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Synthesis and Stereochemistry of the Metabolites of 2α-Cyano-4α,5α-epoxy-17β-hydroxyandrostan-3-one

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The preparation of the metabolites of 2α-cyano-4α,5α-epoxy-17β-hydroxyandrostan-3-one (trilostane) is described, including a novel oxidation with pyridinium chlorochromate.

Pyridinium chlorochromate oxidation of trilostane gave the α,β-unsaturated cyano-ketone (**2**), which was hydrogenated to yield metabolite **M-1** (**3**). The compound **3** was converted to the cyano-acetates (**11**), (**12**), and (**13**) and their stereochemistries were established by nuclear magnetic resonance analysis.

The cyano-acetate (**11**) was transformed into **M-3** triacetate *via* **M-2** diacetate by successive enol acetylation, epoxidation, acid-catalyzed rearrangement, reduction, and acetylation. The metabolites **M-2** and **M-3** were established to have the structures 2α-cyano-3α,16α-dihydroxy-4α,5α-epoxyandrostan-17-one and 2α-cyano-4α,5α-epoxy-3α,16α,-17β-trihydroxyandrostane, respectively.

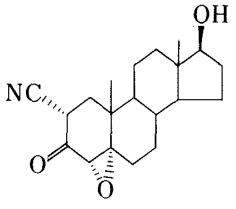
Keywords——2α-cyano-4α,5α-epoxyandrostan-3-one; adrenal steroidogenesis inhibitor; synthesis of metabolites; pyridinium chlorochromate; NMR

Trilostane, 2α-cyano-4α,5α-epoxy-17β-hydroxyandrostan-3-one (**1**), is one of the androstanes first sythesized by Neumann *et al.*[1], and shows inhibition of adrenal steroidogenesis. The drug has a novel structure in which cyano, carbonyl, and epoxy groups are assembled on the steroid A-ring.

In the preceding paper,[2] we reported the metabolism of trilostane in rats and the isolation of five metabolites, three of which were found to be 3,17-dioxo (**M-1**), 3,16-dihydroxy-17-oxo (**M-2**), and 3,16,17-trihydroxy (**M-3**) derivatives of trilostane on the basis of their infrared (IR), nuclear magnetic resonance (NMR), and mass spectra. The stereochemical relationships of substituents of the me tabolites have, however, remained ambiguous.

On the other hand, the experiments to investigate the metabolism required trilostane labeled with deuterium, which is important in the detection of metabolites by use of the ion cluster technique[3] of gas chromatography–mass spectrometry (GC–MS).

The present paper describes the synthesis of trilostane metabolites to establish their stereochemistries. During the course of this investigation, we have found an unusual pyridinium chlorochromate oxidation of an α-cyano-ketone to generate the corresponding α,β-unsaturated compound. This oxidation behavior was effectively applied to the preparation of deuterium-labeled trilostane.



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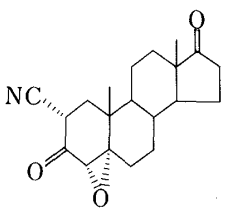
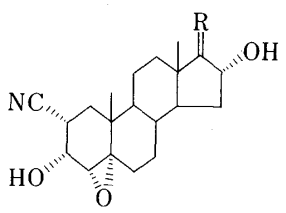


M−1



M−2: R=O

M−3: R=$\overset{OH}{H}$

Chart 1

Our initial study was directed to the preparation of **M-1**, as a first intermediate leading to the other metabolites.

Oxidation of trilostane (**1**) to **M-1** with Jones reagent[4] was attempted at first to give a mixture of products. Extensive chromatography gave **M-1** in very low yield, as well as a mixture of highly polar substances which were not identified. Oxidation of **1** with 4 eq. of pyridinium chlorochromate[5] in dichloromethane–pyridine (1 : 1) yielded an unanticipated compound (**2**) as a sole product in 40% yield. The structure of **2** was unequivocally established on the basis of spectral data. The presence of the unexpected conjugated carbonyl group was inferred from the IR spectrum, which showed a band at 1700 cm^{-1}, as well as absorption at 1730 cm^{-1} due to a five-membered ketone. The NMR spectrum of **2** indicated the presence of one olefinic proton (C-1-H) at δ 7.75 as a singlet and one methine proton (C-4-H) on the epoxide ring at δ 3.65 (singlet).

