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Catalytic Hydrogenations of Several 18-Oxygenated 3-Keto-Δ^4-Steroids

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Hydrogenations of the monoketal *2*, dihydroaldosterone acetate *4a* and aldosterone in the presence of palladium on charcoal are surprisingly easily carried to the tetrahydro stage; this is the basis of a facile synthesis of 3β,5α- and 3β,5β-tetrahydroaldosterone.

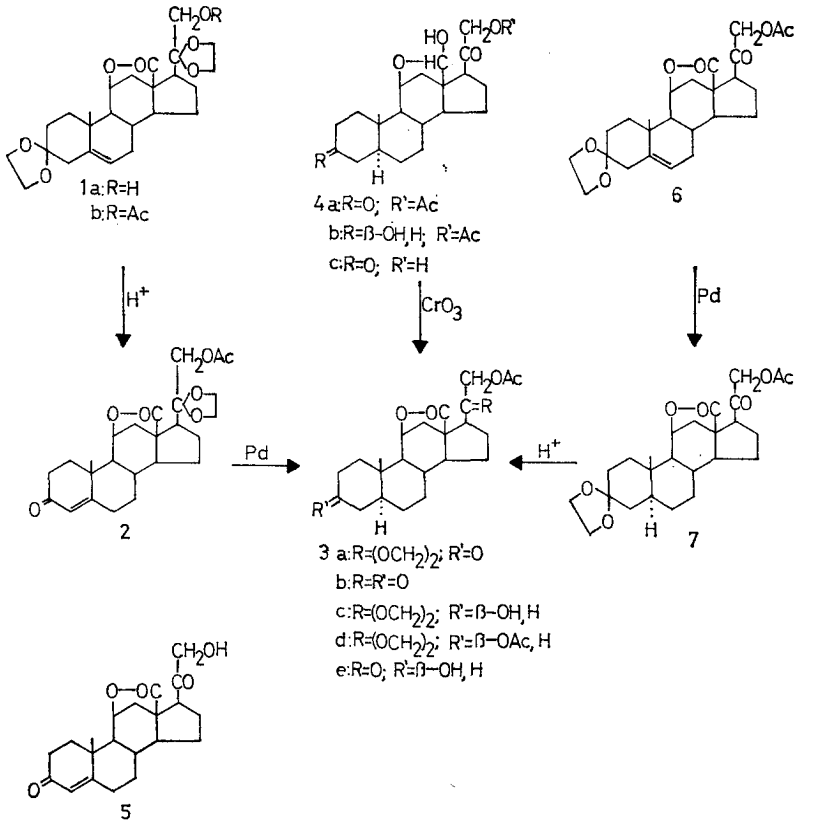
In connection with other studies of C_{18}-oxygenated steroids, the hydrogenation products of the unsaturated ketolactone *2* were needed. The latter compound was now made available by acetylation of the known (1) diketal *1a*, followed by a selective hydrolysis of one ketal grouping in *1b*. It was expected that preferential splitting off would take place at the 3, rather than the 20 position of the acetate *1b*, in line with other precedents (2). With hydrochloric acid in dioxane there were obtained the two lactones *2* and *5*, separated by crystallization and chromatography.

Hydrogenation of the monoketal *2* with 5% palladium charcoal in ethyl acetate for 2 hr caused not only the expected predominant formation of the 5α-dihydrolactone *3a*, but also produced a more polar component, mp 160–163°. When the time of hydrogenation was extended to 15 hr, none of the 5α-compound *3a* could be isolated, but the above polar product was obtained in 20% yield by direct crystallization. That the material on hand (*m/e* 448) was the 3β,5α-tetrahydro derivative *3c* was shown by the following transformations (Table 1).

