STEROIDS AND STEROIDASES

I. LONG-RANGE EFFECTS AND THE PREPARATION OF A-HOMOSTEROIDS

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ABSTRACT

The direction of the diazomethane ring enlargement of 17β-hydroxy-5α-androstan-3-one (I, R = OH) appears to be controlled by the long-range effect of the 17β-hydroxyl group, and proceeds with migration of the C_3—C_4 bond to give the A-homo-3-one III (R = OH). This is in direct contrast to the rearrangement of 5α-cholestan-3-one (I, R = C_8H_{17}), which gives the A-homoketone II (R = C_8H_{17}) as a result of C_2—C_3 bond migration. An analogous directing effect was not observed for the reaction of diazomethane with 17β-hydroxy-5β-androstan-3-one (VII). Oxidation of the A-homo-17β-alcohols gave the corresponding diones III (R = =O), X, and XI.

The need to prepare various A-homosteroids arose from the initiation of investigations designed to evaluate the electronic and structural specificities of the enzymes of steroid metabolism. The main objective of the programme is to gain further information on the mechanism of action of various steroidases, and on the structural and other features of steroids which contribute to biological activity. In addition, it is hoped that the factors responsible for the carcinogenic properties of certain steroids will become apparent.

Until recently, very little information was available concerning the stereochemical and mechanistic pathways of reactions catalyzed by steroidases. However, studies during the past 10 years by several groups have provided evidence regarding some of the steps involved in oxygenation (1, 2), isomerization of Δ^5-3-ketones (2, 3), and dehydrogenation (2).

Studies on the stereochemical pathway for Δ^1-dehydrogenation were carried out independently by several workers (2); after an elegant series of experiments, Ringold *et al.* (4) were able to describe the stereochemical pathway for the Δ^1-dehydrogenase of *Bacillus sphaericus* in terms of the mechanism summarized in Fig. 1, in which A represents an acidic or electrophilic binding site at the active center, and B a basic amino acid side chain.

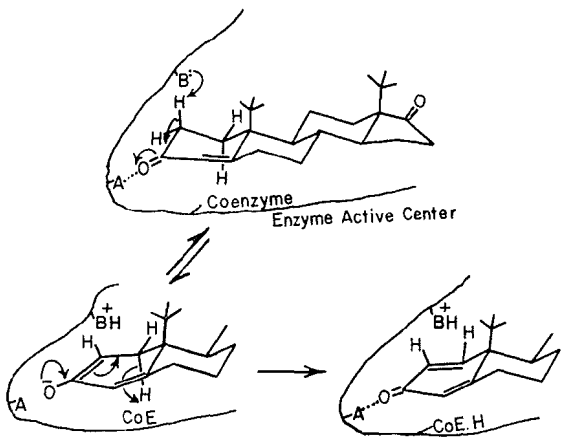
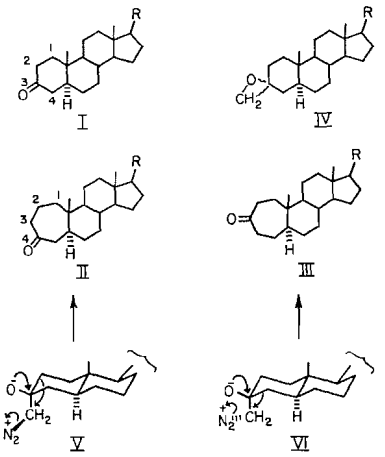


FIG. 1. Mechanism of action of Δ^1-dehydrogenase of *Bacillus sphaericus* (after Ringold *et al.* (4)).

When the present investigation was contemplated, the mechanistic pathway was not known in such detail for any other steroidase. Accordingly, the Δ^1-dehydrogenase of *Bacillus sphaericus* was selected for the initial specificity studies, as it was considered that the structural factors important in the binding and orientation of ring A of steroidal substrates at the active center could be analyzed in terms of the mechanism formulated in Fig. 1.

