ON STEROIDAL SAPOGENINS (VIII) DIRECTING EFFECTS OF THE C_{11}-SUBSTITUENTS ON THE ADDITION REACTION OF 25D,5β-SPIROST-2-ENE[1]

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Received June 18, 1963

We have examined the addition reaction with peracid and osmium tetroxide on some C_{11}-substituted 25D,5β-spirost-2--enes. In contrast to the well-known fact that the addition of the reagent takes place from the β-side in the case of 25D,5β-spirost-2-ene without C_{11}-substituent, it was found that 11α-hydroxy- or 11-keto-25D,5β-spirost-2-ene gave products in which the double bond at C_2-C_3 was attacked mainly by the above-mentioned reagents from the α-side.

On the addition reaction to the Δ^2-double bond in unsubstituted steroidal sapogenins, it is well known that the reagent attacks from the α-side in the 5α-series and from the β-side in the 5β-series: for instance, in the 5α-series, epoxidation by peracid and cis-hydroxylation by osmium tetroxide give α-epoxide and 2α,3α-cis-diol respectively,[2] and in the 5β-series, the analogous reactions give β-epoxide and 2β,3β-cis-diol.[3] Our previous studies[4] also gave the following results: osmium tetroxide addition of the 11-keto, 11α- and 11β-hydroxy 5α-spirost-2-ene derivatives gave

2α,3α-cis-diols, and epoxidation by peracid of 5α,25D-spirost--2-en-11-one gave α-epoxide. In the case of the 5β-series,[5] it was found that epoxidation by peracid of 11α-acetoxy--25D,5β-spirost-2-ene gave β-epoxide.

In addition to these experiments, we examined the epoxidation of 11α-acetoxy- and 11α-hydroxy-25D,5α-spirost--2-ene. On epoxidation with perbenzoic acid in chloroform solution, 11α-acetoxy- and 11α-hydroxy-25D,5α-spirost-2-ene, (I) and (III), gave epoxides (II), m.p. 188°, and (IV), m.p. 197°, respectively in quantitative yield. By converting them into the same 3α,11α-diol (V), m.p. 214°, (its diacetate (VI), m.p. 188°) with lithium aluminium hydride and oxidizing this diol to the known 3,11-diketone (VII) with chromium trioxide-pyridine, it was proved that the configuration of the epoxide ring in both epoxides is α. Summarizing the above and the already reported results, the addition reaction to the Δ^2-double bond in 5α-spirostenes proceeds from the α-side regardless of the 11-substituent. (Chart 1)

But, on the other hand, from the O.R.D. curve[6] of 25D,5β-spirostane-2,11-dione the presence of some interaction between these two positions can also be expected. Then we studied the epoxidation and cis-hydroxylation of the 11α- and 11β-hydroxyl and the 11-keto 25D,5β-spirost--2-enes, and found that the 11α-hydroxyl and 11-keto sub-

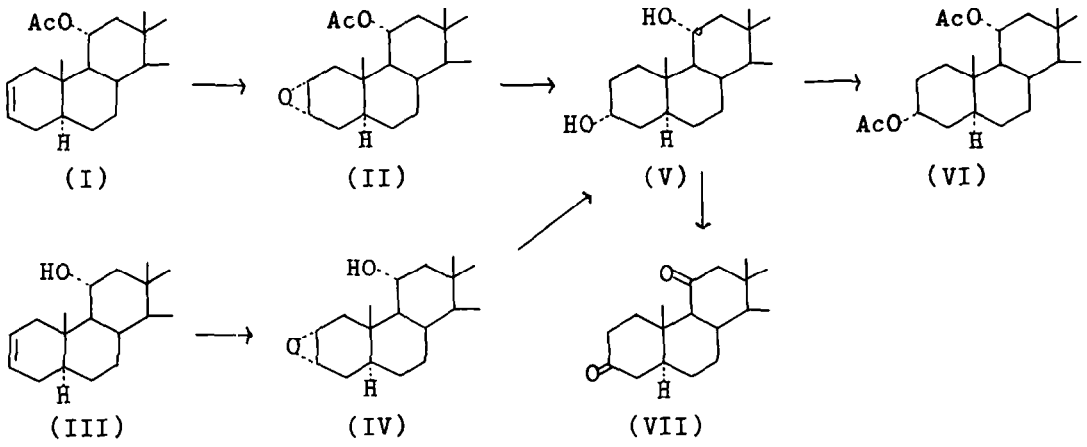


Chart 1

stituents had a directing effect on the addition of reagents.

