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Mechanism of the Color Reaction of Deoxycorticosterone with Sulfuric Acid[1)]

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The mechanism of the color reaction of deoxycorticosterone (**1**) with sulfuric acid was elucidated. Two rearranged products, 13ξ(or 14ξ)-hydroxy-17β-methyl-18-nor-13ξ,14ξ,17α-pregn-4-ene-3,20-dione (**4**) and 17β-methyl-18-nor-17α-pregna-4,13-diene-3,20-dione (**5**), were isolated from the reaction mixture of **1** with 97% sulfuric acid at room temperature in 46 and 32% yields, respectively. Proton nuclear magnetic resonance (^{1}H-NMR) spectra of 97% sulfuric acid solutions of **1**, **4** and **5** revealed that they are present in the acid as the diprotonated form (**7**) of **5**, showing an absorption maximum at 288 nm. The acid solution of **7** was heated for 90 min at 60 °C to give two intermediary species (χ-372 and χ-436) showing maxima at 372 and 436 nm, respectively. Dilution of the mixture with ethanol reduced these species and produced a new chromophore (χ-600) showing a maximum at 600 nm. From the diluted solution, 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (**13**) was isolated in 13% yield. Dissolution of **13** in a 2 : 3 mixture of sulfuric acid and ethanol immediately gave χ-600, which was identified from its ^{1}H-NMR data as the steroidal carbocation (**14**) having a hydroxyalkatetraenyl cation moiety. On the other hand, dissolution of **13** in 90% sulfuric acid gave χ-372, which was shown to be in acid–base equilibrium with χ-600. χ-372 was also identified from its ^{1}H-NMR data as the dication (**15**) having a hydroxyalkenyl cation moiety in ring A and an alkadienyl cation moiety across rings B, C and D.

Keywords——deoxycorticosterone; color reaction; sulfuric acid; ^{1}H-NMR; steroidal tetraenone; steroidal carbocation; dication; hydroxyalkatetraenyl cation; deconjugation; mechanism

Color and fluorescence reactions of corticosteroids with strong acids have been widely used for qualitative and quantitative determinations, though their mechanisms have been relatively little studied[2,3)] and have remained obscure. Most steroidal 4-en-3-ones, such as testosterone, progesterone and several corticosteroids, produce chromo- and fluorophores showing absorption maxima at around 600 nm when they are dissolved in concentrated sulfuric acid, followed by dilution with water or ethanol.

In this series of studies, the reaction mechanisms of testosterone,[4)] progesterone,[5)] and 17α-hydroxyprogesterone[1b,6)] with sulfuric acid have been elucidated, and in all cases the chromo- and fluorophores were shown to be steroidal carbocations having hydroxyalkatetraenyl cation (protonated form of a conjugated tetraenone) moieties.

This paper deals with the mechanism of the color reaction of deoxycorticosterone (21-hydroxypregn-4-ene-3,20-dione, **1**), one of the corticosteroids, with sulfuric acid.

Results and Discussion

When **1** was dissolved in 97% sulfuric acid at room temperature, three chemical species were formed as shown in Fig. 1. The main species (abbreviated as χ-288) showed an absorption maximum at 288 nm with an apparent molar absorptivity (ε′) of 19200, and two

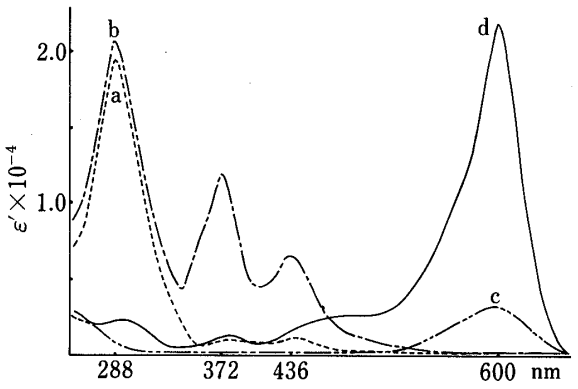
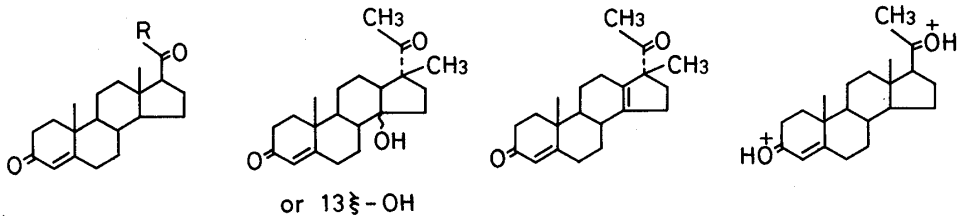


