

Optical Rotatory Dispersions of β -Hydroxylated 5β -Spirostanones¹⁾MASAHIKO TOHMA, AKIHARU FUJINO, KATSUHIKO YAMAZAKI
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The β -axial hydroxylated 5β -spirostan-1- and 3-ones were prepared from the steroidal sapogenins in *Convallaria* and diosgenin, and the optical rotatory dispersion curves of these ketones were measured in methanol or dioxane. The 5-hydroxy-25(*R*), 5β -spirostan-3-one (XIII) showed a weak positive amplitude (α : +16.0) in contrast to the negative one of the parent 3-ketone (I, α : -16.6).

The hydroxyl groups substituted on the symmetrical β -axial positions to the carbonyl groups led to marked changes in the amplitude of Cotton effect in the 5β -spirostanones, and opposed to the prediction from the octant rule.

In the previous papers, it was reported that new steroidal sapogenins, convallagenin-A and B, were obtained from the flowers of *Convallaria keiskei* Miq., Japanese lily of the valley,³⁾ and their structures were elucidated as $1\beta,3\beta,5$ -trihydroxy-^{3b)} and $1\beta,3\beta,4\beta,5$ -tetrahydroxy-25(*S*), 5β -spirostane,^{3c)} respectively.

Optical rotatory dispersion curves of most of steroidal ketone are characterized by a simple Cotton effect, and provide valuable structural informations by application of the octant rule.⁴⁾ For example, 5α -cholestan-3-one exhibits a positive Cotton effect (α : +54), contrary to the 5β -isomer, coprostan-3-one, showing a negative one (α : -27). In the course of the above studies, 5-hydroxy-25(*R*), 5β -spirostan-3-one (XIII) was synthesised from diosgenin and showed an abnormal optical rotatory dispersion curve as described follows.

The present paper deals with the syntheses of β -hydroxylated 1- and 3-ketones in 5β -spirostane series, and the optical effect of axial hydroxyl groups substituted on the β -position to the carbonyl groups.

An initial project was directed to the preparation of 5β -spirostan-3-one series. The parent 3-ketone (I)⁵⁾ was synthesised from smilagenin by Jones oxidation, and partial oxidation of isorhodeasapogenin (IV)⁶⁾ with N-bromosuccinimide in aqueous acetone gave 1β -hydroxy-25(*R*), 5β -spirostan-3-one (V)⁷⁾ which was confirmed by acidic elimination to 25(*R*), 5β -spirost-1-en-3-one (VI) showing a specific ultraviolet absorption band at 232 nm different from that at 225 nm in the 2-en-1-one. The 5β -hydroxy ketone (XIII) was prepared as follows. Epoxidation of diosgenone (VII) with alkaline hydrogen peroxide afforded the β -oxide (VIII)⁸⁾ exhibiting a strong positive Cotton effect,⁹⁾ and lithium aluminum hydride reduction gave a mixture of the epimeric alcohols (IX and XI), which were isolated by chromatography on alumina and decided to the 5β -hydroxy compounds giving only the corre-

1) This paper represents Part XIII of the series: "Constituents of *Convallaria*"; Part XII: M. Kimura, M. Tohma, I. Yoshizawa, and A. Fujino, *Chem. Pharm. Bull.* (Tokyo), **16**, 2191 (1968).

2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo, 060, Japan.*

3) a) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 50 (1966); b) *Idem, ibid.*, **15**, 1204 (1967); c) *Idem, ibid.*, **15**, 1713 (1967).

4) W. Moffitt, R.B. Woodward, A. Moscovitz, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).

5) M.E. Wall and S. Serota, *J. Am. Chem. Soc.*, **78**, 1747 (1956).

6) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 55 (1966).

7) K. Morita, *Bull. Chem. Soc. Japan*, **32**, 791, 796 (1959).

8) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).

