The Synthesis of 17β-(4(5)imidazolyl)-5α-androstane-3β,11β-diamine: a Water Soluble Steroid with a Potentially Catalytic Substituent

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Received June 12, 1972

The title compound, **9**, has been synthesized in an eight-step process, starting with corticosterone (**1**). The Weidenhagen reaction on **1** converted the 17-$COCH_2OH$ to an imidazole. Reduction with lithium–ammonia–methanol gave 17β-(4(5)-imidazolyl)-5α-androstane-3β, 11β-diol (**3**) which could be selectively oxidized to the 3β-ol, 11-one, which was then converted to the oxime and hydrogenated to the 3β-ol, 11β-amine, **6**. Oxidation of the 3β-ol to the 3-one, oximation and sodium–propanol reduction gave **9**.

Le produit **9** a été synthétisé en huit étapes en partant de la corticostérone (**1**). La réaction de Weidenhagen sur **1** convertit le $COCH_2OH$-17 en un imidazole. La réduction par le lithium–ammoniac–methanol conduit à l'(imidazolyl-4(5))-17β 5α-androstane diol-3β,11β (**3**), qui peut être oxydée sélectivement en ol-3β one-11, cette dernière étant transformée ensuite en oxime puis hydrogénée en ol-3β amine-11β **6**. L'oxydation du ol-3β en one-3, l'oximation et la réduction par le sodium dans le propanol donnent **9**.

[Traduit par le journal]

Canadian Journal of Chemistry, **50**, 3993 (1972)

Introduction

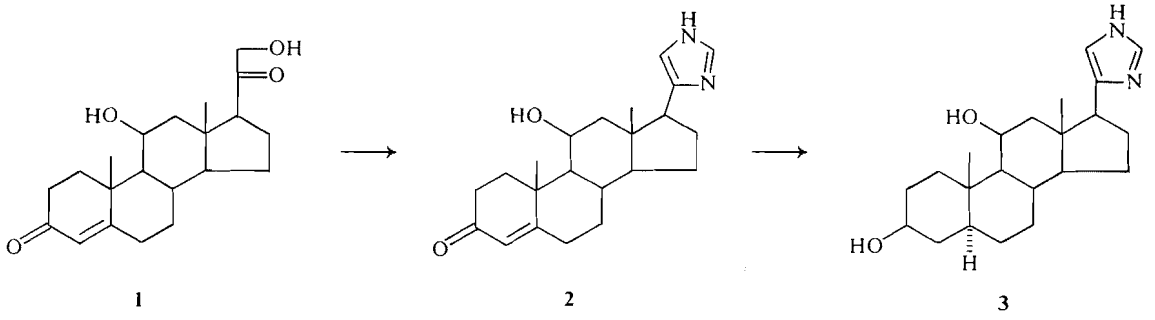
There has been considerable interest (1–13) in the study of enzyme models which show both substrate binding and catalytic properties. Such compounds can provide information bearing on two questions of crucial importance to the development of a full understanding of enzymic catalysis: (*1*) the requirements for non-covalent binding of substrate from aqueous solution (14); (*2*) the orientational requirements for efficient catalysis (15–19). The latter question has traditionally been investigated using intramolecular systems (15, 20, 21), but suffers from the limitation that if the reaction is very rapid, the reactive form of the compound must be generated in solution; already cases are known where even stopped flow techniques are barely adequate to follow the kinetics of the intramolecular reaction (22). Thus it would be preferable to study questions of orientation by looking at reactions of two compounds held together in a non-covalent complex, provided that complex formation was favorable, so that reaction occurred only within the complex, *and* provided that complex formation was specific enough that one could be assured of the orientation of the two molecules within the complex. This should be attainable if one uses relatively large, rigid molecules.

As a step towards the goals outlined above, I wish to report the preparation of 17β-(4(5)-imidazolyl)-5α-androstane-3β,11β-diamine (**9**), which was designed to be water-soluble at p*H* below 10, by virtue of the two amino groups, and to have a quasi-equatorial imidazolyl group, which can act as a catalyst for the hydrolysis of active esters. The steroid nucleus provides a large rigid framework, and has the additional advantage that one can draw upon the immense body of synthetic chemistry performed on steroids. With two ionic substituents, micelle formation should not occur (23). The α surface of **9** is unhindered, and available for hydrophobic binding.