On epoxidation by perbenzoic acid in chloroform solution, 11β-hydroxy-25D,5β-spirost-2-ene (VIII) gave an epoxide (IX), m.p. 180-182°, in high yield. This product was proved to be 2β,3β-epoxide by conversion to the known 3β-acetoxy-11--keto compound (X)[7] by lithium aluminium hydride reduction followed by acetylation and chromium trioxide-pyridine oxidation.

The 11α-hydroxyl compound (XI) gave a mixture of α- and β-epoxide on epoxidation by perbenzoic acid in chloroform solution. Lithium aluminium hydride reduction of this mixture gave two diols, m.p. 264° (diacetate (XIV), m.p. 196°), and m.p. 195° (diacetate, m.p. 213°), in yields of 59.7% and 39.8% respectively. As the former gave the known 2,11--diketone (XV),[8] m.p. 205°, by chromium trioxide oxidation,

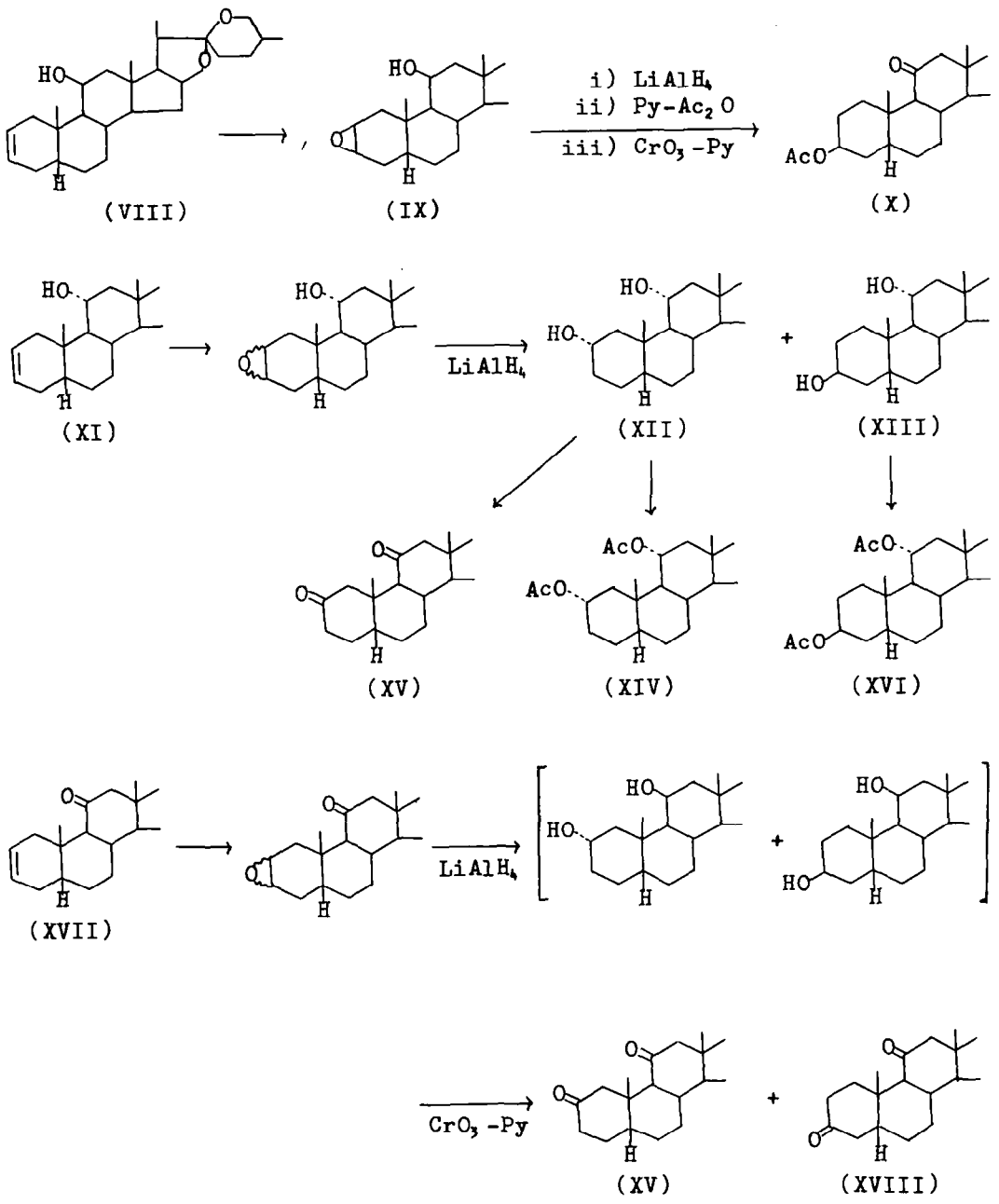


Chart 2

it is confirmed to be the 2α,11α-diol (XII).