9) C. Djerassi, W. Klyne, T. Norin, G. Ohloff, and E. Klein, *Tetrahedron*, **21**, 163 (1965).

sponding monoacetates (X and XII) with acetic anhydride in pyridine. Oxidation of these compounds (IX and XI) with N-bromosuccinimide in aqueous acetone afforded 5-hydroxy-25(*R*),5 β -spirostan-3-one (XIII) as expected. The 1 β ,5 β -dihydroxy-3-ketone (III) was prepared from convallagenin-A (II) by selective oxidation with platinum catalyst and atmospheric oxygen.^{3b)}

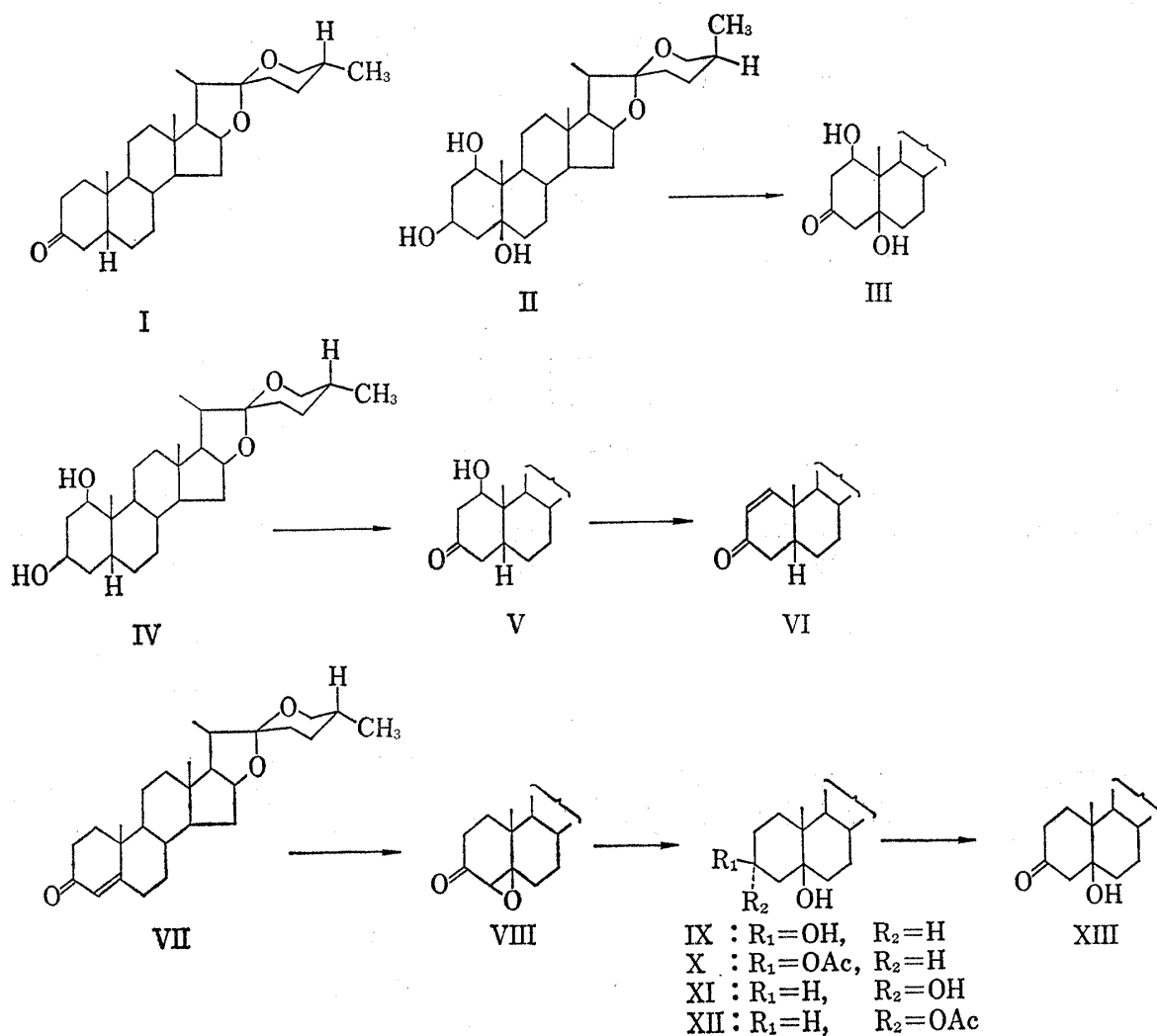


Chart 1

In order to prepare the 5 β -spirostan-1-one series, rhodeasapogenin (XIV)¹⁰⁾ was oxidised with chromium trioxide in pyridine to give 3 β -hydroxy-25(*S*),5 β -spirostan-1-one (XV), a small amount of $\alpha\beta$ -unsaturated ketone (XVI) and the enolate of 1,3-diketone (XVII). The hydroxyl group in XV was readily eliminated to give the $\alpha\beta$ -unsaturated ketone (XVI) with the ultraviolet spectra of known Δ^2 -1-ketosteroids (λ_{max} 225 nm), which was then reduced to 25(*S*),5 β -spirostan-1-one (XVIII) by hydrogenation on palladium charcoal catalyst. Also, 5 β -hydroxy-25(*S*),5 β -spirostan-1-one (XX) was obtained by catalytic hydrogenation of the 5 β -hydroxy unsaturated ketone (XIX), which was prepared from convallagenin-A (II) in the previous paper.^{3b)}

The optical rotatory dispersion curves of these β -axial-hydroxy-1- and 3-ketones were represented in Fig. 1 and 2, and summarized in Table I. These data show that the hydroxyl groups led to marked changes in the magnitude of the Cotton effect, and the 5-hydroxy-3-

10) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 1228 (1968).

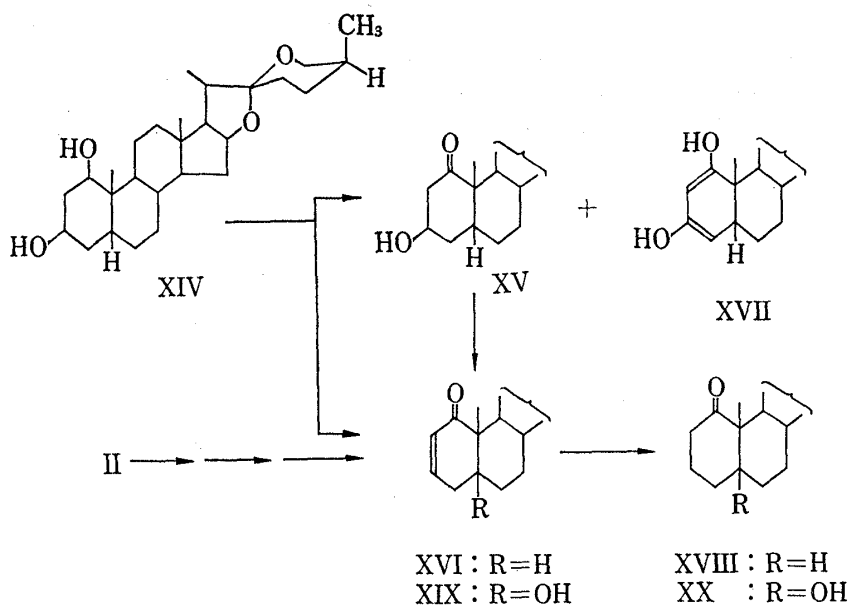


Chart 2

ketone (XIII) exhibited especially a weak positive amplitude ($a: +16.0$), as well as a positive molecular ellipticity ($[\theta]: +1250$) of circular dichroism (Fig. 3) in contrast to the negative one of the parent ketone (I).

