl alcohol were used at room temperature. All these solvents were satisfactory: Cortisone acetate 3-semicarbazone (IV) could be obtained in a yield of about 90%.

In contrast to these results it was found that with isopropyl alcohol and ethylene glycol monomethyl ether the yields of the semicarbazone of cortisone acetate (IV) were 70 to 75%. The products formed in both of these solvents gave absorption spectra in the ultraviolet region which indicated that some substitution at C-4 had occurred (Fig. 1).

With neutral solvents such as dioxane and *t*-butyl alcohol there was a delay of 10 to 15 minutes before a homogeneous solution was obtained. The speed of the reaction was increased by addition of a suitable catalyst, such as salicylic acid, but the final yield was not influenced.

Preparation of the semicarbazone of cortisone acetate from 4-bromo-4,5β-dihydrocortisone acetate (I) can be carried out in *t*-butyl alcohol with a yield which is more than 93% of the theoretical amount. This is appreciably greater than the yield of the 2,4-dinitrophenylhydrazone of cortisone acetate from the same starting material, and in addition a most important advantage is the ease



The probable mechanism of the reaction of 3-keto-4-bromosteroids with phenylhydrazine and with *p*-nitrophenylhydrazine has been studied and will be discussed in another place. The results obtained by Dr. Timothy O'Connor in this Laboratory suggest that the 3-hydrazone of the 4-bromosteroid was formed and that this unstable intermediate compound then lost a bromide ion from C-4 and a proton from the α-nitrogen to give an α,β-unsaturated azo structure. This highly colored product then rearranged to give the Δ^4-hydrazone. When *p*-nitrophenylhydrazine was used the intermediate compound was isolated in crystalline form. With this material it was shown that the rate of rearrangement of the azo structure into the Δ^4-hydrazone was influenced by the solvent and the presence of mineral acid.

with which the semicarbazone can be converted into cortisone acetate. In 70% aqueous acetic acid hydrolysis of the semicarbazide moiety proceeded to the extent of 80 to 85% in 36 hours at room temperature. When *p*-hydroxybenzaldehyde was added as an acceptor for the semicarbazide, from 90 to 93% of the theoretical yield of cortisone acetate was obtained. With two equivalents of pyruvic acid in 70% aqueous acetic acid the recovery of cortisone acetate from the semicarbazone was nearly quantitative.

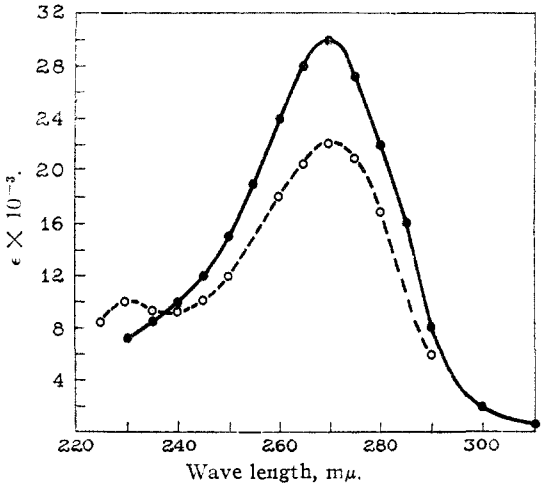


Fig. 1.—Absorption spectrum of cortisone acetate semicarbazone prepared with tertiary butyl alcohol as solvent, ———; reaction product obtained with isopropyl alcohol as solvent — — —.

Separation of cortisone acetate from its 3-semicarbazone can be achieved by crystallization from acetic acid, but crystallization from acetone, ethanol or methanol is unsatisfactory. Small amounts of the semicarbazone can be removed from a chloroform solution of cortisone acetate by washing with *N* hydrochloric acid, but this treatment destroys the semicarbazone taken into the aqueous phase.

Evidence has been obtained that with semicarbazide, under some conditions, the reaction follows a similar course. With semicarbazide and 4-bromo-4,5β-dihydrocortisone acetate (I) dissolved in a mixture of equal parts of acetic acid and chloroform at 0° the semicarbazone of the 4-bromo steroid (II) was formed, but at 0° elimination of hydrogen bromide proceeded very slowly. The rate of dehydrobromination was determined by removal of the insoluble semicarbazide hydrobromide which separated from the solution. Addition of water at any chosen time completed loss of hydrogen bromide from the portion of the 4-bromosteroid that had formed the 3-semicarbazone, and it was observed that the solution, which had been colorless, became yellowish orange. The chloroform which separated from the aqueous phase contained the yellow product (III). The chloroform solution was freed of acetic acid by washing with ice-water and cold sodium bicarbonate solution, and after concentration of the organic phase and addition of petroleum ether the material was precipitated in amorphous form. The absorption spectrum of the unstable yellow product (III) with two maxima—281 mμ and 440 mμ—indicated the presence of the azo structure. When the compound was dissolved in acetic acid, at room temperature, the color rapidly faded and the material then possessed an absorption maximum at 270 mμ, characteristic of cortisone acetate semicarbazone.