The latter and its diacetate, were identified as the

known nogiragenin, 3β,11α-dihydroxy 25D,5β-spirostane (XIII),[5] and its diacetate (XVI)[5] by mixed melting point determination and comparison of I.R. spectra. From the yields of diols, it was estimated that the ratio of α- and β-epoxide is about 6:4.

In the case of the 11-keto compound (XVII), the product obtained by reaction with perbenzoic acid in chloroform was also a mixture of epoxides. In this case, as the diols obtained by lithium aluminium hydride reduction could not be separated, to convert them into diketones the reduction mixture was treated with chromium trioxide in pyridine and the 2,11-diketone (XV), m.p. 205.5°, and the known 3,11-diketone (XVIII)[5], m.p. 207°, were obtained in a yield of 34% and 16% respectively. The yields of these diketones are fairly low. However, judging from the ratio of the diketones thus obtained, it was presumed that the ratio of the α- and β--epoxide is about 7:3. The above-mentioned results are summarized in Table 1. (Chart 2)

Table 1

Product of the epoxidation of 11-substituted 25D,5β-spirost-2-ene with perbenzoic acid in chloroform solution

C-11-Substituent				
H_2[3]	β-OH	α-OAc[5]	α-OH	C=O
			α-Epoxide 6	α-Epoxide 7
β-Epoxide	β-Epoxide	β-Epoxide	β-Epoxide 4	β-Epoxide 3

As mentioned above, the 11α-hydroxyl and the 11-keto substituents showed directing effects on the addition reaction of perbenzoic acid to the C_2,C_3-double bond. In order to confirm these results and to examine the solvent effect, we repeated this reaction on the 11α-hydroxyl and the 11-keto compounds by using perlauric acid in cyclohexane or acetonitrile.

In every experiment, the products, mixtures of α- and β-epoxides, were immediately converted to the 2α,11α- and 3β,11α-diols by the reaction sequence as shown in Chart 2 and 3. The ratio of α- and β-epoxides was inferred from the yields of diols.

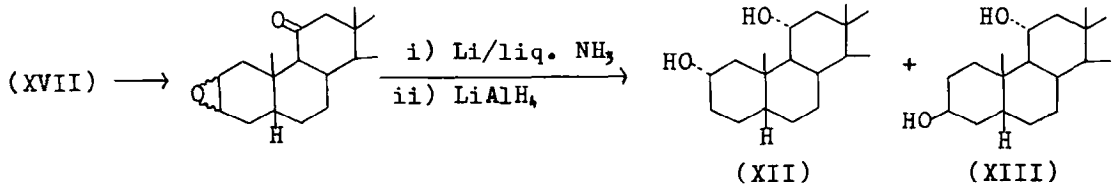


Chart 3

In the case of the 11α-hydroxyl compound,[9] when the reaction was carried out in cyclohexane solution, the yields of 2α,11α- and 3β,11α-diols were about 80% and 3% respectively (the ratio of α:β was 9.6:0.4): the α-epoxide is predominantly produced in cyclohexane solution. However, when acetonitrile was used as a solvent, yields of the 2α,11α- and 3β,11α-diols were about 29% and 46% respectively (the ratio of α:β was

3.9:6.1): the formation of β-epoxide is increased in acetonitrile solution. (Table 2)

In the case of the 11-keto compound,[9] attack of the reagent seems to be effected mainly from the α-side. Thus, in the reaction of cyclohexane solution, the isolated product was only the 2α,11α-diol and no 3β,11α-diol could be detected from the mother solution by paper-chromatography. In the case of acetonitrile solution, the 3β,11α-diol was also obtained, but its yield (ca 26%) was only half of that (ca 53%) of 2α,11α-diol (the ratio of α:β was 6.7: 3.3). From these experiments, the solvent effect was observed to exist between the yield of each epoxide and the used solvent.

Summarizing the above results, we wish to propose the following factors to explain the abnormal addition reaction of the 11α-hydroxyl and 11-keto Δ^2-steroids in the 5β-series.

In the case of the 11α-hydroxyl compound:

As infrared data of 11α-hydroxyl compounds show a weak

Table 2

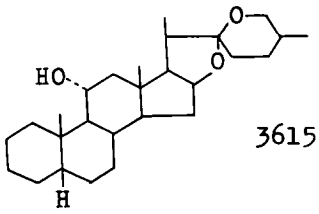
The ratio of epoxides on the epoxidation of 11-substituted 25D,5β-spirost-2-ene with perlauric acid in cyclohexane or acetonitrile solution

	Reaction in cyclohexane α : β	Reaction in acetonitrile α : β
11α-hydroxy compound	9.6 : 0.4	3.9 : 6.1
11-keto compound	10 : 0	6.7 : 3.3

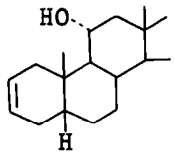
hydrogen bonding between α-hydroxyl group and C_2,C_3-double bond, a close proximity of the 11α-hydroxy group and the Δ^2--double bond is supposed in the 5β-steroids. (Table 3) Further, as mentioned above, the ratio of products depends on the polarity of the solvents. From these points, it is reasonable to consider that the hydrogen bond formation between the 11α-hydroxyl group and the reagent controls the direction of attack of the reagent (Fig. 1). In the non-polar solvent favourable to hydrogen bonding, the proportion of α-epoxide increases.