In both cases of 1- and 3-oxo compounds, the β -axial hydroxyl groups in "upper-left octant" to the carbonyl groups make a negative contribution to the Cotton effect ($\Delta a: V = -30.5$, $XX = -22.6$). On the other hand, the hydroxyl groups in "upper-right octant" have a positive Cotton effect contribution ($\Delta a: XIII = +32.6$, $XV = +31.6$). These results show that the β -axial hydroxyl groups in 5β -spirostanones oppose to the prediction from the octant rule. The $1\beta,5$ -dihydroxy- $25(S),5\beta$ -spirostan-3-one (III), substituted with two axial

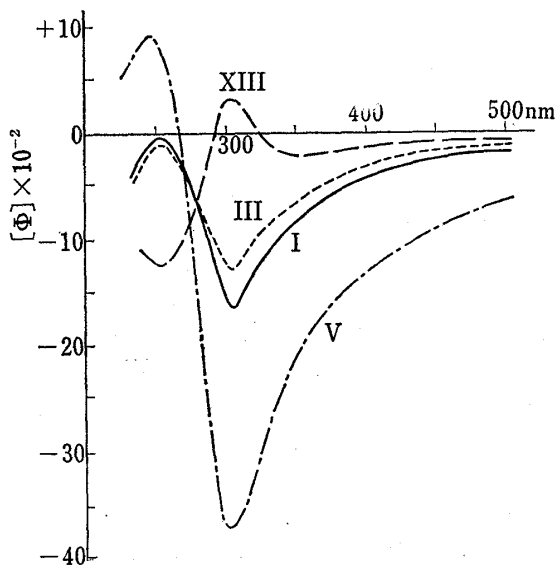


Fig. 1. Optical Rotatory Dispersion Curves of β -Hydroxylated 5β -Spirostan-3-ones in Methanol

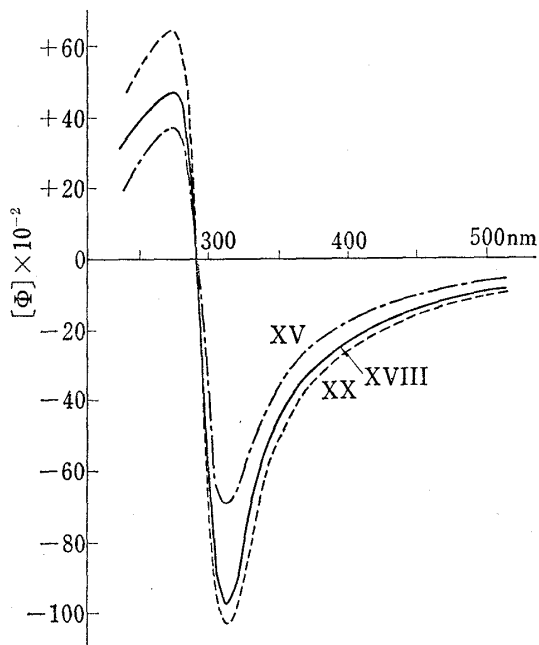


Fig. 2. Optical Rotatory Dispersion Curves of β -Hydroxylated 5β -Spirostan-1-ones in Methanol

hydroxyl groups on the symmetrical β -positions to the carbonyl group, gave a similar magnitude of the molecular amplitude (a: -12.8 , Δa : $+3.8$) to that of the parent ketone (I, a: -16.6). It seems likely that the contributions of two hydroxyl groups are cancelled each other, which are equal in the magnitude but opposite in the direction.