Discussion

The approach used for the preparation of **9** is outlined in Schemes 1 and 2. Corticosterone (**1**) was smoothly converted in excellent yield into 11β-hydroxy-17β-(4(5)-imidazolyl)-4-androsten-3-one (**2**) using a procedure based on the imidazole synthesis devised by Weidenhagen and co-workers (24). The stereochemistry at C-5 was established by reduction of **2** using lithium–ammonia–methanol, to give the diol **3**. This reduction is expected to give the 5α-isomer; the stereochemistry was proved unambiguously by comparison of the ^{13}C n.m.r. spectrum (25) of the corresponding dione **10** with the ^{13}C spectra of the known compounds 5α- and 5β-pregnane-3,11,20-trione. The signal from the 19-methyl carbon is strikingly dependent on the stereochemistry at C-5 (25).

Oxidation of the 11β-hydroxyl was expected



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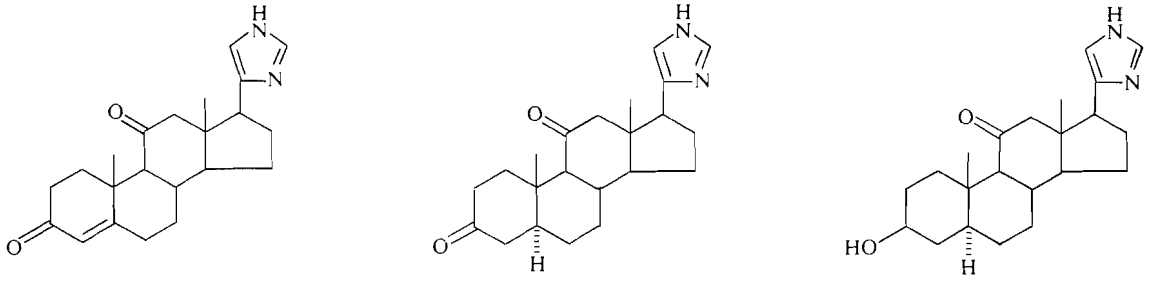
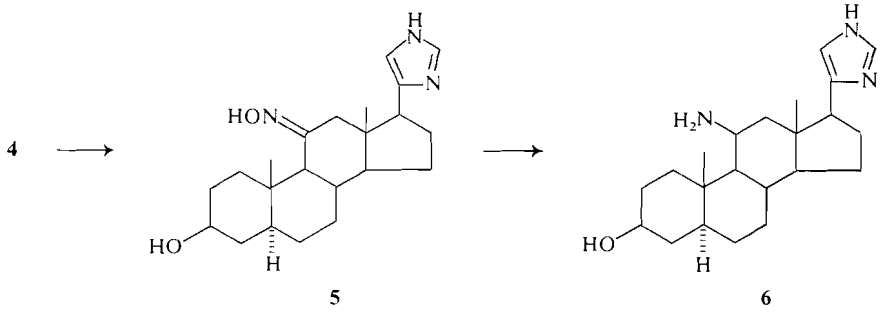


11 10 4

SCHEME 1



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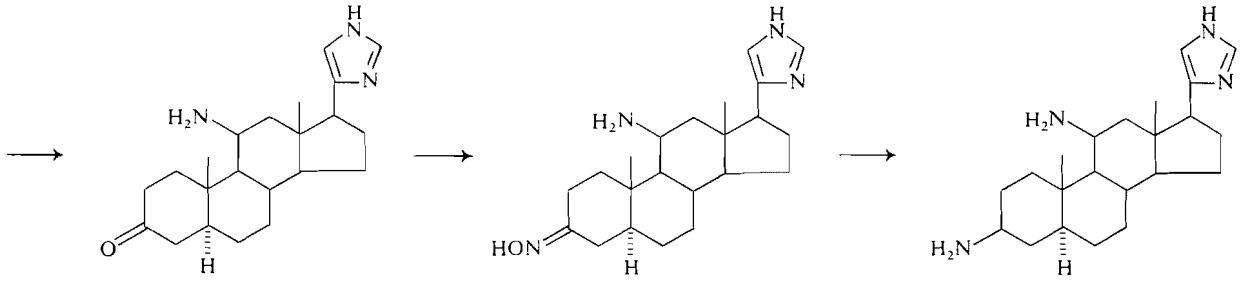