Fig. 1. Color Reaction of Deoxycorticosterone (**1**) in the Sulfuric Acid–Ethanol System

Each absorption spectrum was measured at room temperature at 5 min after preparing the solution. ε': Apparent molar absorptivity. a) Compound **1** (53 μg) was dissolved in 97% H_2SO_4 (5 ml) at room temperature. b) The reaction mixture (a) was heated at 60 °C for 90 min. c) The acid solution (a, 2 ml) was poured into ethanol (3 ml). d) The acid solution (b, 2 ml) was poured into ethanol (3 ml).

minor species (abbreviated at χ-372 and χ-436) showed maxima at 372 and 436 nm. The amounts of the minor species, χ-372 and χ-436, increased when the acid solution was heated for 90 min at 60 °C. Though these three species are rather stable in the acid, dilution of the acid solution with ethanol reduced these species and produced a new chromophoric species (abbreviated as χ-600) showing a maximum at 600 nm. Since the yield of χ-600 after the dilution increased as a result of the preheating the 97% sulfuric acid solution of **1** (Fig. 1), χ-372 and/or χ-436 are assumed to be intermediate(s) for χ-600.

In order to clarify the mechanism of the initial stage of the color reaction, isolation of products from the reaction of **1** with sulfuric acid, and proton nuclear magnetic resonance (^{1}H-NMR) studies of the acid solution were carried out as follows.



1: R=CH_2OH
2: R=CH_2OCOCH_3
3: R=CH_3

4 **5** **8**

Chart 1

1 was dissolved in 97% sulfuric acid and allowed to stand at room temperature for 5 min. The reaction mixture was then poured into excess ice-water, extracted with ethyl acetate and separated by preparative thin-layer chromatography (TLC) to give two rearranged products, 13ξ(or 14ξ)-hydroxy-17β-methyl-18-nor-13ξ,14ξ,17α-pregn-4-ene-3,20-dione (**4**) and 17β-methyl-18-nor-17α-pregna-4,13-diene-3,20-dione (**5**) in 46 and 32% yields, respectively. Schmitt *et al.* isolated **4** and **5** from the reaction mixture of deoxycorticosterone acetate (**2**) with concentrated sulfuric acid.[7] Their physical and spectral data were in good agreement with those of **4** and **5** obtained in the present study. Both **4** and **5** gave χ-288 when they were dissolved in 97% sulfuric acid at room temperature. Since a hydroxyalkenyl cation (protonated form of a conjugated enone) shows an absorption maximum at about 290 nm,[8] both **4** and **5** as well as **1** may be protonated in 97% sulfuric acid to show the maximum at 288 nm. Figure 2a shows the ^{1}H-NMR spectrum of 97% sulfuric acid solution of **4** or **5**. The signal pattern was similar to that given by **5** in $CDCl_3$, though all the signals were shifted to lower field compared with those (Table) of **5** in $CDCl_3$. Thus, this spectrum indicates that **4** and **5** are present as the protonated form (**7**) of **5** in the acid solution. Dissolution of **1** in 77% sulfuric acid gave the dication (**6**), a diprotonated form of **1**, the structure of which was