Djerassi and Klyne¹¹⁾ reported that β -substituents to the carbonyl groups have little influence on the amplitude of its Cotton effect, but 5-substituted 5α -cholestan-3-ones opposed to the octant rule with considerable changes in the dispersion curves, and they concluded that in such compounds there is probably a reorientation of the carbonyl axis caused by some ring distortion. Further conformational change with solvent variation has been demonstrated for several steroids.¹²⁾ Turning now to the 5β -spirostanone series, less-polar solvents caused no significant effect on the amplitude, but a general bathochromic shift of the first extreme as compared with the optical rotatory dispersion curves in methanol (Table I), and these results are similar to those on the solvent effects studied in detail by Kirk, *et al.*¹³⁾

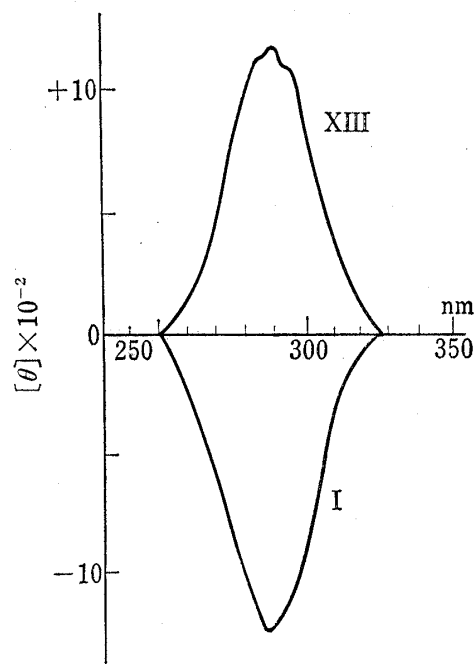


Fig. 3. Circular Dichroism Curves of 5β -Spirostan-3-one (I) and 5-Hydroxy- 5β -spirostan-3-one (XIII) in Methanol

TABLE I. Optical Rotatory Dispersion of β -Hydroxylated 5β -Spirostanones

Compound	Solvent	λ_1 (nm)	λ_2 (nm)	Molecular a	Amplitude Δa
5β -Spirostan-3-one (I)	methanol	306	270	-16.6	
	dioxane	318	276	-15.6	
		308			
1β -Hydroxy- (V)	methanol	304	273	-47.1	-30.5
5β -Hydroxy- (XIII)	methanol	301	258	$+16.0$	$+32.6$
	dioxane	310	266	$+15.1$	
$1\beta,5\beta$ -Dihydroxy- (III)	methanol	303	267	-12.8	$+3.8$
	dioxane	316	282	-10.9	
		306			
5β -Spirostan-1-one (XVIII)	methanol	312	274	-145.0	
3β -Hydroxy- (XV)	methanol	312	273	-113.4	$+31.6$
5β -Hydroxy- (XX)	methanol	311	274	-167.6	-22.6

a) Difference in molecular amplitude from the parent ketones (I or XVIII) in methanol.

Recently, Pao and Santry¹⁴⁾ studied on the optical rotatory strength of methyl cyclohexanone in chair form conformation, and reported on a self-consistent field molecular orbital theory: the sign of the rotatory strength calculated for substitution of the β -axial proton is opposite to that predicted by the octant rule. Snatzke, *et al.*¹⁵⁾ investigated the contribution

11) C. Djerassi and W. Klyne, *J. Chem. Soc.*, 2390 (1963).

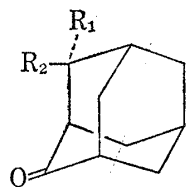
12) C.S. Barnes and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1962 (1962); J.C. Jacquesy and J. Levisalles, *Bull. Soc. Chim. France*, **1965**, 1538.

13) D.N. Kirk, W. Klyne, and S.R. Wallis, *J. Chem. Soc. (C)*, **1970**, 350.

14) Yoh-Han Pao and D.P. Santry, *J. Am. Chem. Soc.*, **88**, 4158 (1966).

15) G. Snatzke and G. Eckhardt, *Tetrahedron*, **26**, 1142 (1970).

of β -substituents on rigid conformational adamantanone derivatives (*e.g.* XXI and XXII) and observed that all equatorial substituents obey the octant rule for ketones, whereas the axial ones show anti-octant behaviour with the exception of hydroxyl group. Their general observations are consistent with our results on the β -axial hydroxyl groups of 5β -spirostanones.