Table 3

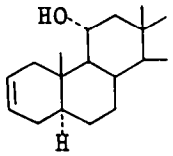
Infrared absorption spectra
of 11α-hydroxyl compounds (in carbon tetrachloride)



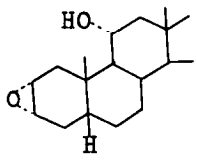
3615 cm^{-1}



3600 cm^{-1}



3616 cm^{-1}



3585 cm^{-1}

In the case of the 11-keto compound:

In the olefin-peracid reaction, it has been proposed[10] that the molecule of peracid forms a spiro, chelated structure in the transition state. However, if the compound has a

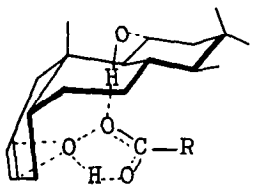


Fig. 1

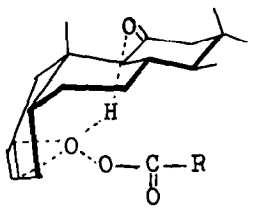


Fig. 2

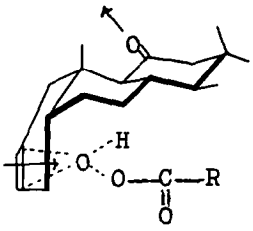


Fig. 3

carbonyl group in the molecule, the carbonyl group is able to make the hydrogen bonding with the peracid. Therefore, the possibility that the hydrogen bond between 11-keto group and the peracid influences the direction of attack of the reagent cannot be excluded (Fig. 2). There also should be considered the existance of the following possibility in this case: Examination of the Dreiding model shows the direction of the dipole of the carbonyl group to be not perpendicular to that of the transition state of the double bond peracid, but to slant slightly. Therefore, rear side attack may be favoured by the relative size of the dipole-dipole interaction between the polar substituent and the polar transition state (Fig. 3). Furthermore, it is probable that distortion of the A ring, due to the trigonal bond of the carbonyl group, makes rear side attack of the reagent easier.

The above-mentioned abnormality was also observed in the osmium tetroxide addition. (Chart 4)

On the reaction in benzene solution containing pyridine,

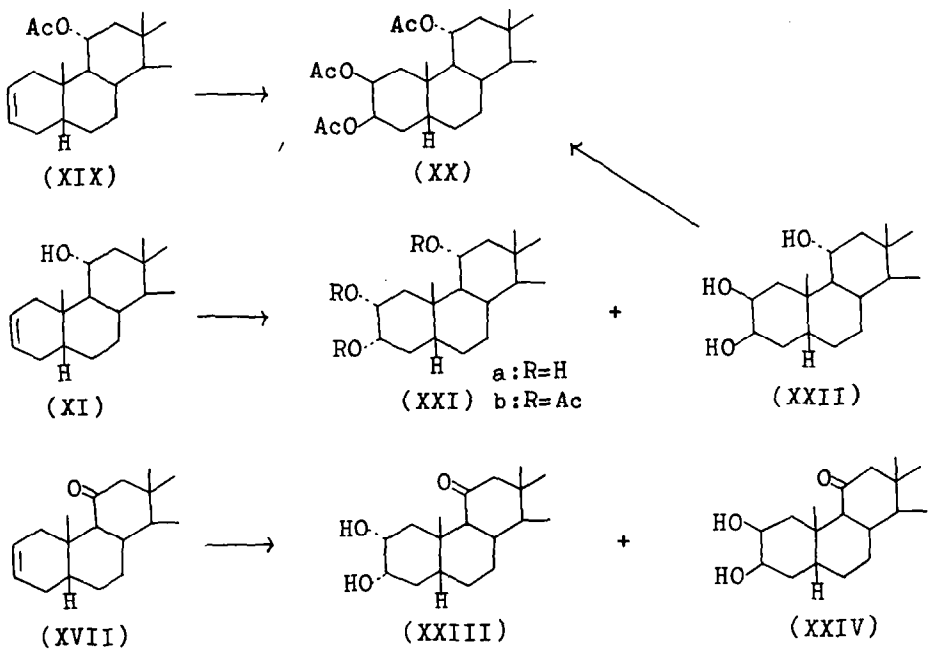


Chart 4

the 11α-acetoxy compound (XIX) gave only the β-cis-diol. After acetylation, the 2β,3β,11α-triacetoxy compound (XX), m.p. 249-252°, was obtained in a 90% yield. With the 11α--hydroxyl- and 11-keto compounds, α-cis- and β-cis-diols were produced in a ratio of 4.5:5.5 and 2:1, respectively. (Table 4)

