[CONTRIBUTION FROM MERCK SHARP & DOHME RESEARCH LABORATORIES]

Transformations of the Cortical Side Chain. Oxygen Elimination at C_{17} and C_{21}

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Through the influence of the 20 semicarbazone group, the oxygen functions at C_{21} and C_{17} of the cortical side chain are activated to elimination by reduction and dehydration, respectively. A convenient method for the synthesis of Δ^{16}-cortical steroids is described.

The stability of the 17-hydroxy-21-acetoxy cortical side chain to a wide range of reaction conditions is well documented.[1] As an example of such stability may be cited the resistance to change under a variety of strongly acidic conditions.[2] In pursuing further the general reaction behavior of the cortical side chain we observed that the 17α-hydroxy-21-acetoxy-20-keto system withstands also the rigors of zinc dust in refluxing acetic acid. These conditions, it is interesting to note, are sufficient in certain instances for effecting reductive removal of α-ketolic hydroxyl functions.[3,4] Further, it has been shown that treatment of a 21-acetoxy-Δ^{16}-pregnene-20-one with zinc and acetic acid even at room temperature results in reductive loss of the 21-acetoxy group.[5]

In view of known activation to elimination of α-haloketones by carbonyl derivatives[6] as well as the recently observed dehydration of α-ketols by dinitrophenylhydrazine[7] our attention was directed to the possible labilization by the 20-semicarbazone group on oxygen functionality at C_{17} and C_{21}. Treatment of the 20-semicarbazone of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione, (I, R = $NNHCONH_2$) with zinc dust in refluxing acetic acid followed by reversal with pyruvic acid produced as the major product 3α-acetoxy-17α-hydroxypregnane-11,20-dione (II, R = O). The reductive loss of the 21-acetoxy function under conditions whereby I itself is stable (see above) is clearly the consequence of labilization by the semicarbazone group. Also indicated in the reaction product was a small amount of 3α-acetoxy-Δ^{16}-pregnene-11,20-dione (III). The latter substance was apparently formed by a secondary reaction involving labilized dehydration at C_{17} of the semicarbazone derivative II, (R = $NNHCONH_2$). This point was confirmed by independently submitting II, (R = $NNHCONH_2$) to refluxing acetic acid whereby III was formed in good yield.

These observations suggested that this mode of formation of Δ^{16} systems might provide a general and convenient means of preparing Δ^{16}-cortical steroids. This suggestion was substantiated through the conversion by this procedure of 3,21-diacetoxy-pregnane-17α-ol-11,20-dione (I) to 3,21-diacetoxy-Δ^{16}-pregnene-11,20-dione (IV) and the transformation of cortisone acetate (V, R = O) to its Δ^{16}-anhydro derivative (VI, R = O). In the latter instance, cortisone acetate (V. R = O) was quantitatively converted to its 3,20-bissemicarbazone derivative (V, R = $NNHCONH_2$)[8] and the latter refluxed for 1 hr. with acetic acid. Removal of the semicarbazone groups produced 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (VI, R = O) in a direct overall yield of 40–45% and a conversion yield of 90% based on recovered cortisone. This substance was first prepared by McGuckin and Mason[9,10] in unspecified yield starting from 3α,21-diacetoxy-12α-bromopregnane-11,20-dione. Recently Allen and Bern-

(1) Rosenkranz and Sondheimer, "Synthesis of Cortisone" in *Fortschritte Chem. Org. Naturstoffe*, Springer-Verlag, Wien 1953, p. 274.

(2) Fried and Sabo, *J. Am. Chem. Soc.*, **75**, 2273 (1953); also Graber, Snoddy, and Wendler, *Chemistry & Industry* **57** (1956).

(3) See for example: Rosenfeld and Gallagher, *J. Am. Chem. Soc.*, **77**, 4368 (1955).

(4) It has recently been reported by Norymberski [*J. Chem. Soc.*, 517 (1956)] that the 17-hydroxyl group is removed from 17α,21-dihydroxy-20-keto steroids by zinc in aqueous acetic acid. Since we have ascertained that this change does not occur to any detectable extent with the corresponding 17α-hydroxy 21-acetoxy-20-keto system, it appears that the loss of the 17-OH reported by Norymberski takes place by elimination in the sense of the Mattox rearrangement [*J. Am. Chem. Soc.*, **74**, 4340 (1952); see also N. L. Wendler and R. P. Graber, *Chemistry & Industry* 549 (1956)] and not by a direct reductive process. It is intimated that reduction occurs at the stage of an intermediate keto aldehyde.