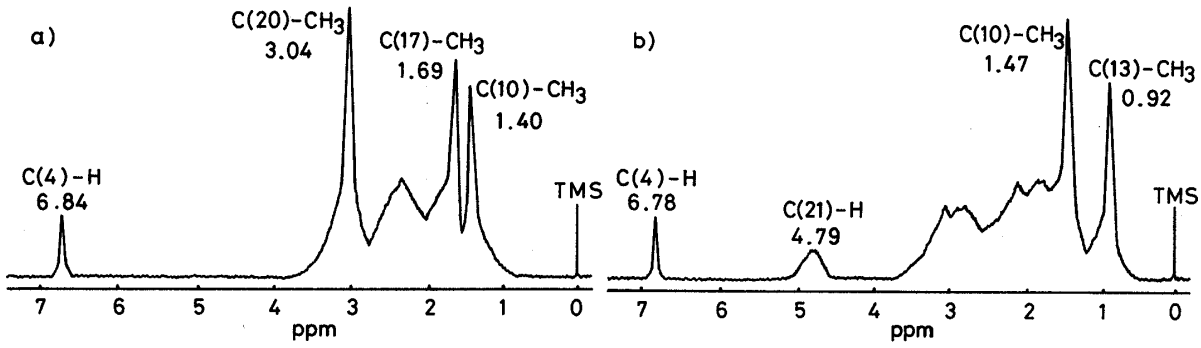


Fig. 2. ^{1}H-NMR Spectra of Deoxycorticosterone (**1**) in 77% and 97% Sulfuric Acid

a) Compounds **1**, **4** or **5** (40 mg) was dissolved in 0.5 ml of 97% H_2SO_4. b) Compound **1** (40 mg) was dissolved in 0.5 ml of 77% H_2SO_4.

TABLE. ^{1}H-NMR Data[a] for Unsaturated Ketosteroids in $CDCl_3$ and in H_2SO_4

	1[b]	**5**[b]	**13**[b]	**8**[c]	**6**[d]	**7**[c]
C(4)-H	5.73	5.80	5.77	6.73	6.78	6.84
C(6)-H			6.05 (d, J=9 Hz)			
C(7)-H			6.66 (d, J=9 Hz)			
C(10)-CH_3	1.19	1.19	1.04	1.40	1.47	1.40
C(13)-CH_3	0.70			0.97	0.92	
C(17)-CH_3		1.19				1.69
C(20)-CH_3		2.06	1.03 (d, J=7 Hz)	3.01		3.04
			1.07 (d, J=7 Hz)			
C(21)-H	4.19 (d, J=5 Hz)				4.79 (m)	
C(21)-OH	3.25 (t, J=5 Hz)					

a) ppm from tetramethylsilane as an internal or external standard. *b*) In $CDCl_3$. *c*) In 97% H_2SO_4. *d*) In 77% H_2SO_4.

confirmed by comparison of ^{1}H-NMR spectrum (Fig. 2b) of the acid solution with those (Table) of **1** in $CDCl_3$ and **8**[5] in 97% sulfuric acid. The structure was also supported by the quantitative recovery of **1** from the acid solution of **1**. On the other hand, dissolution of **1** in 97% sulfuric acid resulted in an ^{1}H-NMR spectrum identical not with that of **6** (Fig. 2b) but with that of **7** (Fig. 2a), indicating that **6** is unstable and is rapidly converted to **7** in the acid solution. These results demonstrate that **7** is χ-288 produced in the reaction of **1** with 97% sulfuric acid. The initial reaction of **1** with 97% sulfuric acid may, therefore, proceed as shown in Chart 2. Protonations of the carbonyl oxygen at C(3) and the hydroxyl oxygen at C(21) of **1** give the dication (**6**), which is unstable and is transformed to **11** and/or **12** through enolization, dehydration and methyl migration. Deprotonation of **12** affords **5** which is in an acid–base equilibrium with χ-288 (**7**), whereas hydration of **12** leads to **4**.

As shown in Fig. 1, the colored solution of χ-600 is obtained when a 97% sulfuric acid solution of **1** is heated for 90 min at 60 °C and then diluted with 1.5 volumes of ethanol. In the same manner, the colored solution of χ-600 was prepared on a preparative scale and poured into excess ice-water. The mixture was extracted with ethyl acetate and separated by preparative TLC to give 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (**13**) in 13% yield. The spectral data were in good agreement with those of the same tetraenone previously isolated from the reaction mixture of progesterone (**3**) with sulfuric acid.[5] As shown in Fig. 3, an absorption maximum at 600 nm appeared immediately with ε' of 51300 when the tetraenone (**13**) was dissolved in a 2 : 3 mixture of sulfuric acid and ethanol. Figure 4 shows the ^{1}H-NMR spectrum of χ-600 obtained by dissolving **13** in a 1 : 2 mixture of D_2SO_4