The aspect of structural specificity that was considered initially was the influence, on binding and catalysis, of variation of the size of ring A; thus, as the first stage of a systematic survey, the synthesis of some androstanes having ring A seven-membered has been effected.

A survey of the literature showed that, in contrast to studies on the expansion of ring D of steroids, relatively little had been reported on the preparation of A-homosteroids (5).



In 1943, Goldberg and Kirchensteiner (6) reported the enlargement of ring A of 17β-acetoxy-5α-androstan-3-one (I, R = OAc) and 5α-cholestan-3-one (I, R = C_8H_{17}) by means of the Tiffeneau–Demjanov rearrangement, which involves conversion of the ketone into the cyanohydrin, followed by reduction to the primary amine and treatment with nitrous acid. The products in both cases were the corresponding A-homo-4-ones II (R = OAc and C_8H_{17}) arising from migration of the C_2—C_3 bond of I. During the course of their investigations on A-homosteroids, Nelson and Schut (7) confirmed the tentative structural assignments of Goldberg and Kirchensteiner for A-homo-5α-cholestan-4-one (II, R = C_8H_{17}) by its synthesis via an independent route. The ring enlargement of 5α-cholestan-3-one (I, R = C_8H_{17}) with diazomethane was also studied by Nelson and Schut (7), and the major product (50% yield) was again the A-homo-4-one II (R = C_8H_{17}) resulting from C_2—C_3 bond migration as under the Tiffeneau–Demjanov reaction conditions. However, a smaller amount (10% yield) of the product of C_3—C_4 migration, A-homo-5α-cholestan-3-one (III, R = C_8H_{17}), was detected. Neither group reported the isolation of an A-homo-3-one III from the Tiffeneau route.

The formation of the same product on ring expansion from both the Tiffeneau and diazomethane reactions was expected (7), as the intermediates and reaction mechanisms involved in the rearrangement step are presumably similar (cf. refs. 8 and 9). Accordingly, in an attempt to prepare 17β-hydroxy-A-homo-5α-androstan-4-one (II, R = OH), diazomethane ring expansion conditions similar to those resulting in C_2—C_3 bond migration in the 5α-cholestane series (7) were applied to 17β-hydroxy-5α-androstan-3-one

(I, R = OH). Gas–liquid chromatographic analysis showed the product to be a mixture of at least six compounds, with one predominating to the extent of 85%. Column chromatographic purification yielded the major component, m.p. 212°, $[\alpha]_D^{24}$ −57.1°, which was obviously different from the expected product II (R = OH), m.p. 197°, $[\alpha]_D^{26}$ +112°, obtained by Goldberg and Kirchensteiner (6). The structural assignment of II (R = OH) by the latter authors was not unambiguous, but it was independently confirmed recently by the preparation of II from 17β-hydroxy-A-homo-5α-androst-1-en-4-one via catalytic hydrogenation (10). The infrared absorption at 1 695 cm^{-1} of the product whose melting point was 212° indicated the presence of a seven membered ring carbonyl, and the elemental analysis and molecular weight (mass spectrum) were in accord with an A-homo-ketone structure. This evidence led inescapably to the conclusion that in the diazomethane ring enlargement of 17β-hydroxy-5α-androstan-3-one (I, R = OH), in contrast to the 5α-cholestane series (which differs only in the nature of the substituent at C_{17}), C_3—C_4 bond migration predominates to give the A-homo-3-one III (R = OH). The many attempts to isolate II (R = OH), the product of C_2—C_3 migration, from the reaction mixture were all unsuccessful. Gas–liquid chromatographic analyses of the reaction mixture showed that the amount of each of the other products expected, the A-homo-4-one II (R = OH), the epoxide IV (R = OH), and A-bishomo compounds, was not greater than 3%.

Investigation of the optical rotatory dispersion (o.r.d.) curve of III (R = OH) confirmed its structure. A comparison of its o.r.d. curve, which shows a strongly negative Cotton effect, with that of A-homo-5α-cholestan-4-one (II, R = C_8H_{17})* is given in Fig. 2*a*.