Table 4

The products of the osmium tetroxide addition of 11-substituted 25D,5β-spirost-2-ene

C-11 Substituents			
H_2	α-OAc	α-OH	C=O
		α-cis-diol 4.5	α-cis-diol 2
β-cis-diol	β-cis-diol	β-cis-diol 5.5	β-cis-diol 1

With the 11α-hydroxyl compound, we examined the solvent effect in this reaction by using cyclohexane and acetonitrile. On the basis of the yields of isolated crystalline products, the ratio did not show a remarkable difference according to the solvents used. Furthermore, as an oily mixture containing both isomers was produced in an appreciable amount, we could not judge correctly the influence of the solvent. At present, we are not yet at the stage where we can explain clearly the abnormality of 11α-hydroxyl and 11-keto compounds in osmium tetroxide addition reaction.

EXPERIMENTAL

All melting points are uncorrected. Infrared spectra were measured with a Koken IR spectrophotometer, Model DS-301. Optical rotations were measured in chloroform solution with a Rudolf Photoelectronic Polarimeter, Model 200.

The following procedure was used unless otherwise noted.

(i) Epoxidation:

(a) With perbenzoic acid: A solution of perbenzoic acid in chloroform was added to a solution of the Δ^2-compound in chloroform. After standing for 24 hours at 3-5°, the reaction mixture was diluted with ether, and the organic layer was washed successively with 5% sodium iodide, 5% sodium thiosulfate, 5% sodium carbonate and then water, dried over sodium sulfate and evaporated.

(b) With perlauric acid in cycloxane solution: The reaction mixture was treated in the same manner as in the case of (a).

(c) With perlauric acid in acetonitrile solution: The reaction mixture was concentrated at room temperature under reduced pressure and treated with water and ether. The ether extract was treated in the same manner as in the case of (a).

(ii) Lithium aluminium hydride reduction:

Lithium aluminium hydride was added to a solution of epoxide in dried ether and the mixture was refluxed for 4 hours.

After destroying the excess reagent with a small amount of water, the reaction mixture was washed successively with 5% hydrochloric acid, water, 5% sodium carbonate and water, dried over sodium sulfate and evaporated.

(iii) Oxidation with chromium trioxide-pyridine complex:

To a solution of a hydroxy compound in pyridine, a solution of chromium trioxide in pyridine was added. After standing for 18 hours at room temperature the mixture was poured into cold water and extracted with ether. The ether solution was washed successively with 5% hydrochloric acid, water, 5% sodium carbonate and water, dried over sodium sulfate and evaporated.

Epoxidation of 11α-acetoxy-25D,5α-spirost-2-ene(I). Two hundred milligrams of (I) and 206 mg of perbenzoic acid gave 207 mg of (II), m.p. 185-187°. Recrystallization from chloroform-hexane gave an analytical sample as prisms, m.p. 188--188.5°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.49; H, 9.46. $IR\nu_{max}^{Nujol}$ cm^{-1}: 1729, 1248 (OAc), 811 (epoxide).

Epoxidation of 25D,5α-spirost-2-en-11α-ol (III) with perbenzoic acid. Two hundred milligrams of (III) and 206 mg of perbenzoic acid gave 205 mg of 2α,3α-epoxide (IV) as needles, m.p. 192-195°. Recrystallization from chloroform-hexane gave an analytical sample, m.p. 195.5-197°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.38; H, 9.69. $IR\nu_{max}^{Nujol}$ cm^{-1}: 3562 (OH), 808 (epoxide).

25D,5α-Spirostane-3α,11α-diol (V) and its diacetate (VI).

(a) From epoxide-alcohol (IV). Reduction of 118 mg of (IV) with 118 mg of lithium aluminium hydride gave 123 mg of crude product. Purification of this product through an alumina column gave 105 mg of (V), m.p. 195-199°. Recrystallization from chloroform-petroleum ether gave an analytical sample, m.p. 212-214°. $[\alpha]_D^{16}$ -74.3° (C 1.049).

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.74; H, 10.43. $IR\nu_{max}^{Nujol}$ cm^{-1}: 3518, 3448, 3366.

