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STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—XXI[1]

THE STRUCTURE OF KOGAGENIN (PART 3) ON ANHYDROKOGAGENIN

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Abstract— In an earlier paper[2] of this series, we assigned the position of the double bond in anhydrokogagenin, a dehydration product of kogagenin, at C-5 and 6 tentatively. This has now been confirmed to be located at C-4 and 5 from the results of the epoxide formation, manganese dioxide oxidation and the glycol fission reaction. It has been found that the reaction of anhydrokogagenin with acetone containing a small amount of *p*-toluenesulphonic acid gives an allylic rearrangement product together with the normal acetonide.

KOGAGENIN is a tetrahydroxysapogenin isolated from the epigeous part of *Dioscorea Tokoro*, Makino and its structure was established as 25D-spirostane-1β,2β,3α,5β-tetrol (Ia).[1,2]

During the course of investigation of this structure, anhydrokogagenin triacetate (IIb), m.p. 171 173°, which was obtained by dehydration of kogagenin triacetate with thionyl chloride in pyridine, played an important role in the structural elucidation of kogagenin.

In the earlier paper,[2] the double bond in anhydrokogagenin triacetate (IIb) was assigned tentatively to the C-5 position merely from the fact that anhydrokogagenin (IIa) gave a negative Rosenheim test. Little attention was paid as to whether its double bond was located at C-4 or at C-5, because this was unnecessary for the elucidation of the structure of kogagenin.

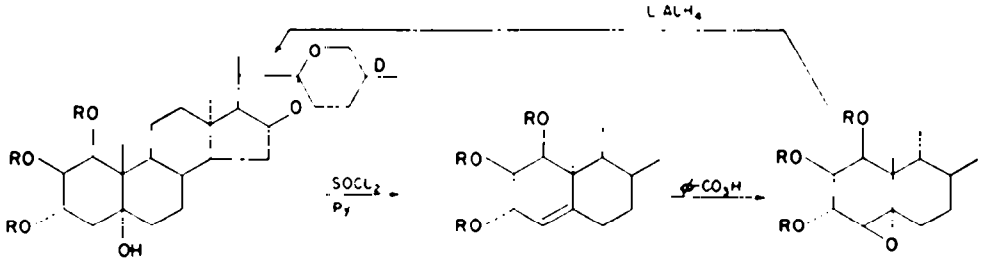
The results of epoxidation of IIb, as described below, showed the possibility of the double bond being at C-4 and prompted investigation to clarify the position of this double bond in anhydrokogagenin (IIa).

Treatment of anhydrokogagenin triacetate (IIb) with perbenzoic acid afforded an epoxide, m.p. 151–153°, which was reduced with lithium aluminium hydride and yielded kogagenin, having the 5β-hydroxyl group as established in the preceding paper,[1] as a sole product.

From these findings it was assumed that the parent triacetate-epoxide must be the β-epoxide. Moreover, in view of the fact that the reduction of steroidal 5β,6β-epoxides with lithium aluminium hydride affords a quantity of 5β-alcohols in addition to 6β-alcohols[3] while 4β,5β-epoxides give only 5β-alcohols,[4] the above results suggested that the double bond in anhydrokogagenin triacetate (IIb) may be located at C-4.

[1] Part XX: T. Kubota, *Chem. Pharm. Bull.* 7, 898 (1959).
[2] K. Takeda, T. Kubota and A. Shimaoka, *Tetrahedron* 7, 62 (1959).
[3] Pl. A. Plattner, H. Heusser and M. Feurer, *Helv. Chim. Acta* 32, 587 (1949); A. S. Hallsworth and H. B. Henbest, *J. Chem. Soc.* 4604 (1957).
[4] Pl. A. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta* 31, 1885 (1948); C. W. Shoppee, M. E. H. Howden, R. W. Killick and G. H. R. Summers, *J. Chem. Soc.* 630 (1959).

On the other hand, Henbest and Wilson[5] reported that the epoxide formation of cyclic allylic alcohols occurs from the same side of this hydroxyl group. On the basis of this fact, perbenzoic acid oxidation of free anhydrokogagenin (IIa) was carried out in order to obtain the anticipated α-epoxide, contrary to the case of IIb. Although there was obtained a single epoxide melting at 274 276°, this product was identical in all respects with the β-epoxide (IIIa) which was obtained on saponification of the triacetate-β-epoxide (IIIb) prepared from IIb. Therefore, epoxidation of anhydrokogagenin (IIa) gave no support to the possibility of this compound possessing the allylic hydroxyl function.



Ia R=H b R=Ac IIa R=H b R=Ac IIIa R=H b R=Ac

CHART I

In order to determine the presence of an allylic hydroxyl group in IIa, this was subjected to manganese dioxide oxidation. When progress of the oxidation was checked by ultra-violet spectra, in the early stage (30 min) of this experiment, it showed a maximum of ε 7000 at 244 mμ indicating the formation of the expected Δ^4-3-ketone system. The intensity of this absorption did not increase with the lapse of oxidizing time and a new absorption at 282 mμ of low intensity appeared. After the oxidation was allowed to proceed for a longer time (6 hr), the absorption at 244 mμ disappeared and that at 282 mμ rose to ε 5600. This observation suggested that the primary product considered to be a Δ^4-3-one derivative must be further attacked by manganese dioxide.