Further mild reduction of the pure dihydro acetate *3a* with palladium charcoal gave an excellent yield of the 163°C melting material, which could be reoxidized with Sarett's reagent to the 3-one *3a*. Acid treatment of the tetrahydro compound *3c* furnished a new lactone, *3e*, mp 225–227°C, which could be oxidized to the dione *3b*, mp 196–198°C; a mild palladium hydrogenation of the latter converted it to the 3β-ol *3e*. The diketolactone *3b* could also be obtained by deketalization of *3a*.

While this series of reactions showed that no deep-seated structural changes in the molecule were taking place, it was implicity assumed that

TABLE 1



the 3-keto group, rather than the lactone, was being reduced by palladium. In order to settle this point unequivocally, 5α-dihydroaldosterone 21-acetate *4a* (3,4) was prepared, and found to be different from the compound *3e*, thus establishing that the site of attack by palladium was at position 3. This conclusion was further supported by NMR spectra of *3e* and *4a*. Chromic acid oxidation of *4a* gave the lactone *3b*, thereby confirming the 5α-configuration of the compounds *3a–e*.

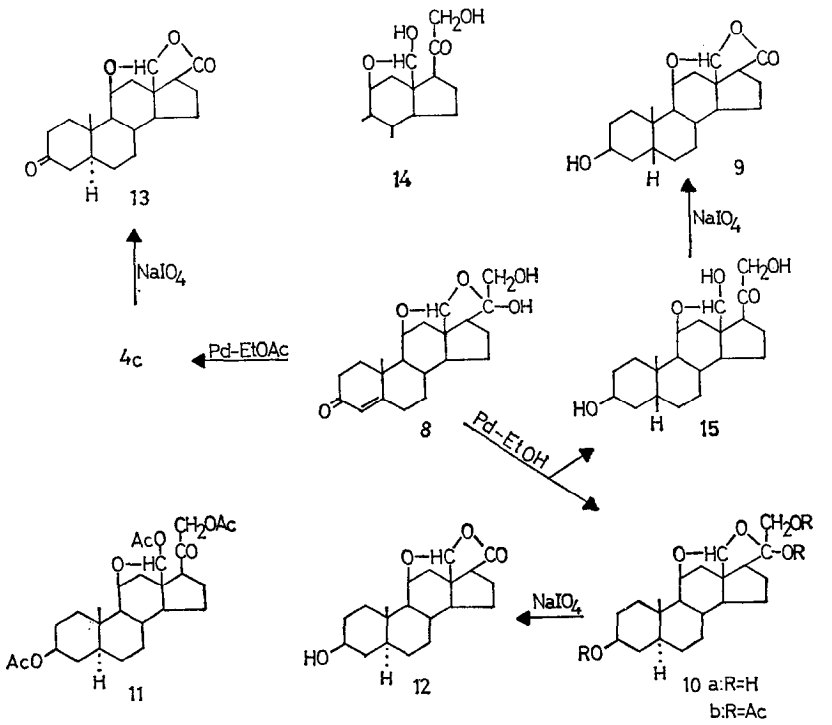
5α-Dihydroaldosterone 21-acetate *4a* mentioned above was prepared by palladium charcoal hydrogenation of aldosterone acetate. The separation of the 5α-dihydro acetate *4a* from the accompanying 5β epimer was laborious, and it was again observed that the reaction mixture contained a more polar component, mp 180–182°C. This was the 3β,5α-tetrahydro derivative *4b* (3), which could also be obtained by hydrogenation of pure *4a* with palladium charcoal. In fact, by checking in tlc the overall re-

action products obtained from hydrogenations of cortisol, 17α-hydroxyprogesterone and other 3-keto-4-ones it was found that they all contained varying amounts of tetrahydro derivatives, in addition to the expected dihydro compounds.

The relatively mild hydrogenation conditions of palladium charcoal in ethyl acetate in the presence of calcium carbonate found application also for the reduction of the 5=6 double bond in the monoketal *6*. Hydrogenation of such double bonds in 3-ketals is at best capricious, but under the present conditions proceeded smoothly, giving *7*. Treatment of the latter with acid furnished the diketolactone *3b*. Unexpectedly, similar hydrogenations of the diketals *1a* and *1b* were without effect.