7 8 9

SCHEME 2

to be much faster than for the 3β-hydroxyl, on the basis of the kinetic studies of Rocek *et al.* (26). In fact, selective oxidation of **3** to **4** was readily achieved. Crude **4**, containing traces of **3** and **10** (t.l.c. analysis), was converted, via its oxime, **5**, to the 11β-amine by hydrogenation with PtO_2 in acetic acid. This hydrogenation proved to be very slow, requiring high pressure, prolonged reaction time, and large amounts of catalyst. However, the desired 11β-amino compound could be obtained in modest yield. Metal hydride reduction, which would also be expected to give the 11β-amine could not be employed because **5** was insoluble in solvents compatible with $LiAlH_4$. That the stereochemistry was indeed 11β is shown by the n.m.r. spectrum, since the methyne signal attributed to the hydrogen on C-11 has the width expected for an equatorial hydrogen.

Finally, the alcohol at C-3 was converted to an amino group, again by way of an oxime. However, since in this case the *equatorial* amine was desired, it was necessary to use sodium–propanol as the reducing agent. Ordinarily the sequence from **6** to **9** was carried out without purifying intermediates, and **9** was crystallized as the trishydrochloride. The n.m.r. spectrum showed a new signal at 3.20 δ, $\Delta\nu_{\frac{1}{2}} = 20$ Hz attributed to the C-3 proton with the width expected for the equatorial amino group.

The overall synthesis requires purifications at only three stages, **3**, **6**, and **9**. Further optimization is almost certainly possible; however the synthesis as reported here makes **9** available in gram quantities, by a route which gives complete control of stereochemistry. Compound **9** is indeed water soluble, to better than 0.1 *M*, at p*H* 8, where the imidazole group is free to act as a nucleophile.

Active esters of 3-(2-naphthyl)-propionic acid (**12**) and 3-(3-phenanthryl)-propionic acid (**13**) react more rapidly with **9** than with imidazole, and the rate enhancement is larger for derivatives of **13**, as would be expected, since the binding area is larger for **13**. Details of these studies of the catalytic properties of **9** will be published shortly.

Experimental

Materials and Methods

Mallinckrodt 2847 silicic acid was used for column chromatography and Camag silica gel was used for t.l.c. Proton n.m.r. spectra were recorded on a Varian HA100. ^{13}C n.m.r. spectra were recorded on a Varian XL100 with noise decoupling of protons; methyl groups were identified by recording off-resonance decoupled spectra. Imidazole-containing compounds were detected using diazotized sulfanilic acid (Pauly reagent (27)).

11-Hydroxy-17β-(4(5)-imidazolyl)-4-androsten-3-one (2)

Corticosterone (**1**) (Sigma Chemical Corp., m.p. 180–183°) (10 g, 28.9 mmol) was dissolved in 800 ml warm 95% ethanol; to this was added 50 ml formalin (Shawinigan, 37% CH_2O) and a solution of 12 g $Cu(OAc)_2 \cdot H_2O$ (60 mmol) in 30 ml water plus 174 ml concentrated ammonia. The solution was refluxed for 3 h, at which time the solution had turned from blue to green, and t.l.c. (80:20, $CHCl_3$:CH_3OH) showed that the reaction was over. The reaction mixture was allowed to cool and then worked-up by partitioning between saturated aqueous sodium chloride and ethyl acetate. The combined ethyl acetate extracts (total volume about 2 l) were dried over Na_2SO_4, and then evaporated to dryness. The residue was extracted with 46 ml of hot methanol, made alkaline with ammonia, and brought to saturation with water. The first crop was 5.44 g. The residue was extracted with methanol till no more Pauly positive material dissolved. These extracts and the crystallization mother liquors were pooled and evaporated. Similarly using 23 ml of hot methanol a second crop of 2.11 g was obtained. The process was repeated to give 1.89 g in the third crop. Total yield of crude product: 9.43 g (92%). Recrystallization from methanol–water (with a few drops of concentrated ammonia added) gave material of m.p. 177° (dec.); n.m.r. (as HCl salt) δ (D_2O), 0.76 (C-18 methyl), 1.37 (C-19 methyl), 4.30 ($\Delta\nu_{\frac{1}{2}} = 9$ Hz, C-11 methine), 5.58 (singlet, —C*H*=C), 7.14, 8.46 (C—H protons of the imidazolium ring).