Pyridinium chlorochromate has been used as a mild oxidizing reagent, compatible with a variety of acid-sensitive functional groups. In addition, a novel oxidative rearrangement was found in the work of Dauben[6] and Herz,[7] who demonstrated independently that oxidation of tertiary and some secondary allylic alcohols with pyridinium chlorochromate led to the formation of the transposed α,β-unsaturated carbonyl compounds. However, the formation of an α-cyano-α,β-unsaturated ketone from an α-cyano-ketone by pyridinium chlorochromate oxidation is unprecedented. This reaction is a new type of oxidation of Corey's reagent.[5] Further work to determine the scope and limitations of this oxidation will be necessary.

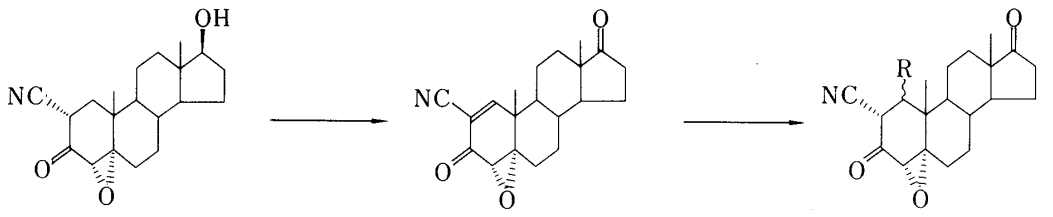


Chart 2

Catalytic hydrogenation of **2** in the presence of 5% Pd–C gave a single product **3**, mp above 300°C, which was found to be identical with the metabolite **M-1**.

The cyano group at C-2 was anticipated to prefer the more stable α-configuration, because the carbonyl group at C-3 existed in both keto and enol forms as deduced from the IR absorptions of the cyano group of **M-1** at 2240 and 2180 cm^{-1}, respectively. When catalytic hydrogenation was carried out under deuterium, the C-1-deuterium derivative (**4**) was obtained. Subsequent enol acetylation, sodium borohydride reduction, and deacetylation of **4** afforded trilostane-1-d_1, which was used in the metabolic study of trilostane (**1**) in rats.[2] Inspection of the molecular ion in the mass spectrum indicated that the isotopic purity of the deuterium-labeled **1** was 93%.

The next object was to convert compound **3** to the metabolite **M-2**. Acetylation of **3** with acetic anhydride in pyridine gave the enol acetate (**5**). As mentioned above, since the carbonyl group at C-3 was much more enolizable than that at C-17 in compound **3**, only the C-3 carbonyl group could be enol acetylated. The enol acetate was converted to the ketal-acetate (**6**) by treatment with ethylene glycol and catalytic amounts of *p*-toluenesulfonic acid in refluxing benzene in good yield. Removal of the acetyl group with sodium methoxide in methanol afforded an epimeric mixture of the keto-ketal (**7**).

Sodium borohydride reduction of **7** did take place at both sides of the C-3 carbonyl group to give an epimeric mixture of products, which were separated by preparative thin-layer