Hydrogenations of aldosterone itself led to convenient preparation of 5α-dihydroaldosterone *4c* and 3β,5α-tetrahydroaldosterone *10a* (Table 2). These compounds had been previously found to be metabolites of aldosterone and prepared by platinum reduction of aldosterone 21-acetate followed by hydrolysis of the acetate group (3). It has now been observed

TABLE 2



that when aldosterone is hydrogenated with palladium charcoal in ethyl acetate at atmospheric pressure, the dihydro compound *4c* can be directly obtained, while hydrogenation of aldosterone for 48 hr at 50 psi in absolute ethanol furnished the highly crystalline tetrahydro compound *10a*, characterized as triacetate and oxidizable to the etiolactone *12*. Chromatography of the mother liquors of *10a* furnished the amorphous 3β,5β-tetrahydroaldosterone *15*, also a metabolite of aldosterone (8–10) which could also be obtained, albeit less conveniently, by raney nickel hydrogenation of aldosterone.

While the infrared spectra (in KBr) of solid aldosterone and solid 3β,5α-tetrahydroaldosterone show the absence of the carbonyl group at position 20 and therefore point to the cyclic structure of the type *10*, the NMR spectra of aldosterone 21-acetate in deuteriochloroform (9) shows the presence of both structures corresponding to *8* and *14*. The NMR spectrum of the triacetate of *10a*, on the other hand, shows a peak at 4.79 ppm (two hydrogens at C-21) corresponding to the open-chain form *11*, but no peak at around 4.1 ppm derived from the cyclic form *10b*.

EXPERIMENTAL

Thin-layer chromatography was carried out on Merck silica gel plates F_{254}, using benzene–acetone, 1:1 (tlc A), benzene–ethyl acetate 1:1 (tlc B), or benzene–methanol–chloroform, 6:3:4 (tlc C). The spots were detected by spraying with phosphomolybdic acid in ethanol, followed by ethanolic sulfuric acid and heating at 120°C. 5% Palladium on charcoal, supplied by Engelhard Industries, was used throughout this investigation. NMR spectra were determined on a Varian A-60 spectrometer.

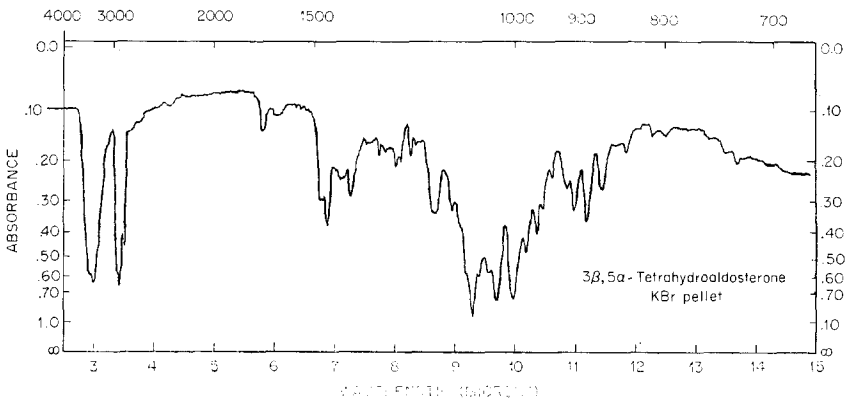


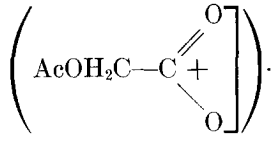
FIG. 1.

11β,21-Dihydroxy-5-Pregnen-3,20-bis-Dioxolane-18-oic Acid-(18 → 11)-Lactone 21-Acetate (1b)

Acetylation of 11.5 g of the 21-hydroxy compound *1a* (5) with a mixture of 80 ml each of pyridine and acetic anhydride at room temperature overnight, followed by quenching with ice and water, furnished 12.4 g of the acetate *1b*, 187–189°C. Recrystallization of a sample from ethyl acetate did not change the mp. λ_{max}^{KBr} 5.65–5.75 μm; R_f 0.53 (tlc A), while that of the starting *1a* is 0.17.