Anal. Calcd. for $C_{22}H_{30}N_2O_2 \cdot CH_3OH$: C, 71.46; H, 8.86; N, 7.24. Found: C, 71.33; H, 8.78; N, 7.32.

17β-(4(5)-Imidazolyl)-5α-androstane-3β,11β diol (3)

A solution of **2** (9 g, 25.4 mmol) in 100 ml methanol was added to 1000 ml of liquid ammonia (giving a turbid suspension). To the stirred suspension was added in portions over 2 h, 15 g lithium metal. The solution was allowed to evaporate overnight under nitrogen. The residue was partitioned between ethyl acetate and saturated aqueous sodium chloride, and the combined ethyl acetate extracts were evaporated to dryness. The residue was dissolved in 250 ml of methanol, cooled, and filtered. Repeated concentration of filtrates led to further crops, giving a total of 4.9 g, 13.8 mmol, 54%. Chromatography of the mother liquors on silicic acid, and crystallization of fractions rich in **3** gave a further 0.583 g, making a total of 5.52 g or 61%. Recrystallization of **3** from methanol–water–ammonia gave material of m.p. 263.5–267.5°; n.m.r. (as HCl salt) δ (D_2O): 0.67 (C-18 methyl), 0.94 (C-19 methyl), 3.50 ($\Delta\nu_{\frac{1}{2}} = 21$ Hz, C-3 methine), 4.25 ($\Delta\nu_{\frac{1}{2}} = 8$ Hz, C-11 methine), 7.11, 8.40 (C—H protons of the imidazolium ring).

Anal. Calcd. for $C_{22}H_{34}N_2O_2$: C, 73.71; H, 9.55; N, 7.82. Found: C, 73.67; H, 9.45; N, 7.72.

3β-Hydroxy-17β-(4(5)-imidazolyl)-5α-androstan-11-one (4)

Crude **3**, (4.50 g, 12.5 mmol) was dissolved in a mixture of 23 ml water and 5.7 ml 6 *N* H_2SO_4. To this was added

225 ml glacial acetic acid. The solution was stirred in an ice bath and 4.3 ml of Jones reagent (2 *M* in CrO_3, 3.3 *M* in H_2SO_4) was added. After 10 min stirring, analytical t.l.c. (80:20 $CHCl_3$:CH_3OH) showed that only traces of starting material remained. Sodium acetate, 4.5 g, in methanol was added to neutralize the excess mineral acid, and the solution was evaporated to dryness. The residue was taken up in water, basified with 50 ml concentrated ammonia, and extracted with ethyl acetate. The extracts were washed with water and the aqueous wash solution was extracted with ethyl acetate. Evaporation of the combined ethyl acetate fractions gave 3.72 g of crude product (10.4 mmol, 84%). Recrystallization of **4** from methanol–water–ammonia gave material of m.p. 301–303°; n.m.r. (as HCl salt) δ (D_2O): 0.41 (C-18, methyl), 0.89 (C-19 methyl); 3.48 ($\Delta\nu_{\frac{1}{2}} = 25$ Hz, C-3 methine), 7.13, 8.40 (C—H protons of the imidazolium ring).

Anal. Calcd. for $C_{22}H_{32}N_2O_2$: C, 74.12; H, 9.05; N, 7.86. Found: C, 74.21; H, 8.88; N, 7.96.

*17β-(4(5)-Imidazolyl)-11-oximido-5α-androstan-3β-ol (**5**)*

Crude **4** (3.73 g, 10.5 mmol) and hydroxylamine hydrochloride, 3.7 g, in refluxing anhydrous pyridine, 25 ml, were allowed to react for 24 h. The reaction mixture was cooled, and poured into 50 ml of water; 1 *N* sodium hydroxide solution was added until the p*H* was 12. The resulting suspension was stirred until the product solidified to a fine powder, and then filtered; yield 3.64 g, 93%.