In *t*-butyl alcohol, at room temperature, formation of the azo compound (III) was indicated by changes in color. An induction period of 15 minutes was followed by an interval during which the solution became yellow. The color reached a maximal intensity at the end of 30 minutes and then slowly faded. After 1 hour the solution was again colorless. It therefore seems probable that the

mechanism of formation of the 3-semicarbazone of cortisone acetate involves an azo intermediate compound and parallels the course suggested for the formation of the Δ^4-*p*-nitrophenylhydrazone from another 3-keto-4-bromosteroid.

Treatment of 4-bromo-4,5β-dihydrocortisone acetate in acetic acid with semicarbazide at room temperature afforded the semicarbazone of cortisone acetate but the solution remained colorless. Either conversion of the yellow intermediate azo compound into the colorless semicarbazone was too rapid to permit the accumulation of a perceptible concentration of the colored compound or the reaction may have taken another course.

Experimental

All melting points were taken on a Fisher–Johns apparatus and recorded as read. The ultraviolet absorption spectra were determined in 95% ethanol with a Beckman model DU spectrophotometer. Specific rotations were taken at a temperature of 27 ± 3° and with a concentration of about 1%. Analyses were made by Mr. J. F. Alicino, Metuchen, New Jersey. We are indebted to Mrs. Grace Dews for the absorption spectra in the infrared region and to Dr. H. L. Mason for their interpretation.

Cortisone Acetate 3-Semicarbazone (IV) from Cortisone Acetate (V).—Cortisone acetate (402 mg.), 225 mg. of semicarbazide base and 1.5 ml. of acetic acid were added to 150 ml. of 95% ethanol at room temperature. Solution was complete, and after 18 hours addition of 100 ml. of water afforded the semicarbazone hemihydrate. Recrystallized from ethanol and dried at 105° the product weighed 448 mg. (96%) and melted at 211–213°; $\lambda_{max}^{ethanol}$ 270 mμ ε 30,000; $[\alpha]_D$ +264 ± 2° (*t*-butyl alcohol); $[\alpha]_D$ +243 ± 2° (0.5% in chloroform).

The semicarbazone lost 1.97% in weight at 65° and 0.1 mm. Re-exposure of the sample to the atmosphere for 8 hours increased the weight 1.91%; theory for a hemihydrate, 1.92%.

Anal. Calcd. for $C_{24}H_{33}O_6N_3 \cdot {}^1/_2H_2O$: C, 61.60; H, 7.32; N, 8.97. Found: C, 61.73; H, 7.54; N, 8.93.

Cortisone Acetate 3-Semicarbazone (IV) from 4-Bromo-4,5β-dihydrocortisone Acetate (I) with *t*-Butyl Alcohol as Solvent.—In a 150-ml. flask at room temperature were placed 966 mg. of I ($[\alpha]_D$ +103 ± 1°, m.p. 223–224°), 300 mg. of semicarbazide base, 50 ml. of *t*-butyl alcohol and 30 ml. of dry, alcohol-free chloroform. The air was displaced with carbon dioxide, the flask was sealed and agitated for 10 to 15 minutes. After 2 hours the solvents were removed by distillation under reduced pressure, and 15 ml. of ethanol was added. The semicarbazide hydrobromide was kept in solution by addition of 10 ml. of water, and the semicarbazone was filtered off, washed with water and air-dried. Halide ion in the filtrate was 98.5% of the theoretical amount. The product weighed 870 mg. (93%) and melted at 200–203°, $[\alpha]_D$ +264 ± 2° (*t*-butyl alcohol); $\lambda_{max}^{ethanol}$ 270 mμ, ε 30,000. Recrystallization gave 801 mg., m.p. 208–210°. The specific rotation and ultraviolet absorption were unchanged. The infrared absorption spectrum was identical with that of a sample of the 3-semicarbazone prepared from cortisone acetate.

IV from I at 0°.—With the same amounts of materials the development and subsequent disappearance of all color in the solution required 3 hours. The semicarbazone of cortisone acetate was separated as described in a yield of 89%.

IV from I with Acetic Acid as Solvent.—A mixture of 30 ml. of acetic acid and 20 ml. of chloroform which contained 300 mg. of semicarbazide was cooled to 0°, the air in the flask was displaced with carbon dioxide, 966 mg. of 4-bromo-4,5β-dihydrocortisone acetate was added and the flask was sealed. After 5 hours at 0° the flask was warmed to, and then maintained at, room temperature for 3 hours. The semicarbazone of cortisone acetate was separated as described in a yield of 83%, m.p. 210–212°, $\lambda_{max}^{ethanol}$ 270 mμ, ε 29,800.