11β,21-Dihydroxy-4-Pregnen-3-one-20-Dioxolane-18-oic Acid-(18 → 11)-Lactone 21-Acetate (2)

To a solution of 10 g of the diketal *1b* in 200 ml of dioxane was added, in one portion, 5 ml of 1 N hydrochloric acid. After letting stand for 40 hr at 25°C, the solution was shaken with 350 ml of ether and 100 ml of aqueous saturated sodium bicarbonate. The aqueous phase was reextracted with 100 ml of ether, and the combined organic phases were washed with 100 ml of water. Evaporation of the solvents on the water bath furnished an oil which consisted (tlc) of about 60% of the desired monoketal *2*, the rest being mostly the lactone *5*. A solution of the oil in methylene chloride containing a trace of pyridine was concentrated to 20 ml. Ether (300 ml) was added and the solution was concentrated to 250 ml. A seed of the lactone *5* was added, which started the precipitation of ball-like clusters of crystals. After 30 min, long needles of the desired monoketal *2* started to crystallize, whereupon the solution was immediately decanted. The next day crystals were collected, the filtrate concentrated, again seeded with *5*, and the procedure repeated, until several crops of *2* and *5* were alternately obtained. The former were recrystallized from methylene chloride–ether to yield 2.5 g of *2*, mp 154–158°C. The pure sample melted at 160–162°C; λ_{max}^{KBr} 5.66, 5.75 and 6.00 μm; R_f 0.37 (tlc B); NMR: 78 (C-19 *Me*), 125 (C-21 acetate *Me*), quartet centered at 178, J = 7 (C-11 H), multiplet centered at 243 (dioxolane H), doublet centered at 288, J = 7 (C-21 H), 347 (C-4 H) cps; mass spectrometry: *m/e* 444; M-73 (CH_2OAc) = 371; 371-44 (CO_2) = 327; 145

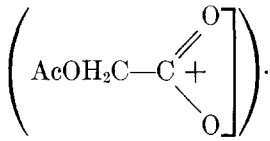


The combined crops of *5* furnished 0.28 g, mp 210–230°C; R_f 0.1 (tlc B), identical (IR, tlc) with authentic material (6).

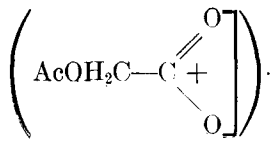
The combined filtrates were chromatographed on 250 g of Florisil. With mixtures of ether and benzene and finally ether, 4 g of pure *2* were eluted while, with 5–30% methanol in ether, a crystalline mixture of *2* and *5* was obtained.

11β,21-Dihydroxy-5α-Pregnane-3-one-20-Dioxolane-18-oic Acid-(18 → 11)-Lactone 21-Acetate (3a) and 3β,11β,21-Trihydroxy-5α-Pregnane-20-Dioxolane-18-oic acid (18 → 11)-Lactone 21-Acetate (3c)

A. By Hydrogenation of 2. A solution of 2.5 g of the ketone *2* in 200 ml of neutral ethyl acetate was stirred for 2 hr under hydrogen in the presence of 2 g of palladium charcoal and 1 g of calcium carbonate powder. The solids were filtered off and washed with methylene chloride which on evaporation gave up to 150 mg of the tetrahydro derivative *3c*, mp 152–155°C. Evaporation of ethyl acetate yielded an oil, tlc of which showed the presence of 15–45% of *3c*, depending on the run. Dissolution in methylene chloride containing a trace of pyridine, concentration to low volume and addition of 10 ml of ether precipitated 400 mg of the 3-one *3a* containing several percent (tlc) of the alcohol *3c*. Further crystallizations from ethyl acetate–ether afforded a sample of *3a*, mp 150–152°C, λ_{max}^{KBr} 5.63, 5.72 and 5.84 μm; R_f 0.47 (tlc B); NMR: 68 (C-19 *Me*), 125 (C-21 acetate *Me*), quartet centered at 178, J = 7 (C-11 H), multiplet centered at 241 (dioxolane H), doublet centered at 288, J = 8 (C-21 H) cps; mass spectrometry: *m/e* 446; M-73 (CH_2OAc) = 373; 373–44 (CO_2) = 329; 145