*11β-Amino-17β-(4(5)-imidazolyl)-5α-androstan-3β-ol (**6**)*

Crude **5**, 5.68 g, in glacial acetic acid, 100 ml, with 5.2 ml of 3 *N* HCl and 2.0 g PtO_2 was hydrogenated at 800 p.s.i. for 48 h. The hydrogenation was twice interrupted and 1.0 g PtO_2 and 2.6 ml 3 *N* HCl were added; after each addition hydrogenation was continued at 1800 p.s.i. for 48 h. After filtering off the catalyst, the solution was evaporated to dryness, taken up in methanol, and adsorbed into 10 g of silicic acid. This material was placed on a column of 60 g of silicic acid, poured in ether. The column was developed with 20% methanol in ether, then 20% 1 *M* ammonia in methanol – 80% ether, then 30% methanolic ammonia, then 40%, finally 100% 1 *M* ammonia in methanol. Elution was followed by t.l.c. Three major bands eluted: 2.50 g of a by-product of unknown constitution, 2.98 g of **6**, and 0.55 g of a more polar material. The n.m.r. of **6** (as HCl salt) δ (D_2O): 0.72 (C-18 methyl), 0.98 (C-19 methyl), 3.18 ($\Delta\nu_{\frac{1}{2}} = 18$ Hz, C-3 methine), 4.02 ($\Delta\nu_{\frac{1}{2}} = 10$ Hz, C-11 methine), 7.34, 8.64 (C—H protons of the imidazolium ring).

*11β-Amino-17β-(4(5)-imidazolyl)-5α-androstan-3-one (**7**)*

Chromatographically purified **6** (0.400 g) was dissolved in 40 ml 6 *N* H_2SO_4. To the stirred solution was added 0.35 ml Jones reagent. After 1 h, an analytical t.l.c. was run to check on the extent of reaction; an appropriate amount of Jones reagent was added and the process was repeated. Reaction was stopped when the ratio of 3-keto to 3-hydroxy-steroid was about 10:1 (attempting to carry the reaction to completion incurs excessive loss of material through over-oxidation). The solution was transferred to a separatory funnel, and basified by the addition of 25 ml of concentrated ammonia solution. Ice was added to cool the solution, which now showed the pink color of ammonia Cr(III) complexes. The solution was extracted four times with 100 ml portions of ethyl acetate; the organic layers were filtered and evaporated to dryness, giving 0.261 g, 67%. Compound **7** was converted to its hydrochloride salt and crystallized from methanol – *n*-propanol; n.m.r. (as HCl salt) δ (D_2O): 0.71 (C-18 methyl), 1.15 (C-19 methyl), 3.94 ($\Delta\nu_{\frac{1}{2}} = 11$ Hz, C-11 methine), 7.13, 8.34 (C—H protons of the imidazolium ring).

Anal. Calcd. for $C_{22}H_{35}N_3OCl_2 \cdot H_2O$: C, 59.18; H, 8.35; N, 9.41. Found: C, 58.87; H, 8.20; N, 9.31.

*17β-(4(5)-Imidazolyl)-3-oximido-5α-androstan-11β-amine (**8**)*

Crude **7** (0.742 g, 2.09 mmol) and 0.75 g of hydroxylamine hydrochloride in 7 ml dry pyridine were heated at *ca.* 90° for 1 h, cooled, poured into 21 g of ice, basified with sodium hydroxide (to p*H* 12), and extracted with ethyl acetate; yield, 0.707 g, 91%.