When manganese dioxide oxidation of anhydrokogagenin (IIa) was stopped after 45 min at 0°, a product expected to be the Δ^4-3-ketone (IV), m.p. 202–204°, showing λ_{max} 244 mμ (log ε 4·10) could be isolated with purification by chromatography on silica gel. This product was identical with the dihydroxy-Δ^4-3-ketone (IV) which was obtained from the previously reported compound (VI)[2] by acid hydrolysis.

Since it has now been proved that the primary oxidation product is the dihydroxy-Δ^4-3-ketone (IV), the further oxidation product showing absorption at 282 mμ was studied. Rosenkranz *et al.*[6] reported that, when Δ^4-3-ketones or Δ^5-3β-ols were treated with manganese dioxide for a long time, each compound afforded $\Delta^{4,6}$-diene-3-ones having the maximum at 284 mμ. The oxidation product of IIa showed

[5] H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.* 1958 (1957).
[6] F. Sondheimer, C. Amendolla and G. Rosenkranz, *J. Amer. Chem. Soc.* 75, 5932 (1953).

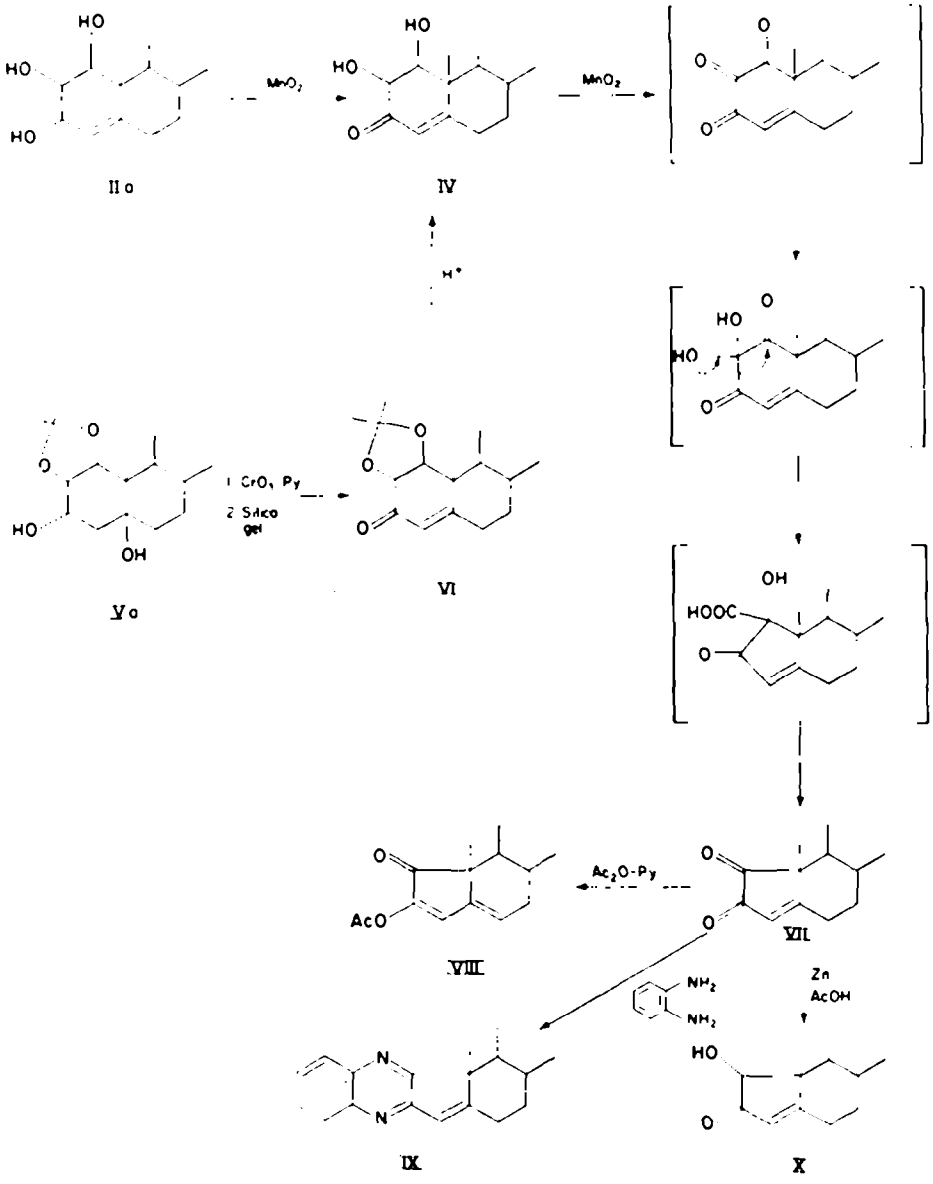


CHART 2

constants differing from those of the expected $\Delta^{4,6}$-diene-3-one compound, as described below. The analytical values agreed with an empirical formula $C_{26}H_{36}O_4$ and suggest the loss of CH_4O from the primary product (IV). Its ultra-violet spectrum showed absorption maxima at 230 and 282 mμ (log ε 3·60 and 3·75, respectively) and the infra-red spectrum exhibited strong bands at 5·69, 5·80 and 6·22 μ but no hydroxyl band. From the basis of these data, the structure of this compound was presumed