The filtrates were chromatographed on 15 g of Florisil. With benzene nonpolar impurities were removed, while 10 and 20% ether in benzene eluted oils which upon scratching with ether crystallized to give a total of 1.2 g of a wide-melting (130–146°C) product, essentially *3a* contaminated probably with the 5β epimer. Crystallizations from ethyl acetate–ether raised the mp to 151–153°C. The solids eluted with 50% ether in benzene and ether furnished 350 mg of a solid, mp 150–152°C, slightly contaminated (tlc) with the 3-ketone *3a*. Recrystallization from a small amount of ethyl acetate raised the mp to 160–163°C, unchanged by further recrystallizations. The 3-ol *3c* thus obtained had the following constants: λ_{max}^{KBr} 2.93, 5.68 and 5.81 μm; R_f 0.33 (tlc B); mass spectrometry: *m/e* 448; M-73 (CH_2OAc) = 375; 375–44 (CO_2) = 331 (metastable 291); 145

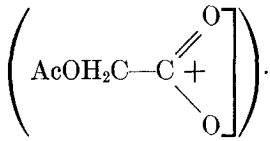


By increasing the time of hydrogenation to 15 hr, a 20% yield of *3c* could be obtained by direct crystallization from ether, and further amounts by chromatography of the filtrates.

B. Hydrogenation of 3a. A solution of 20 mg of the 3-one *3a* in 20 ml of ethyl acetate was hydrogenated for 8 hr with 200 mg of palladium charcoal and 50 mg of calcium carbonate. Filtration, evaporation of solvent and addition of ether precipitated quantitatively a solid, mp 158–160°C, whose infrared spectrum was identical with that of the alcohol *3c*.

Similar results were obtained when palladium was substituted by 50 mg of Adams' catalyst.

The *diacetate 3d*, obtained with acetic anhydride and pyridine, melted at 157–158°C (ether–ethyl acetate); R_f 0.67 tlc B; λ_{max}^{KBr} 5.69 and 5.78 μm; mass spectrometry: *m/e* 490; M-73 (CH_2OAc) = 417; 417–44 (CO_2) = 373; 417–60 (AcOH) = 357; 357–44 (CO_2) = 313; 145



Oxidation of 3c to 3a was carried out using 40 mg of steroid in 2 ml of pyridine and 100 mg of chromic acid. After stirring for 2 hr at 15°C the mixture was diluted with water and ether, the aqueous layer was re-extracted with ether and the combined organic phases were quickly washed with cold dilute hydrochloric acid, then with aqueous bicarbonate. Evaporation of most of the solvent and scratching furnished a solid consisting (tlc) of *3a*, admixed with a small amount of unreacted *3c*. By preparative tlc B it was possible to effect a clean separation, and the product thus obtained (22 mg, mp 147–152°C) was identical with the ketone *3a* obtained above.

3β,11β,21-Trihydroxy-5α-pregnane-20-one-18-oic acid-(18 → 11)-lactone 21-acetate (3e).