*17β-(4(5)-Imidazolyl)-5α-androstane-3β,11β-diamine (**9**)*

Crude **8**, 1.16 g, was dissolved in 140 ml refluxing *n*-propanol, under nitrogen. Sodium metal, 4.5 g, was added in portions. After all the sodium was consumed, t.l.c. showed no remaining **8**. The solution was cooled, concentrated to 50 ml, and then poured into 100 ml half-saturated aqueous sodium chloride solution. This solution was extracted with four portions of ethyl acetate and the combined organic layers were washed with saturated sodium chloride solution, filtered and evaporated to give 1.13 g of crude product. This crude material was converted to its hydrochloride which was crystallized from methanol – *n*-propanol to give 0.674 g of trishydrochloride. A second crop, 0.128 g, was obtained from the mother liquor. The yield of crystallized product was 55%; n.m.r. (as HCl salt) δ (D_2O): 0.73 (C-18 methyl), 0.98 (C-19 methyl), 3.20 ($\Delta\nu_{\frac{1}{2}} = 20$ Hz, C-3 methine), 4.00 ($\Delta\nu_{\frac{1}{2}} = 10$ Hz, C-11 methine), 7.40, 8.71 (C—H protons on imidazolium ring).

Anal. Calcd. for $C_{22}H_{39}N_4Cl_3 \cdot H_2O$: C, 54.60; H, 8.54; N, 11.58; Cl, 21.98. Found: C, 54.73; H, 8.76; N, 11.63; Cl, 22.88.

*17β-(4(5)-Imidazolyl)-5α-androstane-3,11-dione (**10**)*

Crude **3** from lithium–ammonia–methanol reduction of 1 g of **2** was oxidized with excess Jones reagent in 90% acetic acid. The solution was treated with water and concentrated ammonia and then extracted with ethyl acetate. The organic layer was extracted four times with 1 *M* HCl, the aqueous extracts were basified with concentrated NH_3, and then extracted five times with ethyl acetate. Evaporation of the combined ethyl acetate extracts gave 619 mg of crude product, m.p. 230–233°. Two recrystallizations from methanol–water gave material with m.p. 232.5–235°; n.m.r. (on HCl salt) δ (D_2O): 0.46 (C-18 methyl), 1.14 (C-19 methyl), 7.30, 8.61 (C—H protons of the imidazolium ring). ^{13}C n.m.r., δ (pyridine): 11.2, C-19; 14.2, C-18.

Anal. Calcd. for $C_{22}H_{30}N_2O_2$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.35; H, 8.54; N, 7.89.

*17β-(4(5)-Imidazolyl)-4-androstene-3,11-dione (**11**)*

Compound **2** (2 g, 5.7 mmol) in a mixture of 10 ml H_2O, 1.9 ml 3 *M* HCl and 50 ml acetic acid was cooled to 6° and treated with 2.9 ml Jones reagent in two portions. After 10 min 1 ml of isopropanol was added to destroy excess oxidant. The reaction mixture was poured into 500 ml of water, neutralized with Na_2CO_3, and extracted repeatedly with methylene chloride. The combined extracts were

evaporated to dryness and crystallized from hot methanol, giving 0.929 g of product. After recrystallization from methanol–water–ammonia, this material had m.p. 152–155° (dec.); n.m.r. (on HCl salt) δ (D_2O): 0.47 (C-18 methyl), 1.32 (C-19 methyl), 5.67 (—C*H*=C, C-4), 7.14, 8.40 (C—H protons of the imidazolium ring). ^{13}C n.m.r., δ (pyridine): 17.2, C-19; 14.2, C-18.

Anal. Calcd. for $C_{22}H_{28}N_2O_2 \cdot CH_3OH$: C, 71.89; H, 8.38; N, 7.34. Found: C, 71.53; H, 8.41; N, 7.41.

5α- and 5β-Pregnane-3,11,20-triones

These compounds were prepared from 4-pregnene-3,11,20-trione (Sigma) by hydrogenation and separated by crystallization following the procedure of Schmitt *et al.* (28). 5α-pregnane-3,11,20-trione had m.p. 208–215° (lit. m.p. 212–216° (29)); ^{13}C n.m.r., δ (pyridine): 11.1 (C-19) and 14.3 (C-18) p.p.m. 5β-Pregnane-3,11,20-trione had m.p. 155–156° (lit. m.p. 158–160° (30)); ^{13}C n.m.r., δ (pyridine): 14.3 (C-18) and 22.5 (C-19) p.p.m.

Financial support from the National Research Council of Canada, and the University Research Council of the University of Western Ontario is gratefully acknowledged. I wish to thank Dr. J. B. Stothers of this Department for the ^{13}C n.m.r. spectra.

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