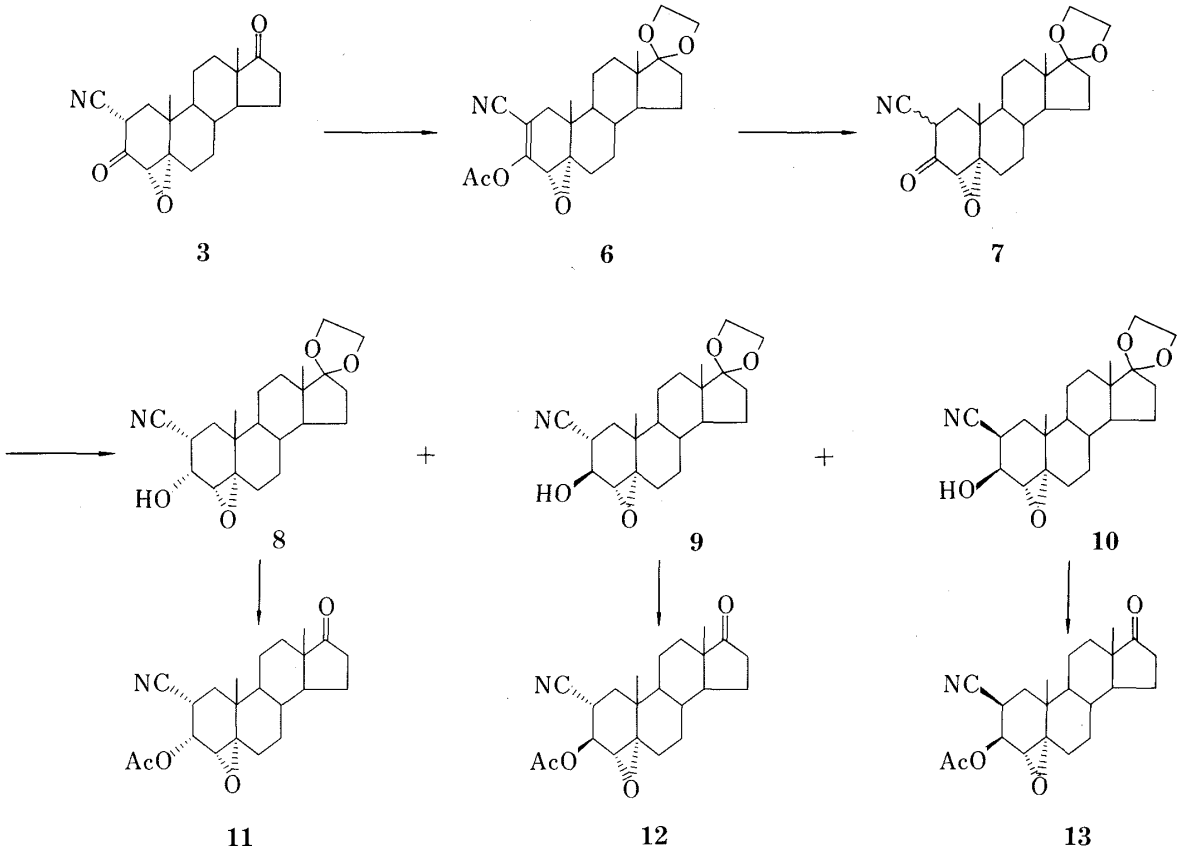


Chart 3

Table I. The PMR Spectral Data of the Keto-acetates (**11**), (**12**), and (**13**), and **M-2** Diacetate (**M-2-Ac**)

Compound	Chemical shift (δ)					Coupling constant (Hz)		
	C-2	C-3	C-4	C-18	C-19	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$
M-2-Ac	2.90	5.32	3.34	0.98	1.12	13, 7.0	6.0	4.0
11	2.98	5.35	3.33	0.90	1.14	12, 4.5	6.0	4.5
12	2.75	5.12	2.95	0.90	1.20	10, 2.5	10.0	0
13	3.45	5.16	3.06	0.90	1.42	5.7, 4.0	8.0	0

chromatography (TLC) into three hydroxy-nitriles (**8**), (**9**), and (**10**) in a ratio of 5: 1: 2. No other isomer could be isolated.

The NMR spectra of the products were too similar to be differentiated. Accordingly, the products (**8**), (**9**), and (**10**) were further transformed into the cyano-acetates (**11**), (**12**), and (**13**), respectively, by acetylation followed by deketalization. The stereochemistries of the cyano-acetates were deduced from a detailed analysis of their NMR spectral data, summarized in Table I.

The NMR spectrum of **11** showed a signal due to the C-4-proton at δ 3.33 as a doublet (J=4.5 Hz), while the signals due to the C-4-protons of **12** and **13** appeared at δ 2.95 and 3.06 as singlets.

As shown in Chart 4, the configuration of C-4-H was determined by the use of molecular models of **12** and **13**, which indicate that the dihedral angle between C-3-H and C-4-H in ring A is nearly 95°C, suggesting that $J_{3,4}$ is 0 Hz.[8] From this finding, it is apparent that the configuration of the acetoxyl groups in **12** and **13** is β-equatorial, while that in **11** is therefore α-

axial. The configuration at C-2-H of **11**, **12**, and **13** was assigned as follows. In the NMR spectrum of **11**, the signal for C-2-H (δ 2.98) appeared as an octet, $J_{1,2}$=12 and 4.5 Hz and $J_{2,3}$=6 Hz, which indicates that the C-2-cyano group is in an equatorial position and that the C-3-acetoxyl group is pseudo-axial. The configuration at C-2 of the isomers (**12**) and (**13**) follows from a comparison of the chemical shift for the C-19-methyl group in **12** (δ 1.20) and **13** (δ 1.42); the downfield shift of the angular methyl resonance in **13** relative to that in **12** must be an effect of deshielding by the axial cyano group. This deshielding behavior is evidence for a 1,3-diaxial methyl-cyano interation.[9] Therefore, the configuration of C-2-CN of **12** is assigned as equatorial and that of **13** as axial.