XXI : $R_1 = \text{OH}$, $R_2 = \text{H}$
XXII : $R_1 = \text{H}$, $R_2 = \text{OH}$

Chart 3

In conclusion, the hydroxyl groups substituted on the symmetrical β -positions to the carbonyl group of 5β -spirostanones seems to show the anti-octant effects with each other, and no considerable solvent effect. These observations are likely to be interpreted by the effects predicted by Pao and Santry¹⁴) rather than the results from the conformational distortion of A-ring, although no other evidence is presently available and further studies are required.

Experimental

Melting points were determined on a micro hot-stage and are uncorrected. Ultraviolet spectra were recorded with a Hitachi EPS-3T and infrared spectra with a Koken-DS-301 Spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Hitachi Model H-60 Spectrometer at 60 Mc by using 5% solution containing $(\text{CH}_3)_4\text{Si}$ as an internal reference in CDCl_3 .

Optical rotatory dispersion and circular dichroism curves were measured on a JASCO Model ORD/UV-5 Optical Rotatory Dispersion Recorder, in 1 or 2 cm cells, concentration: *ca.* 1 mg/ml, temperature: 20–24°.

25(R),5 β -Spirostan-3-one (Smilagenone, I)—Smilagenone (I) was prepared from smilagenin with chromium trioxide in acetone and recrystallized from $\text{MeOH}-\text{CHCl}_3$ as colorless needles, mp 190–190.5°. (*lit.*⁵) mp 188°)

1 β -Hydroxy-25(R),5 β -spirostan-3-one (V)—The *N*-bromosuccinimide (1 g) was added to the solution of isorhodeasapogenin (IV, 914 mg)⁶) in 90% aqueous acetone (100 ml), and the mixture was allowed to stand at 37–40° in a dark place overnight. After the excess reagent was decomposed with 0.1N $\text{Na}_2\text{S}_2\text{O}_3$, the solution was concentrated in a reduced pressure, and extracted with CHCl_3 . The organic layer was washed with 2N H_2SO_4 , 5% NaHCO_3 , water, dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue (754 mg) was recrystallized from MeOH to give the 1 β -hydroxy-3-ketone (V) as colorless needles, mp 231–232° (*lit.*⁷) mp 232–233°, IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3610, 3530 (OH), 1716 (C=O), NMR τ : 9.14 (18- CH_3), 8.77 (19- CH_3). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}_4$: C, 75.31; H, 9.83. Found: C, 75.08; H, 9.59.

A solution of the hydroxy-ketone (V, 34 mg) in 6% $\text{HCl}-\text{MeOH}$ was stirred for 2 hr at room temperature. The reaction mixture was neutralized with 5% NaHCO_3 and extracted with ether. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (26 mg) was recrystallized from $\text{MeOH}-\text{acetone}$ to give 25(R),5 β -spirost-1-en-3-one (VI), mp 237–239°, (*lit.*⁷) mp 191–192°. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1680 (C=O), 1615 (C=C). UV $\lambda_{\text{max}}^{\text{EtOH}}$: 232 nm (log ϵ 3.98). NMR τ : 9.21 (18- CH_3), 8.80 (19- CH_3), 4.14 (d, $J=10$ cps, 2-H), 3.22 (d, $J=10$ cps, 1-H). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{40}\text{O}_3$: C, 78.59; H, 9.77. Found: C, 78.42; H, 9.87.

5-Hydroxy-25(R),5 β -spirostan-3-one (XIII)—To a solution of diosgenone (VII, prepared from diosgenin, 942 mg) in MeOH (100 ml) were added simultaneously 30% hydrogen peroxide (10 ml) and 2N NaOH (10 ml) at 0° and the mixture was kept at 0° in the dark for 4 hr. The resulting suspension was diluted with water and extracted with ether. The organic layer was washed with water and dried over anhydrous Na_2SO_4 , and evaporated to dryness in a reduced pressure. Recrystallization of the residue (847 mg) from $\text{MeOH}-\text{CHCl}_3$ gave 4 β ,5-oxido-25(R),5 β -spirostan-3-one (VIII), mp 205–207° (*lit.*⁸) mp 205–207°. IR $\nu_{\text{max}}^{\text{MeOH}}$ cm^{-1} : 1714 (C=O), 1250 (epoxide). NMR τ : 9.17 (18- CH_3), 8.80 (19- CH_3), 7.00 (4-H). ORD a : +185.6 (MeOH). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{40}\text{O}_4$: C, 75.66; H, 9.41. Found: C, 75.57; H, 9.21.