(5) Nes and Mason, *J. Am. Chem. Soc.*, **73**, 4765 (1951). These authors converted 3α-hydroxy, 21-acetoxy, 12α-bromo-Δ^{16}-pregnene-11,20-dione to 3α-hydroxy-Δ^{16}-pregnene-11,20-dione with zinc in aqueous acetic acid at 15°. This reaction appears to be more or less specific for the compound dealt with by these authors, since we were unable to observe any reductive loss of the 21-acetoxy function when 21-acetoxy $\Delta^{4,16}$-pregnadiene-3,11,20 trione was submitted to the same conditions.

(6) Mattox and Kendall, *J. Am. Chem. Soc.*, **72**, 2290 (1950).

(7) Reich and Samuels, *J. Org. Chem.*, **19**, 1041 (1954); **21**, 65 (1956).

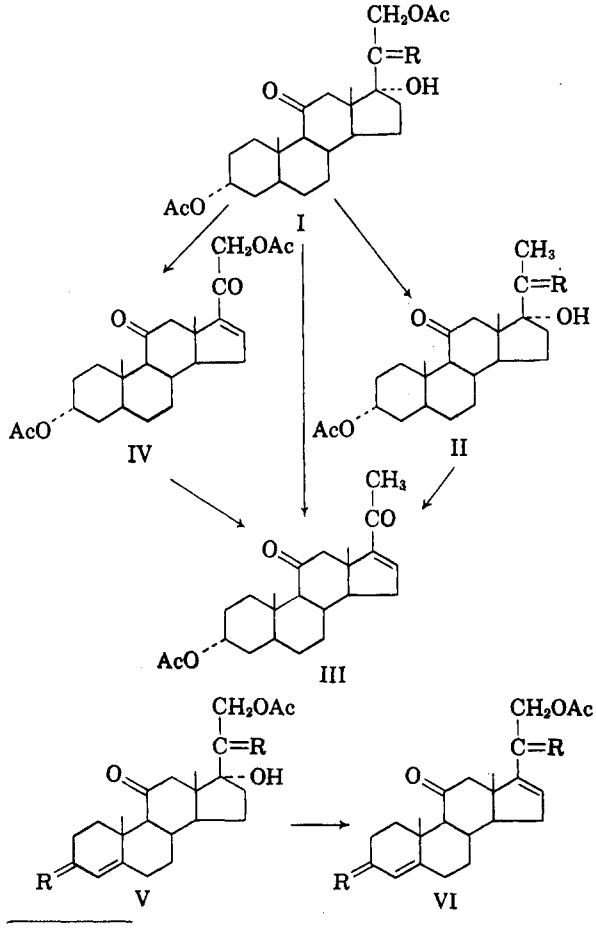
(8) Jones and Robinson, *J. Org. Chem.*, **21**, 586 (1956); Oliveto, Rausser, Weber, Shapiro, Gould, and Hershberg, Hershberg, *J. Am. Chem. Soc.*, **78**, 1736 (1956).

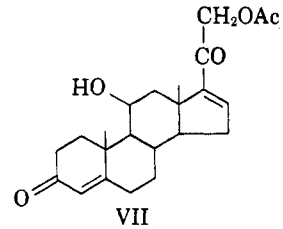
(9) McGuckin and Mason, *J. Am. Chem. Soc.*, **77**, 1822 (1955); see also Colton, Nes, Van Dorp, Mason, and Kendall, *J. Biol. Chem.*, **194**, 235 (1953).

(10) The authors are indebted to Dr. McGuckin of the Mayo Clinic through Dr. Bayler of these Laboratories for making available an infrared comparison sample.

stein[11] have reported the preparation of (VI, R = O) by a more direct route through the dehydration of the 3,20-bisdioxolane derivative of cortisone acetate by means of phosphorus oxychloride in pyridine. This method, however, is made less attractive preparatively because of the poor conversion in the formation of the 3,20-bisdioxolane derivative of cortisone acetate. Further, the method of Szpilfogel and Gerris[12] involving the direct action of phosphorus oxychloride and pyridine on 17-hydroxy-20-ketopregnanes for prolonged periods of time has, in our hands, been found inapplicable to the 21-acyloxy derivatives. Thus, it was observed that 17α-hydroxy-21-acetoxy-pregnane-3,11,20-trione by this method produced no detectable amount of the Δ^{16}-derivative, the product consisting for the most part of intractable tar.[13]

The conversion of 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (VI, R = O) to its corresponding 11β-hydroxy analog (VII) was effected by borohydride reduction of its 3,20-bis-semicarbazone derivative (VI, R = NNHCONH).[14]





VII

R, = O; = NNHCONH₂

EXPERIMENTAL[15]