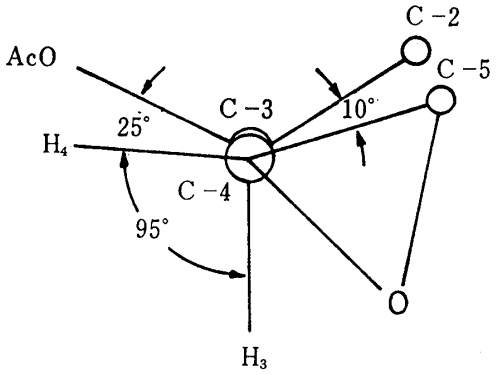


Chart 4

Having assigned the configurations of the cyano and acetoxyl groups of **11**, **12**, and **13**, we turned our attention to the introduction of a C-16-hydroxyl group. As shown in Table I, the NMR spectral data of **11** are so similar to those of **M-2** diacetate (**M-2-Ac**) that we anticipated that only the compound (**11**) could be transformed to **M-2**.

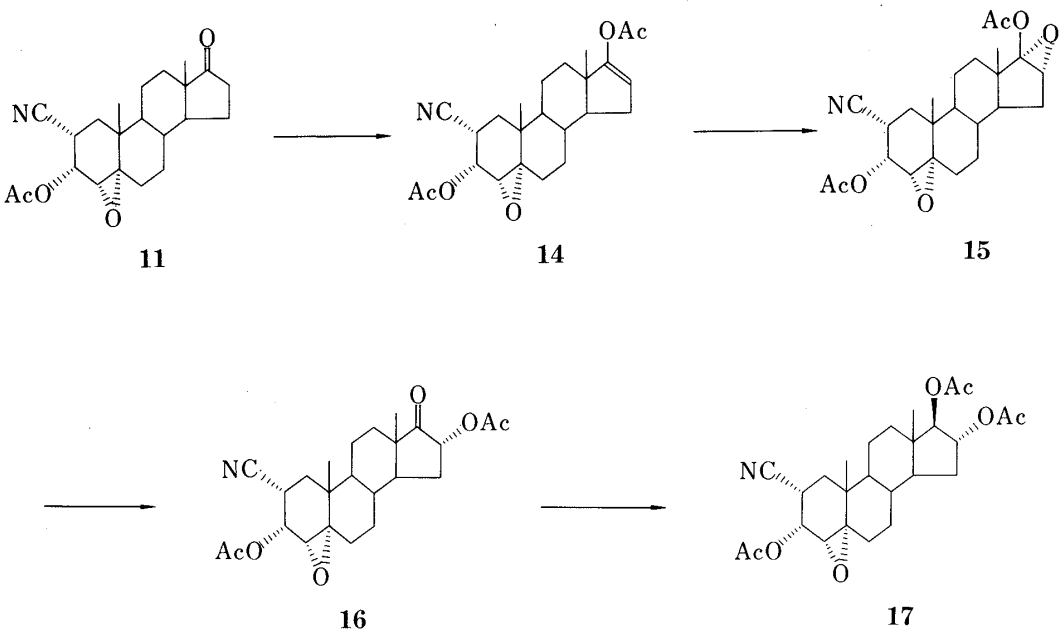


Chart 5

The cyano-acetate (**11**) was converted to the enol acetate (**14**) by refluxing it with isopropenyl acetate in the presence of a catalytic amount of *p*-toluenesulfonic acid. Epoxidation of the enol acetate with *m*-chloroperbenzoic acid gave the 16α,17α-epoxide (**15**). When the epoxide (**15**) was treated with acid, a rearrangement of **15** occurred with opening of the epoxide ring and migration of the C-17-acetyl group to yield the 16α-acetoxy-17-oxo derivative (**16**) with a small amount of the 16α-hydroxy-17-oxo derivative. Acetylation of the crude rearranged products afforded the keto-acetate (**16**) which was identical with a sample of the metabolite **M-2** diacetate.

Reduction of the keto-acetate (**16**) with sodium borohydride followed by acetylation yielded the 3α,16α,17β-triacetate (**17**). The NMR, TLC, and GC–MS data of **17** were identical

with those of the metabolite **M-3** triacetate.

Since the stereochemical outcome of epoxidation of the enol acetate (**14**) followed by rearrangement and reduction was well-established,[10] the configuration of the C-16-acetoxyl-group of **16** is α and that of the C-17-acetoxylgroup of **17** is β.

On the basis of the experiments described above, it was unambiguously determined that the structures of the metabolites **M-2** and **M-3** are 2α-cyano-3α,16α-dihydroxy-4α,5α-epoxy-androstan-17-one and 2α-cyano-4α,5α-epoxy-3α,16α,17β-trihydroxyandrostane, respectively.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 215 spectrometer in $CHCl_3$. Unless otherwise specified, NMR spectra were measured on a Hitachi R-24 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. The abbreviations s, d, t, q, and m signify singlet, doublet, triplet, quartet, and multiplet in the NMR spectra and the coupling constant (J) is given in Hz. Mass spectra (MS) were recorded at 70 eV on a Hitachi RMU-6E mass spectrometer and the abbreviation M^+ signifies a molecular ion. Column chromatographies were performed on silica gel (Mallinckrodt silicic acid, 100 mesh). Silica gel 60 GF_{254} (Merck) was used for thin-layer chromatography (TLC) and silica gel 60 PF_{254} (Merck) for preparative TLC. Unless otherwise mentioned, the extracts were dried over anhydrous magnesium sulfate.