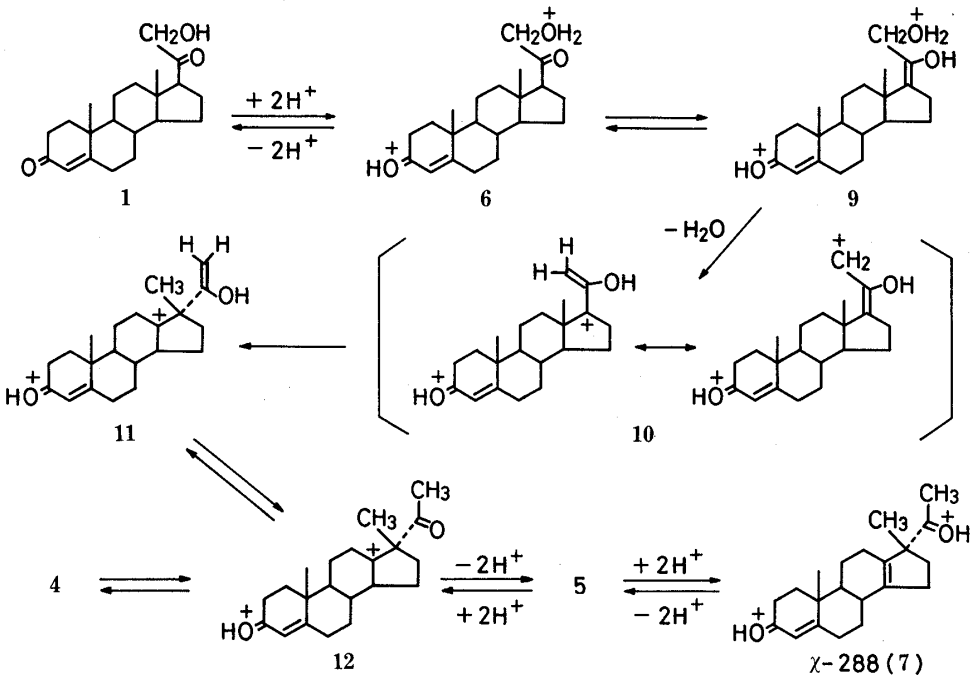


Chart 2

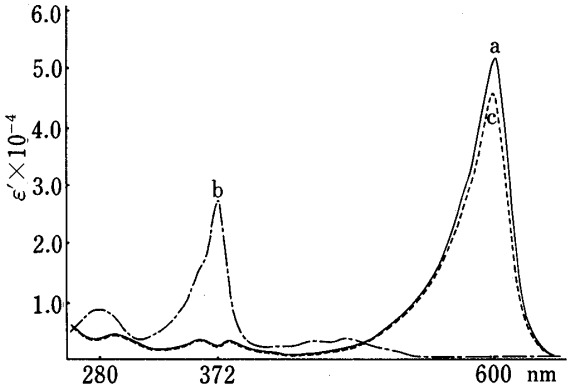


Fig. 3. Absorption Spectra of χ-600 and χ-372

a) Compound **13** (56 μg) was dissolved in a 2:3 mixture of H_2SO_4 and EtOH (5 ml). b) Compound **13** (56 μg) was dissolved in 5 ml of 90% H_2SO_4. c) The acid solution (b, 2 ml) was poured into EtOH (3 ml).

and CD_3OD. The signal pattern was similar to that given by **13** in $CDCl_3$, though all the olefinic proton signals were shifted to lower field and the methyl signals at C(10) and C(20) to higher field compared with those of **13** in $CDCl_3$ (Table). Further, these spectral data of χ-600 closely resemble those of the hydroxyalkatetraenyl cations produced from the reactions of testosterone[4e] and 17α-hydroxyprogesterone[1b] with sulfuric acid. Thus, χ-600 was identified as the steroidal carbocation (**14**) having a hydroxyalkatetraenyl cation moiety.