IV from I with Other Solvents.—At room temperature yields of 90% were obtained when dioxane, tetrahydrofuran and ethylene glycol dimethyl ether were used in place of *t*-butyl alcohol and chloroform was omitted.

With ethylene glycol monomethyl ether and with isopropyl alcohol the yields of the semicarbazone of cortisone acetate were 15 to 20% less. The absorption spectrum was taken in the ultraviolet region (Fig. 1) of the entire reaction product in ethanol. There was an absorption maximum at 270 mμ (cortisone acetate semicarbazone), and a second maximum at 230 mμ indicated the presence of a semicarbazone of a steroid substituted at C-4.

Elimination of Hydrogen Bromide during Formation of Semicarbazone of I.—The procedure for each determination in Table I was as follows: a solution of 483 mg. of I in 15 ml. of acetic acid and 10 ml. of alcohol-free chloroform was cooled to 0°, the air was displaced with carbon dioxide and 150 mg. of semicarbazide base was added. The flask in an ice-bath was shaken for a few minutes until the solid phase dissolved. At the times indicated in Table I the semicarbazide hydrobromide was removed by filtration. Water and chloroform were added to the filtrate and after shaking for a few minutes the aqueous phase was separated and the bromide ion was determined. The semicarbazide hydrobromide was dissolved in water and the bromide ion was determined. The results are given in Table I.

TABLE I

ELIMINATION OF HYDROGEN BROMIDE AT 0°

Reaction time, hours	Semicarbazide hydrobromide isolated, %	Additional bromide ion on addition of water, %	Total bromide ion, %
0.5	0	65	65
1.0	0	78	78
2.0	Trace	92	92
5.0	31	68	99
24.0	83	15	98

Separation of the Δ^3-3-Azo Compound (III).—One millimole of I (483 mg.) in 15 ml. of acetic acid and 10 ml. of alcohol-free chloroform was treated as described in the preceding experiment with 150 mg. of semicarbazide at 0°. After one hour 50 ml. of cold water was added, the chloroform solution was separated, washed with cold sodium bicarbonate solution, dried and concentrated to 5 ml. Addition of 50 ml. of petroleum ether precipitated the yellow compound (400 mg.); $\lambda_{max\,1}^{CHCl_3}$ 281 mμ, ε 13,000; $\lambda_{max\,2}^{CHCl_3}$ 440 mμ, ε 80, $[\alpha]_D$ +112 ± 2°.

In chloroform the yellow product was slowly converted into the colorless semicarbazone. This rearrangement occurred rapidly in methanol. The absorption maxima at 281 mμ and at 440 mμ disappeared and a new absorption maximum appeared at 270 mμ which was characteristic of the semicarbazone of cortisone acetate.

Cortisone Acetate (V) from Cortisone Acetate 3-Semicarbazone (IV) in 70% Acetic Acid.—The hemihydrate of IV (200 mg.) was dissolved in 14 ml. of acetic acid and 6 ml. of water was added. The air in the flask was displaced with carbon dioxide and after 36 hours 20 ml. of water was added and the solution was extracted with chloroform. The combined chloroform extracts were washed with sodium bicarbonate solution and three times with *N* hydrochloric acid to remove unchanged IV. The chloroform solution was dried, concentrated, and V was crystallized from ether–acetone; yield 148 mg. (85%), m.p. 248–249°, $\lambda_{max}^{ethanol}$ 238 mμ, ε 15,800.

V from IV with *p*-Hydroxybenzaldehyde.—A sample of 400 mg. of IV hemihydrate in 14 ml. of acetic acid, 6 ml. of water and 1.6 g. of *p*-hydroxybenzaldehyde was treated as described in the preceding experiment except that a solution of 2% sodium carbonate was used to remove the excess of *p*-hydroxybenzaldehyde. After 24 hours V was separated in a yield of 92%, m.p. 246–247°, $\lambda_{max}^{ethanol}$ 238 mμ, ε 15,800.

V from IV with Pyruvic Acid.—The hemihydrate of IV (459 mg.) was added to 14 ml. of acetic acid, 5 ml. of water and 1.2 ml. of 1.66 *N* aqueous pyruvic acid. Air was displaced with carbon dioxide, the flask was warmed to 40 to 45° until IV was completely dissolved (3 to 4 minutes), and was allowed to stand at room temperature. After 15 hours V was separated as described; yield 389 mg. (99%), m.p. 249–250°, $\lambda_{max}^{ethanol}$ 238 mμ, ε 16,300.

ROCHESTER, MINN.