The epoxide (VIII, 680 mg) in tetrahydrofuran (50 ml) was added dropwise to a slurry of LiAlH_4 (1 g) in ether (30 ml) and the mixture was refluxed for 3 hr. The excess reagent was decomposed with aqueous ether and the solution was acidified with 1N HCl . The organic layer was washed with 5% NaHCO_3 and water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The mixture of alcohols (620 mg) was chromatographed on a neutral alumina (19 g). Elution with benzene gave 25(R),5 β -spirostan-3 β ,5-diol (IX, 171 mg) which was recrystallized from MeOH to give colorless needles, mp 251–252°. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3565, 3480 (OH). NMR τ : 9.20 (18- CH_3), 9.00 (19- CH_3). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_4$: C, 74.96; H, 10.25. Found: C, 74.89; H, 10.24.

A solution of IX (65 mg) in a mixture of pyridine (2 ml) and Ac₂O (2 ml) was allowed to stand for 14 hr at room temperature. After treatment in the usual way, the product was recrystallized from MeOH to give the acetate (X) as colorless needles (60 mg), mp 210—212°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3610 (OH), 1746 (C=O). NMR τ : 9.18 (18-CH₃), 8.97 (19-CH₃), 7.84 (OAc). *Anal.* Calcd. for C₂₉H₄₆O₅: C, 73.38; H, 9.77. Found: C, 73.41; H, 9.67.

Elution with benzene-CHCl₃ (95:5) in the above chromatography gave 25R,5 β -spirostan-3 α ,5-diol (XI, 214 mg) which was recrystallized from MeOH to give colorless needles, mp 262—264°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3460 (OH). NMR τ : 9.21 (18-CH₃), 9.06 (19-CH₃). *Anal.* Calcd. for C₂₇H₄₄O₄: C, 74.95; H, 10.25. Found: C, 74.99; H, 10.04.

The acetate of the diol (XI) was prepared in the same manner as described above, mp 191.5—193°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3600 (OH), 1735 (C=O). NMR τ : 9.20 (18-CH₃), 9.05 (19-CH₃), 7.92 (OAc). *Anal.* Calcd. for C₂₉H₄₆O₅: C, 73.38; H, 9.77. Found: 73.21; H, 9.65.

The 3 α ,5 β -diol (XI, 33 mg) was oxidized with *N*-bromosuccinimide (39 mg) in the same manner to that of isorhodeasapogenin (IV) described above, to give 5-hydroxy-25(*R*),5 β -spirostan-3-one (XIII), which was recrystallized from MeOH as colorless needles (23 mg), mp 265.5—268°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3580, 3370 (OH), 1716 (C=O). NMR τ : 9.15 (18-CH₃), 8.77 (19-CH₃). The hydroxyl group of this compound (XIII) was eliminated readily to diosgenone with alumina. *Anal.* Calcd. for C₂₇H₄₂O₄: C, 75.31; H, 9.83. Found: C, 75.23; H, 9.25.

The 3 β ,5 β -diol (IX) was oxidized more easily with *N*-bromosuccinimide than the 3 α ,5 β -diol (XI) to give the same product (XIII).