As described above, the intermediary species, χ-372 and χ-436, are produced by heating a 97% sulfuric acid solution of **1**. In the same manner, the rearranged product (**5**) also gave χ-372 and χ-436. Attempted identifications of χ-372 and χ-436 by product analysis of the reaction mixtures failed because pouring the mixture of χ-372 and χ-436 into excess ice-water resulted in the conversion of these species to χ-600. Though χ-600 is formed from **5** *via* χ-372 and/or χ-436, the oxidation state of χ-600 is the same as that of **5**. Therefore, an oxidation step is not involved in the formation of χ-600 from **5**. The reactions responsible for the conversion of **5** to χ-372 and/or χ-436, and the formation of χ-600 from χ-372 and/or χ-436, are thus assumed to be dehydration, methyl migration and acid-base-catalyzed double bond isomerization. As mentioned above, χ-600 is immediately formed when the tetraenone (**13**) is dissolved in a 2:3 mixture of sulfuric acid and ethanol. On the other hand, dissolution of **13** in 90%

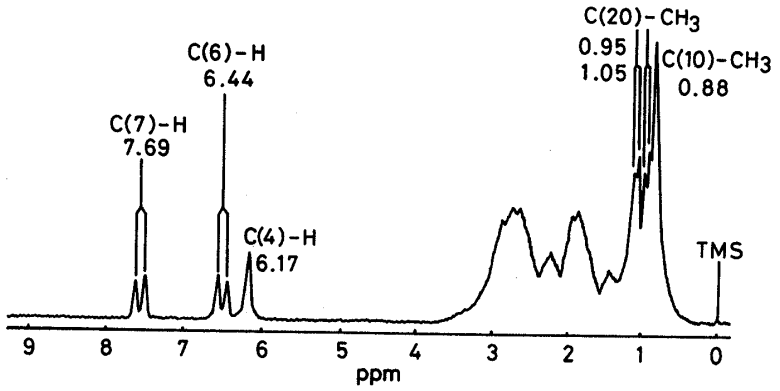


Fig. 4. ^{1}H-NMR Spectrum of χ-600 in a Mixture of Sulfuric Acid-d_2 and Methanol-d_4
Compound **7** (40 mg) was dissolved in a 1 : 2 (v/v) mixture of D_2SO_4 and CD_3OD (0.5 ml).

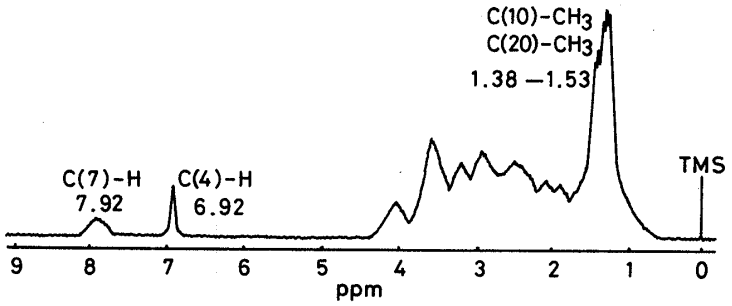
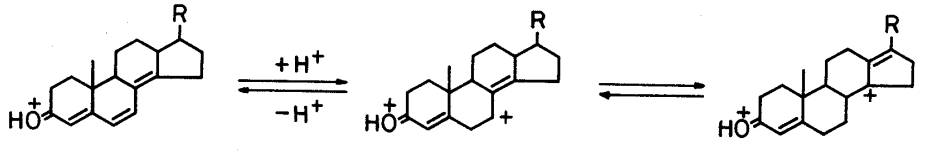


Fig. 5. ^{1}H-NMR Spectrum of χ-372 in 90% Sulfuric Acid
Compound **7** (40 mg) was dissolved in 0.5 ml of 90% H_2SO_4.

sulfuric acid gave χ-372, which was transformed to χ-600 by diluting the acid solution with ethanol as shown in Fig. 3. These findings indicate that χ-600 is in an acid–base equilibrium with χ-372 as well as **13**. Figure 5 shows the ^{1}H-NMR spectrum of χ-372 in 90% sulfuric acid. Compared with the signals of χ-600 (Fig. 4), a remarkable down-field shift was observed for the signal due to the olefinic proton at C(4), and two doublet signals due to olefinic protons at C(6) and C(7) of χ-600 were replaced by a broad one at lower field due to an olefinic proton. Though the signals due to methyl groups at C(10) and C(20) of χ-372 were not well resolved, they were shifted to lower field compared with those of χ-600. These spectral data suggest that χ-372 has two cation moieties produced by further protonation and deconjugation of the hydroxyalkatetraenyl cation moiety of χ-600. Alkadienyl cations are known to show absorption maxima at around 400 nm,[9] and some of them are stable in concentrated sulfuric acid.[10] Thus, χ-372 was tentatively assigned as the dication (**15**) which has the hydroxyalkenyl cation in ring A and the alkadienyl cation across rings B, C, and D. Hydroxyalkatrienyl cations, **16** and **18**, have been shown to deconjugate to the dications, **17** and **19**, respectively, in medium of high acid-strength where they can be further protonated.[4c,5] These deconjugations, therefore, seem to be characteristic of hydroxyalkapolyenyl cations in media of high acid-strength (Chart 3).