Acetylation of this diol with boiling acetic anhydride gave the diacetate, m.p. 187.5-188.5°. $[\alpha]_D^{17}$ - 71.1° (C 0.999).

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.05; H, 9.36. Found: C, 72.06; H, 9.44. $IR\nu_{max}^{Nujol}$ cm^{-1}: 1724, 1248.

(b) From epoxide-acetate (II). Reduction of 185 mg of (II) with 185 mg of lithium aluminium hydride gave 196 mg of crude crystals. Recrystallization from chloroform-petroleum ether

gave a pure (V), m.p. 206-208°. This crystal did not show depression in mixed melting point with the sample obtained from epoxide alcohol.

25D,5α-Spirostane-3,11-dione (VII). Oxidation of 73 mg of (V) with 73 mg of chromium trioxide in pyridine and purification of the product, m.p. 222-232°, through an alumina column gave 41 mg of (VII), m.p. 237-241°. Recrystallization from chloroform-petroleum ether gave an analytical sample, m.p. 241-242°. $[\alpha]_D^{20}$ -16.3° (C 1.036).

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.65; H, 9.40. Found: C, 75.65; H, 9.53. IR ν_{max}^{Nujol} cm^{-1}: 1713, 1694.

Epoxidation of 25D,5β-spirost-2-en-11β-ol (VIII) with perbenzoic acid. Eight hundred milligrams of (VIII) and 840 mg of perbenzoic acid gave 873 mg of product, m.p. 148-157°. Recrystallization from chloroform-hexane gave 675 mg of (IX), m.p. 166-170°. This was further recrystallized to give an analytical sample, m.p. 180-182°.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 75.31; H, 9.83. Found: C, 75.25; H, 10.01. IR ν_{max}^{Nujol} cm^{-1}: 3562 (OH), 812 (epoxide).

25D,5β-Spirostane-3β,11β-diol 3 monoacetate. Six hundred and fifty milligrams of (IX) was reduced with 650 mg of lithium aluminium hydride. Acetylation of the product with acetic anhydride and pyridine followed by purification through an alumina column gave 484 mg of the diol-monoacetate. Recrystallization from methanol gave a pure sample, m.p. 177--178°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.27; H, 9.68. IR ν_{max}^{Nujol} cm^{-1}: 3580 (OH), 1727, 1252 (OAc).

3β-Acetoxy-25D,5β-spirostan-11-one (X). The above diol monoacetate (470 mg) was treated with 470 mg of chromium trioxide in pyridine. Purification of the product, m.p. 160--168°, 424 mg, through an alumina column gave 313 mg of crystals, m.p. 170-174°. Recrystallization from chloroform--methanol gave a pure sample, m.p. 175-177°. This was identified as (X) by comparison of IR spectra and mixed melting point with an authentic sample.

Epoxidation of 25D,5β-spirost-2-en-11α-ol (XI) with perbenzoic acid. The 2-en-11α-ol (XI) (432 mg) and 452 mg of perbenzoic acid gave 475 mg of crude crystals, m.p. 184--192°. Treatment of 200 mg of these crystals with 200 mg of lithium aluminium hydride gave 204 mg of crude crystals, m.p. 236-245°. Recrystallization from chloroform-methanol gave 122 mg of (XII) as needles, m.p. 262-264°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.70; H, 10.25.

From the mother liquor was obtained 82 mg of (XIII), m.p. 192-195°, which was identified as nogiragenin by comparison of IR spectra. After refluxing with acetic anhydride, this gave a diacetate (XVI), m.p. 211-213°, which was identified as nogiragenin diacetate by mixed melting point and comparison of IR spectra.

2α,11α-Diacetoxy-25D,5β-spirostane (XIV). Acetylation of 60 mg of (XII) with acetic anhydride under refluxing gave 65 mg of the crude product. Purification of this product through an alumina column gave 33 mg of (XIV), m.p. 192-193°. Recrystallization from methanol gave a pure sample, m.p. 195--196°. $[\alpha]_D^{16}$ -99.5°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.05; H, 9.36. Found: C, 72.09; H, 9.43. IR ν_{max}^{Nujol} cm^{-1}: 1730, 1738.

25D,5β-Spirostane-2,11-dione (XV). The 2α,11α-diol (XII) (122 mg) was treated with 122 mg of chromium trioxide in pyridine. Purification of the crude product, m.p. 196-200°, 114 mg, through an alumina column gave 102 mg of (XV), m.p. 204-205°. Recrystallization from chloroform-petroleum ether afforded an analytical sample, m.p. 204-205.5°, $[\alpha]_D^{23}$ -30.6°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.65; H, 9.40. Found: C, 75.67; H, 9.24. IR ν_{max}^{Nujol} cm^{-1}: 1710 (C=O).