The observed strongly negative Cotton effect (*a* = −91) of III (R = OH) is in agreement with that quoted (*a* = −60) in ref. 11 for A-homo-5α-cholestan-3-one (III, R = C_8H_{17}). The o.r.d. of 17β-hydroxy-A-homo-5α-androstan-4-one has not been reported, but the circular dichroism spectrum (10) shows a positive maximum, as would be expected by analogy with the positive Cotton effect observed for the o.r.d. curve of II (R = C_8H_{17}).

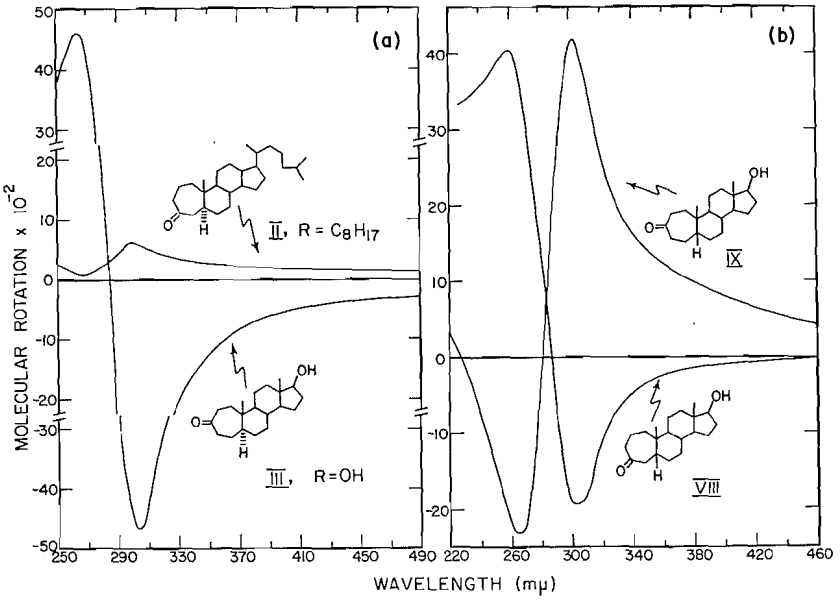


FIG. 2.

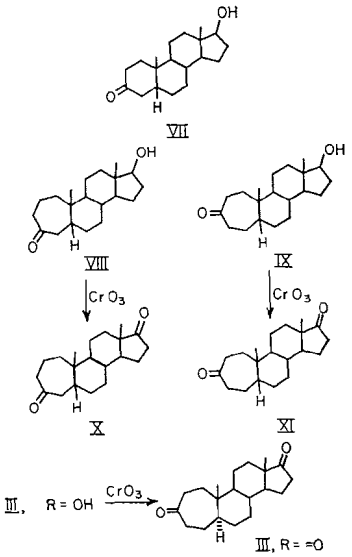
**We thank Drs. G. D. Meakins and D. J. Morris, University of Oxford, for providing us with a pure sample of this compound.*

That the change in the direction of ring enlargement is due to the effect of the hydroxyl group alone, and is not in some way associated with differences between the androstane and cholestane skeletons, has been confirmed by the observation that, for 5α-androstan-3-one (I, R = H), diazomethane ring enlargement proceeds mainly with C_2—C_3 migration, as in the cholestane series, to give 80% of A-homo-5α-androstan-4-one (II, R = H) (a = +94) (12).

The marked influence of the hydroxyl group at C_{17} on the direction of diazomethane ring enlargement appears to be another example of the long-range effects that substituents may exert across widely separated areas of the steroid nucleus (13). As the direction of ring enlargement is almost completely reversed when the polar hydroxyl group replaces the nonpolar substituents at C_{17}, this effect is one of the most powerful yet observed.