16: R = CH_3
18: R = $CH(CH_3)_2$

17: R = CH_3
19: R = $CH(CH_3)_2$

Chart 3

From the results obtained in present study, the color reaction of **1** is concluded to proceed by the mechanisms shown in Charts 2 and 4. Dissolution of **1** in 97% sulfuric acid at room temperature gives χ-288 (**7**), as shown in Chart 2, and this is then transformed to χ-372 (**15**) through methyl migration, dehydration and an acid–base-catalyzed double bond isomerization on heating of the reaction mixture. The χ-372 (**15**) thus formed is in an acid–base equilibrium with χ-600 (**14**) as well as its conjugated base (**21**), and is stable in the acid solution. However, the equilibrium lies to the side of the chromophoric χ-600 (**14**) when the acid-strength of the medium is reduced by diluting the reaction mixture with ethanol or water. Another intermediary species, χ-436, seems to be one of the intermediates in the formation of χ-600 (**14**) from χ-288 (**7**), probably the dication (**20**) having another alkadienyl cation, though no definitive evidence for the structure was obtained in the present study.

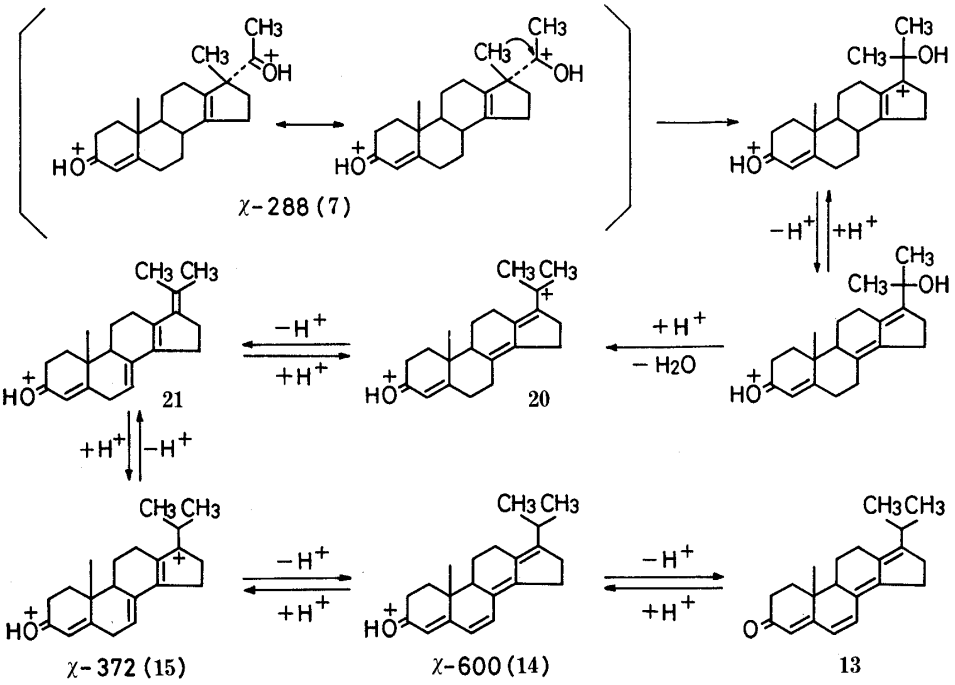


Chart 4

Experimental

General Methods——Infrared (IR) spectra were measured with a JASCO A-102 recording spectrometer. Mass spectral (MS) measurements were run on a JEOL JMS-D300 spectrometer. Absorption spectra were measured with a Shimadzu UV-220 recording spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-FX100 FT spectrometer at 100 MHz or a Hitachi R-20B spectrometer at 60 MHz with tetramethylsilane (TMS) as an internal standard. For preparative TLC, silica gel (Wakogel B-5F) was used as an adsorbent. The melting point of **4** was taken on a micro-hot stage apparatus and is uncorrected.