This compound did not show depression in mixed melting point with an authentic sample.

Epoxidation of 25D,5β-spirost-2-en-11-one (XVII) with perbenzoic acid. The 2-en-11-one (XVII) (327 mg) and 343 mg of perbenzoic acid gave 348 mg of a mixture of epoxides, m.p. 189-193°. Treatment of 200 mg of this mixture with 200 mg of lithium aluminium hydride gave 215 mg of a non-crystalline product. Oxidation of 178 mg of this product with 178 mg of chromium trioxide in pyridine followed by purification through an alumina column gave 113 mg of crystals, m.p. 178-185°. By recrystallization from chloroform-petroleum ether this gave 67 mg of (XV), m.p. 203-204°, and 31 mg of (XVIII), m.p. 204-207°. Both products did not show depression in mixed melting point with their respective authentic samples.

Epoxidation of 25D,5β-spirost-2-en-11α-ol (XI) with perlauric acid. (a) In cyclohexane solution. To a solution of 50 mg of (XI) in 26 ml of dried cyclohexane, 29 mg of perlauric acid (purity, 96.8%) was added and the mixture was allowed to stand for 95 hours at room temperature. The obtained product (48 mg) was reduced in dried tetrahydrofuran and ether with 50 mg of lithium aluminium hydride. By recrystallization and alumina chromatography, the obtained residue (46 mg) was separated into 1.8 mg of the starting

material (XI), 37 mg of (XII), m.p. 262-264°, 1.5 mg of (XIII), m.p. 188-191° and 5.5 mg of the mixture of (XII) and XIII).

(b) In acetonitrile solution. A solution of 100 mg of (XI) in 25 ml of acetonitrile was treated with 108 mg of peracid for 152 hours at room temperature. The product, 112 mg, was reduced in dried tetrahydrofuran and ether with 115 mg of lithium aluminium hydride. By alumina chromatography and recrystallization, 105 mg of crystalline product was separated into 6 mg of starting material (XI), 27.5 mg of (XII), m.p. 248-253°, and 42.5 mg of (XIII), m.p. 187-190°.

Epoxidation of 25D,5β-spirost-2-en-11-one (XVII) with perlauric acid. (a) In cyclohexane solution. A solution of 51.5 mg of (XVII) in 16 ml of dried cyclohexane was treated with 52.4 mg of perlauric acid (purity, 82.4%) for 93 hours at room temperature and 62 mg of crystalline residue, m.p. 169-173°, was obtained.

To a lithium liq. ammonia solution (prepared from 59.2 mg of lithium in 20 ml of dried liq. ammonia) was added dropwise a solution of 61 mg of epoxide in 5 ml of abs. ether and 0.3 ml of abs. ethanol over a period of 2 min. After 15 min. the excess of lithium was destroyed by 0.4 ml of abs. ethanol. After evaporation of ammonia, the reaction mixture was treated with water and ether. Evaporation of the ether solution gave 63 mg of residue.

By alumina chromatography and recrystallization, 63 mg of residue was separated into 7 mg of (XI), m.p. 150-165°, 35 mg of (XII), m.p. 260-268°, 7 mg of oily product containing of (XI) and 8 mg of oily product containing of (XII).

(b) In acetonitrile solution. A solution of 70 mg of (XVII) in 20 ml of acetonitrile was treated with 73.4 mg of perlauric acid for 161 hours at room temperature and 74 mg of product was obtained.

A solution of 74 mg of epoxide in 5 ml of abs. ether and 0.4 ml of abs. ethanol was added dropwise to a solution of lithium liq. ammonia (prepared from 71.8 mg of lithium and 30 ml of dried liq. ammonia) over a period of 2 min. under stirring. After 5 min. 0.4 ml of abs. ethanol was added. After an additional 5 min. the excess of lithium was destroyed with abs. ethanol. After treating in the same manner as in the case of (a) the reaction mixture gave 76.5 mg of residue. This was reduced in dried tetrahydrofuran and ether with 95 mg of lithium aluminium hydride. By recrystallization, alumina chromatography and preparative paper chromatography, 79 mg of product was separated into 10 mg of (XI), 32 mg of (XII), m.p. 263-266° and 15.5 mg of (XIII), m.p. 180-190°.