At present, the factors determining the direction of ring enlargement, in both the diazomethane and Tiffeneau reactions, are not well defined. Explanations for the differences in bond migration, based either on equatorial nucleophilic attack of the steroid-3-ketone leading to one migration, and axial addition to the other, or on differences in conformations of the intermediate arising by axial attack of the nucleophile, e.g. V and VI for C_2—C_3 and C_3—C_4 migration, respectively, are not completely satisfactory. The latter rationale is suggested from a consideration of the data available on the stereochemistry of additions of nucleophiles, with steric dimensions similar to those of diazomethane and cyanide ions, to cyclohexanones in general (14). It seems probable that axial addition (product development control (15)) should predominate; further experiments designed to provide information on this point, and on the nature of the long-range effect exerted by the hydroxyl group on the nucleophilic addition and subsequent rearrangement steps, are in progress.

As A-homo-androstanes with an A/B *cis* ring fusion were also required for the specificity studies, the reaction of 17β-hydroxy-5β-androstan-3-one (VII) with diazomethane was carried out. Ring expansion to the A-homo compounds occurred in 72% yield, but no overriding influence of the 17β-hydroxyl on the direction of migration was observed in this reaction and the products of both C_2—C_3 and C_3—C_4 migration, VIII and IX,



respectively, were formed in equivalent amounts. The properties of 17β-hydroxy-A-homo-5β-androstan-3-one (IX) were in agreement with those reported by Müller (11) for IX obtained by hydrogenation of 17β-hydroxy-A-homo-androst-4*a*-en-3-one. The o.r.d. curve showed a positive Cotton effect (Müller reports a positive circular dichroism curve), whereas that for the isomeric A-homo-4-one VIII showed a strongly negative Cotton effect (see Fig. 2*b*). Oxidation of both alcohols with chromium trioxide gave the corresponding diones X and XI in quantitative yields. Oxidation of 17β-hydroxy-A-homo-5α-androstan-3-one (III, R = OH) with chromium trioxide also gave the corresponding diketone III (R = =O) in good yield.

Since enzymes are, in general, stereospecific in their catalytic action, before any meaningful specificity and inhibition studies of the A-homo-androstanes with the Δ^1-dehydrogenase of *Bacillus sphaericus* can be carried out, it is necessary to establish the conformation of the seven-membered ring. A discussion of the conformation of ring A of A-homosteroids will be reported shortly.

EXPERIMENTAL

Infrared spectra were obtained on a Beckmann model 11 spectrometer, and optical rotations on a Bendix–Ericsson polarimeter. Optical rotary dispersions were measured on a Jasco–Durrum ORD 5 spectropolarimeter. Melting points were determined on a Fisher–Johns apparatus and are corrected. All solvents for column chromatography were distilled before use; alumina refers to British Drug Houses chromatographic alumina deactivated by shaking with 5% of its own weight of 10% acetic acid. Gas–liquid chromatographic analyses were effected with columns containing 3.8% SE 30 on silanized "Diatoport S" and 1% QF-1 on silanized "Chromosorb G" by using a F and M model 400 biomedical unit. Merck silica gel G was used as the thin-layer chromatography adsorbent, and benzene – 50% ether as the developing solvent. The spots were visualized by spraying the chromatograms with 50% aqueous toluene – *p*-sulfonic acid followed by heating them at 100°. All compounds that are described were purified until no impurity could be detected by thin-layer chromatography or by gas–liquid chromatography on both SE 30 and QF-1 columns.

17β-Hydroxy-5α-androstan-3-one (I, R = OH)

17β-Hydroxyandrost-4-en-3-one was reduced with lithium in liquid ammonia as described by Brutcher and Bauer (16). The crude product (80% yield) was purified by column chromatography on alumina prepared in petroleum ether, b.p. 40–60°, and was eluted with benzene. Recrystallization from acetone gave needles, m.p. 181–182° (lit. m.p. 181.5–182.5° (17)).