A. From Ketal 3c. A solution of 220 mg of ketal *3c* in a mixture of 8.5 ml of 70% perchloric acid and 91.5 ml of acetic acid was held for 10 min at 22°C, then cooled in ice and treated with 13.75 ml of 1 M potassium acetate in acetic acid. To the resulting suspension was added 200 ml of methylene chloride, 50 ml of ice water and, in small portions, 25 g of sodium bicarbonate. Water (150 ml) was added and the mixture shaken in a separatory funnel. The aqueous layer was reextracted with 150 ml of methylene chloride and the combined phases washed with aqueous bicarbonate and evaporated. The residue crystallized on addition of ether and the

solid was recrystallized from ethyl acetate to afford 78 mg of needles, mp 215–220°C. The pure material, mp 225–227°C, was obtained by preparative tlc B on silica gel, and elution of the appropriate zone with acetone in a sohxlet; λ_{max}^{KBr} 2.90, 5.61, 5.67 and 5.76 μm; R_f 0.27 (tlc B); NMR: 55 (C-19 *Me*), 130 (C-21 acetate *Me*), multiplet centered at 175 (C-11 H), 289 (C-21 H) cps; mass spectrometry: *m/e* 404; M-18 = 386; M-73 (CH_2OAc) = 331; 331–18 = 313.

B. From Diketone 3b. Hydrogenation of 20 mg of the dione *3b* in 50 ml of neutral ethyl acetate in the presence of 300 mg of palladium charcoal for 8 hr gave material which was recrystallized from ethyl acetate. The product (7 mg, mp 210–215°C) was identical (tlc, ir) with the one obtained under *A*.

11β,21-Dihydroxy-5α-Pregnane-3,20-dione-18-oic Acid-(18 → 11)-Lactone 21-Acetate (3b)

A. From the 20-Dioxolane 3a. The ketal (30 mg) was hydrolyzed as described for the compound *3c*. The desired 20-one *3b* (15 mg), mp 188–193°C, obtained by trituration with ether, was identical with the product obtained below.

B. From 5α-Dihydroaldosterone 21-Acetate (4a). A solution of 55 mg of *4a*, mp 174–178°C, in 1 ml of acetic acid was treated with 0.4 ml of a 5% chromic acid solution in acetic acid. After 2 hr at 0°C the reaction mixture was diluted with four drops of methanol, and after further 30 min worked up in the usual manner with methylene chloride, water and aqueous bicarbonate. The product, obtained by trituration with ether, melted at 188–193°C, 29 mg. The pure sample (ethyl acetate) melted at 196–198°C; λ_{max}^{KBr} 5.70 and 5.85 μm; R_f 0.43 (tlc B); NMR: 67 (C-19 *Me*), 129 (C-21 acetate *Me*), multiple centered at 185 (C-11 H), triplet centered at 288, J = 3 (C-21 H) cps; mass spectrometry: *m/e* 402; M-18 = 384; 384–28 (CO_2) = 364; M-60 (AcOH) = 342; M-73 (CH_2OAc) = 329.

C. From the alcohol 3e. Oxidation of 40 mg of *3e* was carried out exactly as described above for *4a*. There was obtained 22 mg of material, mp 191–204°C, whose tlc and IR showed it to be *3b*.

11β,21-Dihydroxy-5α-Pregnane-18-al-3,20-dione-11,18-hemiacetal 21-Acetate (5α-Dihydroaldosterone 21-Acetate) (4a) and 3β,11β,21-Trihydroxy-5α-Pregnane-20-one-18-al-11,18-Hemiacetal 21-Acetate (4b)

Aldosterone 21-acetate (2.34 g), suspended in 300 ml of neutral ethyl acetate, was hydrogenated at atmospheric pressure for 2 hr in the presence of 2 g of palladium charcoal. The filtered solution was concentrated to 40 ml and stored at room temperature for 36 hr. The collected crystals

(crop A, 0.76 g), melted at 178–180°C; NMR: doublet at 65 and 68 (C-19 *Me*), doublet at 126 and 128 (C-21 acetate *Me*), 184 (OH), doublet centered at 246 (C-21 H, form C[7]), doublet centered at 294 (C-21 H, form B[7]), 325 (C-18 H) cps.