Treatment of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione-20-semicarbazone (I, R = $NNHCONH_2$) *with zinc and acetic acid.* To a solution of 1.0 g. of I (R = $NNHCONH_2$)[8] in 30 ml. of stirred refluxing acetic acid was added 3.0 g. of zinc dust over a period of 35 min. The reaction mixture was filtered, concentrated *in vacuo*, and dissolved in chloroform. The chloroform solution was washed with dilute potassium bicarbonate solution, water, and finally dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was dissolved in 12 ml. of acetic acid and treated with 6 ml. of water and 3 ml. of pyruvic acid. After standing at room temperature overnight and at 60° for 2 hr., the reaction mixture was diluted with water and extracted with chloroform. The chloroform solution was washed successively with water, 5% aqueous potassium bicarbonate solution, water, and dried over magnesium sulfate. Removal of the solvent afforded a crystalline residue which was chromatographed on 40 g. of neutral alumina and eluted with benzene and benzene-chloroform mixtures. The initial eluates yielded a few milligrams of material with $\lambda_{max}^{CH_3OH}$ 236 mμ (E = 3,700) indicating the presence of 3α-acetoxy-Δ^{16}-pregnene-11,20-dione (III). From the benzene eluates there was obtained after crystallization from ether-petroleum ether, 355 mg. of II (R = O), m.p. and mixed m.p. with an authentic sample 198–203°. From the eluates corresponding to 20–50% chloroform-benzene there was obtained 180 mg. of I, m.p. 226–229°, undepressed on admixture with an authentic specimen.

3α-Acetoxy-Δ^{16}-pregnene-11,20-dione (III). A solution of 0.55 g. of II (R = $NNHCONH_2$)[8] in 30 ml. of acetic acid containing 0.6 ml. of acetic anhydride was refluxed in a nitrogen atmosphere for 16 hr. The reaction mixture was concentrated *in vacuo* to a volume of *ca.* 6 ml. and treated with 3 ml. of water and 1.5 ml. of pyruvic acid and allowed to stand at room temperature overnight and 2 hr. at 60°. After dilution with water, the product was extracted with chloroform, and the chloroform solution was washed with water, 5% potassium bicarbonate solution, water, and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was chromatographed on 20 g. of neutral alumina and eluted with petroleum ether-benzene-chloroform mixtures. The fractions corresponding to 50% petroleum ether–benzene afforded, after crystallization from ether, 200 mg. of III, m.p. 167–169°, $\lambda_{max}^{CH_3OH}$ 235 mμ, E = 9,500. Identity was established by comparison with a sample prepared by another method.[16]

From the fractions corresponding to 1–5% chloroform-benzene there was obtained, after crystallization from ether-petroleum ether, 155 mg. of II, m.p. 198–203°.

3α,21-Diacetoxy-Δ^{16}-pregnene-11,20-dione (IV). A solution of 1.0 g. of I (R = $NNHCONH_2$)[8] in 60 ml. of acetic acid containing 3 ml. of acetic anhydride was refluxed in a nitrogen atmosphere for 1 hr. The pale red reaction mixture was concentrated *in vacuo* to a volume of *ca.* 15 ml. and treated

(11) Allen and Bernstein, *J. Am. Chem. Soc.*, **77**, 1028 (1955).

(12) Szpilfogel and Gerris, *Rec. trav. chim.*, **74**, 1402 (1955).

(13) Observation of C. S. Snoddy, Jr., of these laboratories.

(14) Wendler, Huang-Minlon, and Tishler, *J. Am. Chem. Soc.*, **73**, 3818 (1951).

(15) All melting points recorded were taken on a micro hot stage.

(16) Beyler and Hoffman, *J. Org. Chem.*, **21**, 572 (1956); also Schork and Karpel, U. S. Patent **2,684,963**; Julian and Karpel, U. S. Patent **2,671,794**.

with 7 ml. of water and 3 ml. of pyruvic acid and allowed to stand at room temperature overnight and at 60° for 2 hr. After dilution with water, the product was extracted with chloroform and the chloroform solution was washed with water, 5% potassium bicarbonate, water, and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was chromatographed on 50 g. of neutral alumina and eluted with benzene-chloroform mixtures. From the benzene eluates there was obtained 200 mg. of IV, m.p. 126–131°. Recrystallization from acetone–petroleum ether gave material of m.p. 131–132°, $\lambda_{max}^{CH_3OH}$ 237 mμ, E = 9,900.

Anal. Calcd. for $C_{25}H_{32}O_6$: C, 70.08; H, 7.53. Found: C, 69.75; H, 7.78.

From the fractions corresponding to 1–20% chloroform-benzene there was recovered 400 mg. of I (R = O), m.p. 226–229°.