Reaction of 17β-Hydroxy-5α-androstan-3-one with Diazomethane

Potassium hydroxide (14.2 g) followed by 17β-hydroxy-5α-androstan-3-one (10 g) was dissolved in methanol (400 ml) and anhydrous ether (300 ml). N-Nitrosomethylurea (10.6 g) was added in portions during 20 min to the stirred, cooled (0°) solution; after the addition was complete, stirring was continued for a further 5 h. Hydrochloric acid (200 ml, 2 *N*) was then added, the mixture filtered, and the solid material washed well with ether. The combined filtrate and ether washings were concentrated under reduced pressure to remove the organic solvents, and the resulting mixture was extracted with ether (3 × 75 ml). The ether solution was washed with water (50 ml), dried ($MgSO_4$), and evaporated. The residual solid (10.8 g) was chromatographed on alumina (300 g), and elution with benzene – 10% ether gave 17β-hydroxy-A-homo-5α-androstan-3-one (III, R = OH) (8.4 g). Repeated chromatography followed by recrystallization from acetone gave needles (5.1 g), m.p. 212–213°, $[\alpha]_D^{25}$ −57.1° (*c*, 0.7 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 3 600 (hydroxyl) and 1 695 cm^{-1} (seven membered ring carbonyl); nuclear magnetic resonance ($CDCl_3$) δ 0.73 and 0.80 (C_{18}, C_{19}, CH_3, singlets), 1.9 (OH, singlet), and 3.65 p.p.m. (C_{17}—α-H, triplet); o.r.d. (Fig. 2*a*) (*C*, 0.004 in CH_3OH) 24°; $[\phi]_{700}$ −166°; $[\phi]_{589}$ −227°; $[\phi]_{304}$ −4 489°; $[\phi]_{260}$ +4 599°; $[\phi]_{220}$ +444°.

Anal. Calcd. for $C_{20}H_{32}O_2$ (304.5): C, 78.90; H, 10.59. Found: C, 78.88; H, 10.51; mol. wt. (mass spectrum) 304.

A-Homo-5α-androstan-3,17-dione (III, R = =O)

17β-Hydroxy-A-homo-5α-androstan-3-one (450 mg) was dissolved in the minimum amount of acetone (60 ml), and 8 *N* chromic acid (Jones' reagent (18)) was added dropwise to the stirred, cooled (5°) solution until the solution just retained the brown-orange color of the chromic acid. After the mixture was stirred for a further 30 min at 5°, it was poured into water (600 ml) and extracted with ether (3 × 100 ml). The combined ether extracts were washed first with saturated sodium bicarbonate and then with water, and dried ($MgSO_4$). Evaporation of the ether gave a solid (450 mg) which, on recrystallization from hexane,

yielded A-homo-5α-androstan-3,17-dione as needles (430 mg), m.p. 112.5–113.5°, $[\alpha]_D^{25}$ 22.5° (*c*, 0.8 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1 735 (five membered ring carbonyl) and 1 698 cm^{-1} (seven membered ring carbonyl).

Anal. Calcd. for $C_{20}H_{30}O_2$ (302.5): C, 79.42; H, 9.99. Found: C, 79.20; H, 9.96; mol. wt. (mass spectrum) 302.

17β-Hydroxy-5β-androstan-3-one (VII)

17β-Hydroxy-5β-androstan-3-one was prepared in 95% yield by catalytic hydrogenation of 17β-hydroxy-androst-4-en-3-one in the presence of potassium hydroxide, based on the procedure described by Djerassi *et al.* (19). After purification by chromatography on alumina and recrystallization from cyclohexane, a product with m.p. 141–142° was obtained (lit. m.p. 139–141° (20)).