2-Cyano-4α,5α-epoxyandrost-1-ene-3,17-dione (**2**)——A solution of 100 mg of trilostane (**1**) in 5 ml of pyridine was added to a stirred suspension of 500 mg of pyridinium chlorochromate in 5 ml of CH_2Cl_2. The mixture was stirred for 2.5 h at 25°C, then EtOAc (50 ml) and ether (20 ml) were added. The mixture was filtered through Celite and the precipitate was washed with EtOAc–ether (1: 1). The filtrate and washings were combined, washed with water, dried and concentrated to give 40 mg of a crystalline solid. Recrystallization of the solid from $CHCl_3$–ether (1: 1) gave 25 mg of the α,β-unsaturated ketone (**2**) as colorless needles, mp above 300°C. IR ν_{max} cm^{-1}: 2230 (CN), 1730 (CO), 1700 (CO). NMR δ: 0.83 (3H, s, CH_3), 1.14 (3H, s, CH_3), 3.56 (1H, s, C_4–H), 7.75 (1H, s, C_1–H). MS m/z: 325 (M^+), 41 (base peak). *Anal.* Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.61; H, 7.07; N, 4.26.

2α-Cyano-4α,5α-epoxyandrostane-3,17-dione (**3**)——A solution of 30 mg of **2** in 20 ml of EtOAc was catalytically hydrogenated over 20 mg of 5% Pd–C at room temperature and atmospheric pressure for 2 h. The catalyst was filtered off and filtrate was evaporated to dryness under reduced pressure to leave 30 mg of a crystalline solid. Recrystallization of the solid from $CHCl_3$–Et_2O (1: 5) gave 20 mg of the diketone (**3**) as colorless prisms, mp above 300°C. IR ν_{max} cm^{-1}: 2180 (CN), 1723 (CO). NMR δ: 0.89 (3H, s, CH_3) 0.94 (3H, s, CH_3), 3.53 (1H, s, C_4–H). MS m/z: 327 (M^+), 79 (base peak). The IR, NMR, and mass spectra of the diketone (**3**) were identical with those of metabolite **M-1**.[3]

1-Deuterio-diketone (**4**) **and 1-Deuterio-trilostane**——In the same way as described for **3**, 200 mg of **2** was hydrogenated under a deuterium atmosphere to give 190 mg of the compound (**4**) as colorless prisms, mp 237°C. MS m/z: 328 (M^+) (95.5% d_1).

A mixture of **4** (190 mg), acetic anhydride (1 ml), and pyridine (1 ml) was allowed to stand overnight at room temperature. The reaction mixture was extracted with EtOAc. The extract was washed successively with 2% HCl, 5% $NaHCO_3$, and water, dried and evaporated to dryness to leave 220 mg of the acetate. Sodium borohydride (16 mg) was added to a solution of the acetate in MeOH (7 ml) and tetrahydrofuran (THF) (1 ml). After being stirred for 1 h at room temperature, the mixture was extracted with EtOAc. The extract was washed with water, dried and evaporated to dryness to leave a crystalline residue. A solution of the residue in MeOH (2 ml) was treated with 1 N NaOH (0.1 ml). The mixture was stirred for 1 h, made acidic with 2% HCl, and extracted with EtOAc. The extract was washed with water, dried and concentrated. The residue was purified by preparative TLC (10% MeOH–$CHCl_3$) to give 136 mg of 1-deuterio-trilostane as a colorless amorphous substance, mp 245—249°C. MS m/z: 330 (M^+) (93.5% d_1).

3-Acetoxy-2-cyano-4α,5α-epoxyandrost-2-en-17-one (**5**)——A mixture of 480 mg of the diketone (**3**), acetic anhydride (1 ml), and pyridine (1 ml) was allowed to stand overnight at room temperature. The mixture was quenched with water and extracted with EtOAc. The extract was washed with 2% HCl, 5% $NaHCO_3$, and water, then dried and concentrated to leave a crystalline residue. Recrystallization of the residue from Et_2O–hexane (3: 1) gave 437 mg of the enol acetate (**5**) as colorless plates, mp 174—177°C. IR ν_{max} cm^{-1}: 2225 (CN), 1775 (CO), 1735 (CO), 1665, 1190. NMR δ: 0.90 (3H, s, CH_3), 1.06 (3H, s, CH_3), 2.29 (3H, s, $COCH_3$), 3.20 (1H, s, C_4–H). MS m/z: 369 (M^+), 43 (base peak). *Anal.* Calcd for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.47; H, 7.44; N, 3.73.