Concentration of the filtrate to 10 ml furnished crop B, 0.31 g, mp 165–175°C, and then crop C, 0.32 g, mp 152–158°C. Evaporation to dryness and scratching with petroleum ether gave 0.65 g of a crude solid which undoubtedly contained appreciable amounts of the 5β epimer of *4a*.

Examination by tlc A showed that crops A and B contained 10– 20% of *4b*. Their chromatography on a column of Florisil using 10 and 20% ether in benzene yielded a solid mixture of two compounds, presumably the 5α and 5β isomers of dihydroaldosterone acetate showing very close mobilities in tlc A. The melting point was erratic (172–182°C), but a small amount, melting at 180–182°C, could be obtained [reported for *4a* 178–184°C; 167–170°C (4)]; λ_{max}^{KBr} 2.90, 5.75 and 5.85 μm. Elution with benzene–ether 1:1 and with ether furnished 25 mg of the tetrahydro compound *4b*, mp 180–182°C (ethyl acetate) [reported (3) 194–198°C]; R_f 0.60 (tlc A); λ_{max}^{KBr} 2.88, 3.00 and 5.77 μm; mass spectrometry: *m/e* 406; M-18-388; 388–28 (CO_2) = 360; M-60 (AcOH) = 346; M-73 (CH_2OAc) = 333. Column chromatography of crop C afforded additional amounts of *4a*, melting at 174–178°C (methylene chloride–ether, then ethyl acetate); R_f 0.83 (tlc A).

11β,21-Dihydroxy-5α-Pregnane-20-one-3-Dioxolane-18-oic Acid-(18 → 11)-Lactone 21-Acetate (7) and Its Conversion to 3b

A solution of 100 mg of 11β,21-hydroxy-5-pregnen-20-one-3-dioxolane-18-oic acid-(18 → 11) lactone 21-acetate (*6*) (1) in 50 ml of neutral ethyl acetate was hydrogenated for 18 hr in the presence of 300 mg of palladium charcoal and 200 mg of calcium carbonate. After distillation of the filtered solution and addition of ether the product crystallized to afford 97 mg, mp 202–208°C. Further crystallization from ethyl acetate raised the mp to 214–215°C; λ_{max}^{KBr} 5.565, 5.72 and 5.79 μm; R_f 0.60 (tlc B).

Treatment of *7* with a solution of perchloric acid in acetic acid, as described for the conversion of *3c* into *3e*, yielded *3b* (tlc, IR).

5α-Dihydroaldrosterone (4c)

A solution of 2 g of aldosterone in 400 ml of neutral ethyl acetate was hydrogenated in the presence of 1.5 g of palladium charcoal at room temperature and atmospheric pressure over a 3-hr period. Evaporation *in vacuo* of the filtered solution and trituration of the semisolid residue with 15 ml of neutral ether afforded 1.1 g of a solid, mp 150–165°C.

Recrystallization from 10 vol of neutral ethyl acetate furnished small needles, mp 156–161 [reported (3) 150–158°C], R_f 0.40 (tlcA), R_{ald} 1.1.

Periodate oxidation furnished 3-keto-5α-dihydroaldosterone-γ-etiolactone 13, mp 290–292°C [reported (3) 278–288°C], whose infrared spectrum was identical with that reported (8).

3β,5α-Tetrahydroaldosterone (10a)

A solution of 2 g of aldosterone in 400 ml of absolute ethanol was hydrogenated in a Parr shaker at room temperature and 50 psi for 48 hr in the presence of 4 g of palladium charcoal. After evaporation of solvent the residue was dissolved in 40 ml of acetone and allowed to stand overnight. The precipitated tetrahydro compound *10a* was filtered off and the filtrate concentrated several times. The crops thus obtained (490 mg) were recrystallized by dissolving in 100 ml of neutral ethyl acetate and concentrating to 40 ml. In several crops there was obtained a total of 350 mg of *10a* (mother liquor-A), mp varying from 180–183°C to 180–190°C, $(\alpha)_D$ + 46.5° (c, 1.0 in dioxane) [reported (3) mp 115–120°C, $(\alpha)_D$ + 45° (*c*, 0.291 in chloroform)], *m/e* 364, R_{ald} 0.72 (tlc A).