Osmium tetroxide addition of 11α-acetoxy-25D,5β-spirost--2-ene (XIX). One hundred milligrams of osmium tetroxide was added to a solution of 100 mg of (XIX) in 3 ml of pyridine and 5 ml of abs. benzene and the mixture was allowed to stand for 72 hours at room temperature. The reaction mixture was evaporated under reduced pressure and refluxed with a benzene--methanol solution of mannitol and potassium hydroxide. After refluxing for 4 hours, the organic layer was separated and the water solution extracted with benzene. Evaporation of the combined benzene solution gave 106 mg of product, m.p. 267-269°. This was acetylated with boiling acetic anhydride. Purification of the product through an alumina column gave 113 mg of (XX), m.p. 244-247°, which was identified as metagenin triacetate[11] by mixed melting point and comparison of IR spectra.

Osmium tetroxide addition of 25D,5β-spirost-2-en-11α--ol (XI). (a) In benzene solution containing pyridine. To a solution of 100 mg of (XI) in 5 ml of benzene and 3 ml of pyridine, 100 mg of osmium tetroxide was added. The mixture was treated in the same manner as in the case of (XIX) and the obtained crude triol, 109 mg, m.p. 258-265°, was purified through a column of alumina. The chloroform--methanol (9:1) eluate (79 mg) was separated into 34 mg of (XXIa),[12] m.p. 216-218°, and 42 mg of metagenin (XXII),[11] m.p. 265-269°, by recrystallization from chloroform-methanol. Both compounds were acetylated with pyridine-acetic anhydride and gave triacetates (XXIb),[12] m.p. 236-237°, and metagenin triacetate (XX), m.p. 245-249°, respectively. All these products were identified by mixed melting point and comparison of IR data with authentic samples.

(b) In cyclohexane solution. To a solution of 70 mg of (XI) in 25 ml of cyclohexane, 49 mg of osmium tetroxide was added. After standing 41 hours at room temperature, the solvent was evaporated without heating and the residue was treated in the same manner as in the case of (XIX). The product (84 mg) was chromatographed through a column of Florisil. The benzene-chloroform (2:1, 1:1) and chloroform eluates (53 mg) gave 13.5 mg of (XXII), m.p. 258-263°, by recrystallization from ether and then chloroform-methanol. From the mother liquor, 9 mg of (XXIa), m.p. 211-214°, was obtained by recrystallization from ether-hexane. The ether and chloroform--methanol (10:1) eluates gave 20 mg of (XXII), m.p. 254-263°. All these products were identified by paper chromatography and comparison of IR spectra with authentic samples.

(c) In acetonitrile solution. A solution of 70 mg of (XI) in 25 ml of acetonitrile was treated with 49 mg of osmium tetroxide in the same manner as in the case of (b). The product (68 mg) was crystallized from ether and recrystallized

from methanol-chloroform to give 31 mg of (XXII), m.p. 257--273°, and 11 mg of a crystalline mixture. The former was identified as metagenin by comparison of IR spectra with an authentic sample. Its mother liquor gave 3 mg of (XXIa), m.p. 211-213°, by crystallization from ether-hexane, which was also identified by comparison of IR spectra and paper chromatography with an authentic sample.
Eleven milligrams of a crystalline mixture was separated into 2.7 mg of (XXIa) and 4 mg of (XXII) by preparative paper chromatography (solvent system: xylene 150: acetic acid 8) and both products were identified by paper chromatography respectively.

Osmium tetroxide addition of 25D,5β-spirost-2-en-11-one (XVII). To a solution of 51 mg of (XVII) in 2.5 ml. of abs. benzene and 1.5 mlof pyridine, 51 mg of osmium tetroxide was added. After standing for 65.5 hours, hydrogen sulfide gas was induced into the reaction mixture for 1.25 hours. After filtration, the filtrate was washed with 5% hydrochloric acid, water, 5% sodium carbonate and then water, dried over sodium sulfate and evaporated to give 49 mg of residue. This residue was chromatographed through a column of alumina (Woelm III). The benzene and benzene-chloroform (3:1) eluates gave 6 mg of oily product. The benzene-chloroform (1:1), chloroform and ether eluates gave 10 mg of crude crystals, which was recrystallized from acetone-petroleum ether to give 4 mg of (XXIII), m.p. 222-226°.[12] The ether eluate gave 23 mg of crude product, which was crystallized from petroleum ether to give 3 mg of metagenone (XXIV),[11] m.p. 237-242°. The above oily fraction and the mother liquor of recrystallization were combined and rechromatographed.

The benzene-chloroform (1:1) eluate gave 11 mg of crystals of (XXIII). The benzene-chloroform (1:2) and chloroform eluates gave a mixture of (XXIII) and (XXIV), in which the ratio of both compounds was estimated to be about 5:3 on the basis of the area of spots of the paper chromatogram.

The chloroform-ether and ether eluates gave 2 mg of (XXIV) as crystals. Both products, (XXIII) and XXIV), were identified by comparison of IR spectra and paper chromatography with authentic samples respectively.

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