The Ketal-acetate (**6**)——A solution of 520 mg of the enol acetate (**5**) in 70 ml of dry benzene was treated with 1 ml of ethylene glycol and 5 mg of p-toluenesulfonic acid. The mixture was refluxed for 7 h while water was azeotropically removed with a Dean-Stark apparatus. After cooling, the solvent was evaporated off under reduced pressure. The residue was made alkaline with 5% $NaHCO_3$ and extracted with EtOAc. The extract was washed with water, dried and concentrated to leave a crystalline solid. Recrystallization

of the solid from $CHCl_3$–Et_2O–hexane (1: 3: 1) gave 400 mg of the ketal-acetate (**6**) as colorless plates, mp 178—179°C. IR ν_{max} cm^{-1}: 2228 (CN), 1775 (CO), 1664, 1190. NMR δ: 0.87 (3H, s, CH_3), 1.03 (3H, s, CH_3), 2.28 (3H, s, $COCH_3$), 3.17 (1H, s, C_4–H), 3.88 (4H, s, OCH_2CH_2O). MS *m/z*: 413 (M^+), 99 (base peak). *Anal.* Calcd for $C_{24}H_{31}NO_5$: C, 69.71; H, 7.56; N, 3.39. Found: C, 69.42; H, 7.59; N, 3.36.

The Keto-ketal (**7**)——A solution of 0.54 ml of 2 M NaOMe in MeOH was added to a solution of 405 mg of the ketal-acetate (**6**) in 22 ml of dry MeOH. The mixture was stirred for 3.8 h at room temperature, then the solvent was removed under reduced pressure. The residue was made neutral with 2% HCl and extracted with EtOAc. The extract was washed with water, dried and evaporated to dryness to leave 359 mg of the keto-ketal (**7**). IR ν_{max} cm^{-1}: 2200 (CN), 1715 (CO). MS *m/z*: 371 (M^+), 99 (base peak). This compound was used for the next reaction without further purification, because **7** was easily deprotected during purification.

The Hydroxy-nitriles (**8**), (**9**), **and** (**10**)——A solution of 359 mg of the keto-ketal (**7**) in 17 ml of MeOH was treated with 28 mg of $NaBH_4$ at 0° under stirring. The mixture was stirred for 3.7 h then concentrated under reduced pressure. The residue was extracted with EtOAc and the extract was washed with water, dried and evaporated to dryness to leave 400 mg of a crystalline residue. The residue was purified by preparative TLC (1% MeOH–$CHCl_3$, four developments) to give three products (A; 128 mg), (B; 25 mg), and (C; 50 mg).

Recrystallization of A from $CHCl_3$–Et_2O (1: 5) gave 104 mg of the hydroxy-nitrile (**8**) as colorless plates, mp 296—299°C. IR ν_{max} cm^{-1}: 3540 (OH), 2240 (CN). NMR δ: 0.86 (3H, s, CH_3), 1.07 (3H, s, CH_3), 2.74 (1H, d, J=11 Hz, OH), 2.77 (1H, m, C_2–H), 3.25 (1H, d, J=5 Hz, C_4–H), 3.89 (4H, s, OCH_2CH_2O), 4.22 (1H, t of d, J=11 and 5 Hz, C_3–H). MS *m/z*: 373 (M^+), 99 (base peak). *Anal.* Calcd for $C_{22}H_{31}NO_4$: C, 70.75; H, 8.37; N, 3.75. Found: C, 70.79; H, 8.48; N, 3.79.

Recrystallization of B from $CHCl_3$–Et_2O (1: 5) gave 9 mg of the hydroxy-nitrile (**9**) as colorless needles, mp 241—243°C. IR ν_{max} cm^{-1}: 3590 (OH), 3440 (OH), 2270 (CN). NMR δ: 0.86 (3H, s, CH_3), 1.14 (3H, s, CH_3), 2.60 (1H, m, C_2–H), 2.96 (1H, s, C_4–H), 3.89 (4H, s, OCH_2CH_2O), 4.14 (1H, d, J=9.5 Hz, C_3–H). MS *m/z*: 373 (M^+), 99 (base peak). *Anal.* Calcd for $C_{22}H_{31}NO_4$: C, 70.75; H, 8.37; N, 3.75. Found: C, 70.70; H, 8.40; N, 3.60.