Oxidation of a sample with sodium metaperiodate gave pure 3β,5α-tetrahydroaldosterone-γ-etiolactone 12, whose infrared spectrum was identical with that reported 8.

3β,5α-Tetrahydroaldosterone 3,18,21-triacetate (11)

A mixture of 250 mg of triol *10a*, 5 ml of pyridine and 5 ml of acetic anhydride was heated at 60°C for 3 hr. The solvents were removed in high vacuum and 5 ml of water was added. The resulting solid (325 mg, mp 154–160°C) was recrystallized from methylene chloride–ether and gave, in several crops, 230 mg, mp 163° (sinter 158°C), R_f 0.47 (tlc B); λ_{max}^{KBr} 5.70–5.80, 6.85, 6.95, 7.18, 7.27, 7.88, 8.05, 8.15, 9.88 and 10.10 μm; $(\alpha)_D$ + 123° (*c*, 1.0 in chloroform); *m/e* 490.

3β,5β-Tetrahydroaldosterone (15)

Chromatography of 900 mg of a gum obtained by evaporation of the filtrate A (see preparation of *10a*) was carried out on 60 g of silica gel 60 (mesh 70–230, E. Merck, Darmstadt), using the "dry column" method and benzene–acetone 1:1 as solvent (fractions of 40 ml each). Flasks 1 and 2 gave mixtures only, while flask 3 afforded practically pure compound *15* (150 mg), in the form of an uncrystallized glass (8–10), R_{ald} 0.86 (tlc A). Further elution gave mixtures of different tetrahydro compounds.

Oxidation of *15* with sodium metaperiodate in the usual manner gave the corresponding crystalline etio-γ-lactone 9, whose infrared spectrum

was identical with that reported (8) for the lactone derived from 3β,5β-tetrahydroaldosterone.

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REFERENCES

1. HEUSLER, H., KALVODA, J., MEYSTRE, CH., WIELAND, P., ANNER, G., WETTSTEIN, A., CAINELLI, G., ARIGONI, D., AND JEGER, O. (1961) *Helv. Chim. Acta* **44**, 502.
2. (a) ANTONUCCI, R., BERNSTEIN, S., HELLER, M., LENHARD, R., LITTELL, R., AND WILLIAMS, J. H. (1953) *J. Org. Chem.* **18**, 70; (b) ANTONUCCI, R., BERNSTEIN, S., AND LENHARD, R. H. (1954) *J. Amer. Chem. Soc.* **76**, 2956; (c) BARTON, D. R. H., AND BEATON, J. M. (1962) *J. Amer. Chem. Soc.* **84**, 199.
3. KOHLER, H., HESSE, R. H., AND PECHET, M. M. (1964) *J. Biol. Chem.* **239**, 4117.
4. Brit. Patent 941,140 [*Chem. Abstr.* **60**, 8099 (1964)].
5. VON EUW, J., NEHER, R., AND REICHSTEIN, T. (1955) *Helv. Chim. Acta* **38**, 1423.
6. SIMPSON, S. A., TAIT, J. F., WETTSTEIN, A., NEHER, R., VON EUW, J., SCHINDLER, O., AND REICHSTEIN, T. (1954) *Helv. Chim. Acta* **37**, 1200.
7. WIELAND, P., HEUSLER, K., AND WETTSTEIN, A. (1960) *Helv. Chim. Acta* **43**, 2066.
8. ULICK, S., ROSE, H. C., AND RAMIREZ, L. C. (1970) *Steroids* **16**, 183.
9. KELLY, W. G., BANDI, L., AND LIEBERMAN, S. (1962) *Biochemistry* **1**, 792.
10. ULICK, S., AND VETTER, K. K. (1962) *J. Biol. Chem.* **237**, 3364.