Materials——Deoxycorticosterone (**1**) was purchased from Sigma Chemical Co. and used after recrystallization. Sulfuric acid (super special grade, 97.2% w/w) was obtained from Wako Pure Chemical Industries Ltd.

^{1}H-NMR Spectra in Sulfuric Acid Solutions——Sulfuric acid (97, 90 or 77% w/w, 0.5 ml) was added to a dried sample (40 mg), and the mixture was shaken vigorously to obtain a homogeneous solution. The ^{1}H-NMR spectra of the solutions were recorded at 35 °C, with TMS in a capillary as a reference.

Isolation of 13ξ(or 14ξ)-Hydroxy-17β-methyl-18-nor-13ξ,14ξ,17α-pregn-4-ene-3,20-dione (4) and 17β-Methyl-18-nor-17α-pregna-4,13-diene-3,20-dione (5)——A mixture of sulfuric acid (97%, 3 ml) and **1** (180 mg) was shaken vigorously to give a homogeneous solution and then allowed to stand at room temperature for 5 min. The reaction mixture was gradually poured into excess ice-water under vigorous stirring and the whole was extracted with ethyl acetate (200 ml × 3). The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was subjected to preparative TLC and developed with benzene–acetone (9 : 1, × 2). Elution of the adsorbent corresponding to the spot of *Rf* 0.32 with chloroform gave **4** (82 mg) as a crystalline residue, which was

recrystallized from acetone to afford colorless needles, mp 161—164 °C (lit.[7] 165 °C). *Anal.* Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.27; H, 9.15. λ_{max}^{EtOH} nm (ε): 238 (12200). MS *m/z*: 330 (M^+). IR (0.005 M in CCl_4) cm^{-1}: 3440 (intramolecularly hydrogen-bonded OH), 1678 (shoulder) and 1687 (intramolecularly hydrogen-bonded C=O at C(20) and conjugated C=O at C(3)), 1622 (C=C). ^{1}H-NMR (in $CDCl_3$): 1.15 (3H, s, C(10)–CH_3), 1.42 (3H, s, C(17β)–CH_3), 2.20 (3H, s, –$COCH_3$), 3.80 (1H, s, C(13ξ)- or C(14ξ)–OH. This signal disappeared on addition of D_2O to the sample), 5.76 (1H, s, C(4)–H). Elution of the adsorbent corresponding to the spot of *Rf* 0.48 with chloroform gave **5** (55 mg) as a slightly yellow oil, attempted crystallization of which was unsuccessful. λ_{max}^{EtOH} nm (ε): 237 (16800). MS *m/z*: 312 (M^+). IR ν_{max}^{neat} cm^{-1}: 1670 (C=O at C(3)), 1700 (C=O at C(20)), 1620 (C=C). The ^{1}H-NMR data for **5** are shown in the Table. Compound **4** was converted quantitatively to **5** by dissolving it in a 2 : 5 mixture of 6 N HCl and MeOH at room temperature for 10 min.

Isolation of 17-Isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (13)——A mixture of sulfuric acid (97%, 5 ml) and **1** (372 mg) was shaken vigorously to make a homogeneous solution, which was then heated for 90 min at 60 °C. The reaction mixture was diluted with ethanol (7.5 ml) under ice-cooling to give a deep purple solution. The colored solution was worked-up and subjected to preparative TLC in the same manner as described above, except that benzene–acetone (6 : 1) was used as the developing solvent. Elution of the adsorbent corresponding to the spot of *Rf* 0.58 with chloroform gave **13** (42 mg) as a red-brown oil. λ_{max}^{EtOH} nm (ε): 412 (19800). MS *m/z*: 294 (M^+). IR ν_{max}^{neat} cm^{-1}: 1650 (C=O at C(3)), 1560 (C=C). The ^{1}H-NMR data for **13** are shown in the Table.

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