Recrystallization of C from $CHCl_3$–Et_2O (1: 5) gave 24 mg of the hydroxy-nitrile (**10**) as colorless needles, mp 235—236°C. IR ν_{max} cm^{-1}: 3580 (OH), 3420 (OH), 2260 (CN). NMR δ: 0.86 (3H, s, CH_3), 1.38 (3H, s, CH_3), 2.83 (1H, d, J=7 Hz, OH), 3.04 (1H, d, J=1.5 Hz, C_4–H), 3.29 (1H, m, C_2–H), 3.88 (4H, s, OCH_2CH_2O), 4.26 (1H, d of t, J=7 and 1.5 Hz, C_3–H). MS *m/z*: 373 (M^+), 99 (base peak). *Anal.* Calcd for $C_{22}H_{31}NO_4$: C, 70.75; H, 8.37; N, 3.75. Found: C, 70.41; H, 8.36; N, 3.72.

3α-Acetoxy-2α-cyano-4α,5α-epoxyandrostan-17-one (**11**)——A solution of 110 mg of the hydroxy-nitrile (**8**) in 0.5 ml of acetic anhydride and 0.5 ml of pyridine was allowed to stand overnight. The solution was poured into ice-water and extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$, 2% HCl and water, then dried and evaporated to dryness to leave a crystalline residue. A solution of the residue in 2 ml of acetone was treated with 4 mg of *p*-toluenesulfonic acid. After being stirred for 4 h at room temperature, the mixture was made alkaline with 5% $NaHCO_3$ and extracted with EtOAc. The extract was washed with water, dried and concentrated. Recrystallization of the residue from $CHCl_3$–Et_2O (1: 2) gave the cyano-acetate (**11**) as colorless needles, mp 200—203°C. IR ν_{max} cm^{-1}: 2240 (CN), 1730 (CO). NMR δ: 0.90 (3H, s, CH_3), 1.14 (3H, s, CH_3), 2.20 (3H, s, $COCH_3$), 2.98 (1H, oct, J=12, 6, and 4.5 Hz, C_2–H), 3.33 (1H, d, J=4.5 Hz, C_4–H), 5.35 (1H, q, J=6 and 4.5 Hz, C_3–H). MS *m/z*: 371 (M^+), 43 (base peak). *Anal.* Calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77. Found: C, 70.80; H, 7.97; N, 3.78.

3β-Acetoxy-2α-cyano-4α,5α-epoxyandrostan-17-one (**12**)——In the same manner as described for **11**, 24 mg of the hydroxy-nitrile (**9**) was converted to 4.5 mg of the cyano-acetate (**12**), colorless prisms, mp 271—272°C. IR ν_{max} cm^{-1}: 2240 (CN), 1735 (CO). NMR δ: 0.90 (3H, s, CH_3), 1.20 (3H, s, CH_3), 2.16 (3H, s, $COCH_3$), 2.75 (1H, t of d, J=10 and 2.5 Hz, C_2–H), 2.95 (1H, s, C_4–H), 5.12 (1H, d, J=10 Hz, C_3–H). MS *m/z*: 311 (M^+-60), 41 (base peak). *Anal.* Calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77. Found: C, 70.77; H, 7.65; N, 3.67.

3β-Acetoxy-2β-cyano-4α,5α-epoxyandrostan-17-one (**13**)——In the same manner as described for **11**, 47 mg of the hydroxy-nitrile (**10**) was converted to 16 mg of the cyano-acetate (**13**), colorless needles, mp 159—160°C. IR ν_{max} cm^{-1}: 2240 (CN), 1730 (CO). NMR δ: 0.90 (3H, s, CH_3), 1.42 (3H, s, CH_3), 2.20 (3H, s, $COCH_3$), 3.06 (1H, s, C_4–H), 3.45 (1H, oct, J=8, 5.7, and 4 Hz, C_2–H). MS *m/z*: 371 (M^+), 43 (base peak). *Anal.* Calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77. Found: C, 70.86; H, 7.83; N, 3.72.