3 β -Hydroxy-25(*S*),5 β -spirostan-1-one (XV)—Rhodeasapogenin (XIV, 500 mg)¹⁰ in pyridine (3 ml) was added at 0° to the complex prepared from chromium trioxide (100 mg) and pyridine (20 ml) and the mixture was allowed to stand at room temperature for 20 hr. The product was diluted with ice-water (100 ml), and extracted with ether. The organic layer was washed with 2*N* HCl, 5% NaHCO₃ and water, dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue (435 mg) was chromatographed on a neutral alumina (12 g).

Elution with hexane-benzene (1:1) gave 25*S*,5 β -spirost-2-en-1-one (XVI, 61 mg) which was recrystallized from MeOH-acetone as colorless plates, mp 234.5—235°. UV $\lambda_{\max}^{\text{EtOH}}$: 225 nm (log ϵ 3.90). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1670 (C=O), 1618 (C=C). NMR τ : 9.22 (18-CH₃), 8.90 (19-CH₃), 4.06 (d, *J*=11 cps, 2-H), 3.35 (m, 3-H). *Anal.* Calcd. for C₂₇H₄₀O₃: C, 78.59; H, 9.77. Found: C, 78.39; H, 9.68.

Elution with benzene gave 3 β -hydroxy-25(*S*),5 β -spirostan-1-one (XV, 324 mg) which was recrystallized from acetone-cyclohexane as colorless needles, mp 242—243.5°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3610, 3460 (OH), 1703 (C=O). NMR τ : 9.21 (18-CH₃), 8.81 (19-CH₃). *Anal.* Calcd. for C₂₇H₄₂O₄: C, 75.31; H, 9.83. Found: C, 75.30; H, 9.80.

Elution with benzene-CHCl₃ gave 1,3-dihydroxy-25*S*,5 β -spirosta-1,3-diene (XVII, 50 mg) which was recrystallized from CHCl₃-MeOH as colorless needles, mp 279.5—280.5°. UV $\lambda_{\max}^{\text{EtOH}}$: 257 nm. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3440 (OH), 1607 (C=C). *Anal.* Calcd. for C₂₇H₄₀O₄: C, 75.66; H, 9.41. Found: C, 75.29; H, 9.35.

25(*S*),5 β -Spirostan-1-one (XVIII)—The 3 β -hydroxy-1-ketone (XV, 126 mg) in 6% HCl-MeOH was stirred for 2 hr at room temperature. The reaction mixture was treated in the same way to that of the 1-en-3-one (VI) and gave the $\alpha\beta$ -unsaturated ketone (112 mg), mp 233—235°, which was identical in all respects with 25(*S*),5 β -spirost-2-en-1-one (XVI) as described above.

The $\alpha\beta$ -unsaturated ketone (XVI, 70 mg) in MeOH (20 ml) was reduced with atmospheric hydrogen and 10% palladium charcoal (50 mg) for 3 hr at room temperature. After treatment in the usual way, recrystallization from MeOH-acetone gave 25(*S*),5 β -spirostan-1-one (XVIII, 57 mg) as colorless plates, mp 213—214°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1705 (C=O). NMR τ : 9.21 (18-CH₃), 8.82 (19-CH₃). *Anal.* Calcd. for C₂₇H₄₂O₃: C, 78.21; H, 10.21. Found: C, 78.00; H, 10.18.

5-Hydroxy-25(*S*),5 β -spirostan-1-one (XX)—The 5-hydroxy-25(*S*),5 β -spirost-2-en-1-one (XIX, 56 mg)^{3b} prepared from convallagenin-A (II) was hydrogenized in the same manner to that of the 2-en-1-one (XVI) described above. The product was recrystallized from MeOH to give 5-hydroxy-25(*S*),5 β -spirostan-1-one (XX, 51 mg) as colorless needles, mp 201—203°. UV $\lambda_{\max}^{\text{EtOH}}$: 281 nm. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3590, 3420 (OH), 1692 (C=O). NMR τ : 9.22 (18-CH₃), 8.75 (19-CH₃). *Anal.* Calcd. for C₂₇H₄₀O₄: C, 75.66; H, 9.41. Found: C, 75.59; H, 9.31.

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