Reaction of 17β-Hydroxy-5β-androstan-3-one with Diazomethane

Potassium hydroxide (3.6 g) was added to 17β-hydroxy-5β-androstan-3-one (5 g) in methanol (200 ml) and anhydrous ether (150 ml), and the resulting solution was cooled to 0°. N-Nitrosomethylurea (2.7 g) was added, with stirring, during 20 min, and the cold mixture was stirred for a further 5 h at 0°. Hydrochloric acid (50 ml, 2 *N*) was then added, and the insoluble material filtered off and washed with ether. The combined filtrate and ether washings were evaporated under reduced pressure, and the residue was extracted with ether (3 × 50 ml). The ether solution was washed with water (50 ml), dried ($MgSO_4$), and evaporated, and the crude product (5.0 g) was then chromatographed on alumina (500 g). Elution with benzene – 1% ether gave 17β-hydroxy-A-homo-5β-androstan-4-one (VIII) (1.6 g) which, after repeated recrystallization from *n*-hexane, gave needles, m.p. 172.5–173°, $[\alpha]_D^{25}$ 12.7° (*c*, 0.8 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 3 597 and 3 436 (hydroxyl), and 1 695 cm^{-1} (seven membered ring carbonyl); o.r.d. (Fig. 2*b*) (*C*, 0.004 in CH_3OH) 24°; $[\phi]_{700}$ +1°; $[\phi]_{589}$ 44°; $[\phi]_{303}$ −1 932°; $[\phi]_{260}$ +4 049°; $[\phi]_{220}$ +3 660°.

Anal. Calcd. for $C_{20}H_{32}O_2$ (304.5): C, 78.90, H, 10.59. Found: C, 78.87; H, 10.46.

Subsequent elution with benzene – 10% ether gave 17β-hydroxy-A-homo-5β-androstan-3-one (IX) (1.6 g). Repeated recrystallization from *n*-hexane gave needles, m.p. 162–162.5° (lit. m.p. 156–157° (11)), $[\alpha]_D^{25}$ 84.8° (*c*, 0.6 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 3 597 and 3 436 (hydroxyl), and 1 698 cm^{-1} (seven membered ring carbonyl); o.r.d. (Fig. 2*b*) (*C*, 0.003 in CH_3OH) 24°; $[\phi]_{700}$ +150°; $[\phi]_{589}$ +177°; $[\phi]_{302}$ +4 160°; $[\phi]_{264}$ −2 314°; $[\phi]_{220}$ +362°.

Anal. Calcd. for $C_{20}H_{32}O_2$ (304.5): C, 78.90; H, 10.59. Found: C, 78.93; H, 10.43.

A-Homo-5β-androstan-4,17-dione (X)

17β-Hydroxy-A-homo-5β-androstan-4-one (150 mg) was dissolved in acetone (10 ml), and 8 *N* chromic acid (Jones' reagent (18)) was added dropwise, with stirring, to the cooled (ice bath) solution until a permanent brown color remained. After the solution was stirred for a further 30 min, it was poured into water (100 ml) and extracted with ether (3 × 50 ml). The ethereal extract was washed with saturated sodium bicarbonate and then with water. The dried ($MgSO_4$) ether solution was evaporated and the residue (150 mg) was recrystallized several times from petroleum ether (b.p. 40–60°) to give A-homo-5β-androstan-4,17-dione as needles (98 mg), m.p. 127–128°, $[\alpha]_D^{21}$ 85.7° (*c*, 0.25 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1 733 (five membered ring carbonyl) and 1 695 cm^{-1} (seven membered ring carbonyl).

Anal. Calcd. for $C_{20}H_{30}O_2$ (302.5): C, 79.42; H, 9.99. Found: C, 79.36; H, 9.95.

A-Homo-5β-androstan-3,17-dione (XI)

17β-Hydroxy-A-homo-5β-androstan-3-one (150 mg) was oxidized with Jones' reagent as described above to give a quantitative yield of A-homo-5β-androstan-3,17-dione, which, after several recrystallizations from petroleum ether (b.p. 40–60°), gave rhombs (94 mg), m.p. 80–81° (lit. m.p. 87–88° (11)), $[\alpha]_D^{24}$ 150.7° (*c*, 0.35 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1 736 (five membered ring carbonyl) and 1 698 cm^{-1} (seven membered ring carbonyl).

Anal. Calcd. for $C_{20}H_{30}O_2$ (302.5): C, 79.42; H, 9.99. Found: C, 79.53; H, 9.94.

ACKNOWLEDGMENTS

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