21-Acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (VI).[9] A solution of 5.0 g. of cortisone acetate-3,20-bissemicarbazone[8] (V) in 100 ml. of acetic acid containing 5 ml. of acetic anhydride was refluxed in a nitrogen atmosphere for 1 hr. The pale yellow to red reaction mixture was concentrated *in vacuo* to a volume of 60 ml. and treated with 30 ml. of water and 15 ml. of pyruvic acid, and allowed to stand at room temperature for 40 hr. and at 60° for 2 hr. After dilution with water, the product was extracted with chloroform and the chloroform solution was washed with water, 5% potassium bicarbonate, water, and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was chromatographed on 200 g. of neutral alumina and elution with benzene afforded after crystallization from acetone-ether, 1.37 g. (38%) of VI (R = 0), m.p. 186–187°, $\lambda_{max}^{CH_3OH}$ 237–238 mμ, E = 25,200, identical in the infrared spectrum with an authentic sample.[10]

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.96; H, 7.26.

Further elution of the chromatograph column with 50% benzene–ethyl acetate gave 2.47 g. (61%) cortisone acetate (V), thus affording the Δ^{16} compound VI in *ca.* 91% yield based on recovered cortisone. In several typical preparations a direct yield of 40 to 46% of VI has been attained.

11β,21-Dihydroxy-$\Delta^{4,16}$-pregnadiene-3,20-dione (VII).[11] 21-Acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione was converted to the corresponding 3,20-bissemicarbazone derivative[8] and reduced with lithium borohydride by the method of Wendler, Huang-Minlon, and Tishler.[14] The reversal of the semicarbazone was carried out as described above with pyruvic acid to give VII, m.p. 153–156°. A sample for analysis was chromatographed on neutral alumina and eluted with chloroform to afford VII crystallized from acetone–petroleum ether, m.p. 159–161°, $\lambda_{max}^{CH_3OH}$ 241 mμ, E = 20,900.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.83; H, 8.25.

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Synthesis and Antimicrobial Activity of Some Alkyl 3-Phenanthridinols

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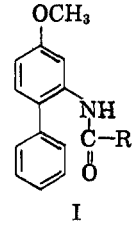
Three 6-alkyl-3-phenanthridinols have been synthesized for antimicrobial testing. A convenient method for the synthesis of the requisite 2-amino-4-methoxybiphenyl was found through the reaction of sodium amide on 3-bromo-4-methoxybiphenyl.

As a result of the observation by Steinberg[2] that 3- and 2-phenanthrols possess marked fungistatic activity toward *Aspergillus niger*, research was initiated to determine whether greater activity might be achieved by introduction of substituents into the phenanthrol nucleus[3] or by alteration of the aromatic system.[4] As part of the latter program, we decided to undertaken the synthesis of some 3-phenanthridinols (II). Copp and Walls[5] synthesized the first 6-substituted 3-phenanthridinol derivative (III, R = *p*-$NO_2C_6H_4$) because of their interest in possible trypanocides. The only 6-alkyl-3-phenanthridinol known (II, R = CH_3)



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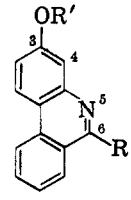

II, R′ = H
III, R′ = CH_3

was prepared by Mitsuhashi.[6] It appears that Mitsuhashi followed Copp and Walls[5] both with regard to the cyclization procedure and to the method used in preparing the 2-amino-4-methoxybiphenyl (V). The latter synthesis involved the preparation of 2-nitro-4-methoxybiphenyl by the Gomberg method (in unspecified yield) followed by reduction.

We have found that the desired amine (V) may be obtained in 60% yield from the easily prepared 3-bromo-4-methoxybiphenyl (IV), in only one step, by reaction with sodium amide in liquid

(1) Allied Chemical and Dye Corp. Fellow, 1953–1954. Taken in part from a thesis submitted by P. H. Leake in partial fulfillment of the requirements for the Ph.D. degree at Duke University, 1954. This work was supported in part by the Chemical Corps, Fort Detrick, Md., under contract with Duke University.

(2) Steinberg, *J. Agr. Research*, **60**, 765 (1940).

(3) Bradsher, Brown, and Leake, *J. Am. Chem. Soc.*, **78**, 4400 (1956).

(4) Bradsher, Brown, and Porter, *J. Am. Chem. Soc.*, **76**, 2357 (1954).

(5) Copp and Walls, *J. Chem. Soc.*, 311 (1950).

(6) Mitsuhashi, *J. Pharm. Soc. Japan*, **II**, 1232 (1951); *Chem. Abstr.*, **46**, 5593 (1952).