2α-Cyano-3α,17-diacetoxy-4α,5α-epoxyandrost-16-ene (**14**)——A solution of 70 mg of the hydroxy-nitrile (**11**) and 5 mg of *p*-toluenesulfonic acid in 40 ml of isopropenyl acetate was refluxed for 26 h. After cooling, the solution was concentrated and made alkaline with 5% $NaHCO_3$. The mixture was extracted with EtOAc. The extract was washed with water, dried and concentrated to leave a brown oil. The oil was purified by preparative TLC (10% acetone-$CHCl_3$) to give 13 mg of the enol acetate (**14**), which was recrystallized from Et_2O–hexane (2: 1) to afford 12 mg of **15** as colorless needles, mp 210—213°C. IR ν_{max} cm^{-1}: 2240 (CN), 1740 (CO). NMR δ: 0.91 (3H, s, CH_3), 1.12 (3H, s, CH_3), 2.15 (3H, s, $COCH_3$), 2.19 (3H, s, $COCH_3$), 2.84 (1H, m, C_2–H), 3.28 (1H, d, J=4.5 Hz, C_4–H), 5.31 (1H, q, J=6 and 4.5 Hz, C_3–H), 5.53 (1H, m, C_{16}–H). MS *m/z*: 413 (M^+), 43 (base peak).

2α-Cyano-3α,16α-diacetoxy-4α,5α-epoxyandrostan-17-one (16)——A solution of 19 mg of the enol acetate (**14**) in 1 ml of CH_2Cl_2 was treated with 10 mg of *m*-chloroperbenzoic acid. After being stirred for 3 h at room temperature, the mixture was made alkaline with 5% $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with water, dried and concentrated to leave 22 mg of the epoxide (**15**) as a crystalline solid. The solid was dissolved in 15 ml of $CHCl_3$ and *p*-toluenesulfonic acid (2 mg) was added to the solution. After being stirred for 1 h at room temperature, the reaction mixture was extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$ and water, then dried and concentrated to leave 24 mg of a crystalline residue. A mixture of the residue, 0.1 ml of acetic anhydride and 0.1 ml of pyridine was allowed to stand overnight at room temperature. The mixture was extracted with EtOAc. The extract was washed successively with 2% HCl, 5% $NaHCO_3$ and water, then dried and concentrated to leave 25 mg of a residue. Purification of the residue by preparative TLC (10% acetone–$CHCl_3$) gave 20 mg of the α-keto-acetate (**16**) mp 140—143°C ($CHCl_3$: Et_2O=1: 4). IR ν_{max} cm^{-1}: 2240 (CN), 1752 (CO), 1740 (CO). NMR δ: 0.98 (3H, s, CH_3), 1.12 (3H, s, CH_3), 2.10 (3H, s, $COCH_3$), 2.28 (3H, s, $COCH_3$), 2.90 (1H, oct, J=13, 7, and 6 Hz, C_2–H), 3.30 (1H, d, J=4 Hz, C_4–H), 5.32 (1H, q, J=6 and 4 Hz, C_3–H) 5.42 (1H, m, C_{16}–H). MS *m/z*: 369 (M^+-60), 43 (base peak). A sample of the α-keto-acetate (**16**) was identical with a sample of the **M-2** diacetate prepared from M-2 by treatment with acetic anhydride–pyridine.

2α-Cyano-4α,5α-epoxy-3α,16α,17β-triacetoxyandrostane (17)——A solution of 23 mg of of α-keto-acetate (**16**) in 2 ml of MeOH was treated with 1 mg of $NaBH_4$. After being stirred for 45 min at room temperature, the mixture was extracted with $CHCl_3$. The extract was washed with water, dried and concentrated to leave a crystalline residue (23 mg). A solution of the residue in pyridine (0.2 ml) and acetic anhydride (0.2 ml) was allowed to stand overnight at room temperature, then poured into H_2O. The product was extracted with EtOAc and the extract was washed with 2% HCl, 5% $NaHCO_3$, and H_2O, then dried and concentrated. The residue was recrystallized from $CHCl_3$–Et_2O (1: 4) to give 20 mg of the triacetate (**17**) as colorless prisms, mp 130—132°C. IR ν_{max} cm^{-1}: 2240 (CN), 1735 (CO), 1240. NMR δ: 0.82 (3H, s, CH_3), 1.08 (3H, s, CH_3), 2.03 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.19 (3H, s, $COCH_3$), 2.82 (1H, m, C_2–H), 3.26 (1H, d, J=4 Hz, C_4–H), 4.89 (1H, d, J=5 Hz, C_{17}–H), 5.15 (1H, m, C_{16}–H), 5.30 (1H, q, J=6 Hz, C_3–H). *Anal.* Calcd for $C_{26}H_{35}NO_7$: C, 65.94; H, 7.45; N, 2.96. Found: C, 66.18; H, 7.41; N, 2.84. A sample of the triacetate (**17**) was identical with a sample of the **M-3** triacetate prepared from **M-3** by treatment with acetic anhydride–pyridine.

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