to be the five-membered ene-dione (VII). This was supported by the following chemical behaviour: (a) acetylation with refluxing acetic anhydride–pyridine gave an enol acetate (VIII) showing the ultra-violet absorption at 299 mμ (log ε 3·97); (b) reaction with *o*-phenylenediamine afforded a quinoxaline derivative (IX), $C_{32}H_{40}O_2N_2$; (c) reduction with zinc and acetic acid gave an unsaturated ketol (X) which showed ultra-violet absorption maximum at 237 mμ (log ε 4·16), the infra-red bands at 2·86 μ (hydroxyl) and at 5·85 and 6·17 μ (five-membered unsaturated ketone), and a positive triphenyltetrazolium test. The formation of the five-membered ring in VII perhaps resulted from hydration of a triketone, which would be probably produced by oxidation of the ketol function in the dihydroxy-Δ^4-3-ketone (IV) with manganese dioxide, followed by the benzilic acid rearrangement as shown in Chart 2. However such compounds were hitherto unknown and the elucidation of the above-mentioned ultra-violet absorption remained doubtful.

From the above results it became certain that IIa possesses the double bond at C-4. In order to obtain more convincible evidence for the structure IIa of anhydrokogagenin, cleavage reaction of the double bond was carried out. Anhydrokogagenin triacetate (IIb) was *cis*-hydroxylated to a pentol triacetate (XI) with osmium tetroxide as described previously.[2] The newly introduced glycol function in XI has been assigned the β-configuration by analogy to the results on the above-mentioned epoxide formation and catalytic reduction.[2] When the pentol triacetate (XI) was treated with lead tetracetate, one equivalent of the reagent was consumed to yield the 4,5-secoaldehyde-ketone (XIII), m.p. 187 190°.[7] By oxidation with chromium trioxide, this aldehyde-ketone (XIII) led to an amorphous triacetoxy-keto-acid (XVb). When XVb was treated with a 1 per cent methanolic potassium hydroxide solution, besides an amorphous product assumed to be a trihydroxy-keto-acid (XVa), a small amount of a neutral product, m.p. 156 158°, having an empirical formula $C_{23}H_{36}O_3$ was obtained. The infra-red spectrum of this neutral product showed, except for an absorption at 5·84 μ corresponding to a six-membered ketone, neither another carbonyl band nor a hydroxyl band. Also, treatment of this compound with hydroxylamine gave an oxime, $C_{23}H_{37}O_3N$, m.p. 217°. Based on the above results the structure of this ketone has been assigned to be a des-A-5-ketone (XVI) which may be produced via retro-aldol reaction[8] of the trihydroxy-4,5-secoketo-acid (XVa) derived from the Δ^4-compound. When the triacetoxy-keto-acid (XVb) was treated with alkali under more vigorous conditions, it gave the same XVI in good yield, as would be expected. The methyl group at C-10 in XVI has been assigned to the more stable α-configuration (i.e. equatorial) by the analogous example.[9]

Furthermore, the pentol (XII) prepared from saponification of XI underwent glycol fission with lead tetracetate and yielded the expected trisnor-1,5-secoaldehyde-ketone (XIV), $C_{24}H_{36}O_4$, m.p. 222 225° (decomp). Since it is well-known that such formyl ketones are sensitive to alkali or acid,[10] by treatment with alcoholic potassium hydroxide solution, XIV was easily converted to the above-mentioned des-A-5-ketone (XVI).

These results have now confirmed that the double bond in anhydrokogagenin is

[7] This compound was amorphous in the previous experiment.[2]
[8] C. Djerassi and H. G. Monsimmer, *J. Amer. Chem. Soc.* **79**, 2901 (1957).
[9] J. Castells, E. R. E. Jones, G. D. Meakins and R. W. J. Williams, *J. Chem. Soc.* 1159 (1959).
[10] A. L. Wilds and C. Djerassi, *J. Amer. Chem. Soc.* **68**, 1715 (1946).

located at C-4. Therefore, the formulae described in the previous paper for anhydrokogagenin and its derivatives should now be corrected to Δ^4-25D-spirostene-1β,2β,3α-triol (IIa) and its derivatives. Assuming that ionic elimination readily occurs between *trans*-diaxial function, it seems to be reasonable that dehydration of kogagenin triacetate (Ib) with thionyl chloride–pyridine furnished exclusively the Δ^4-compound (IIb), because the 5β-hydroxyl group is axial to ring A but equatorial to ring B.

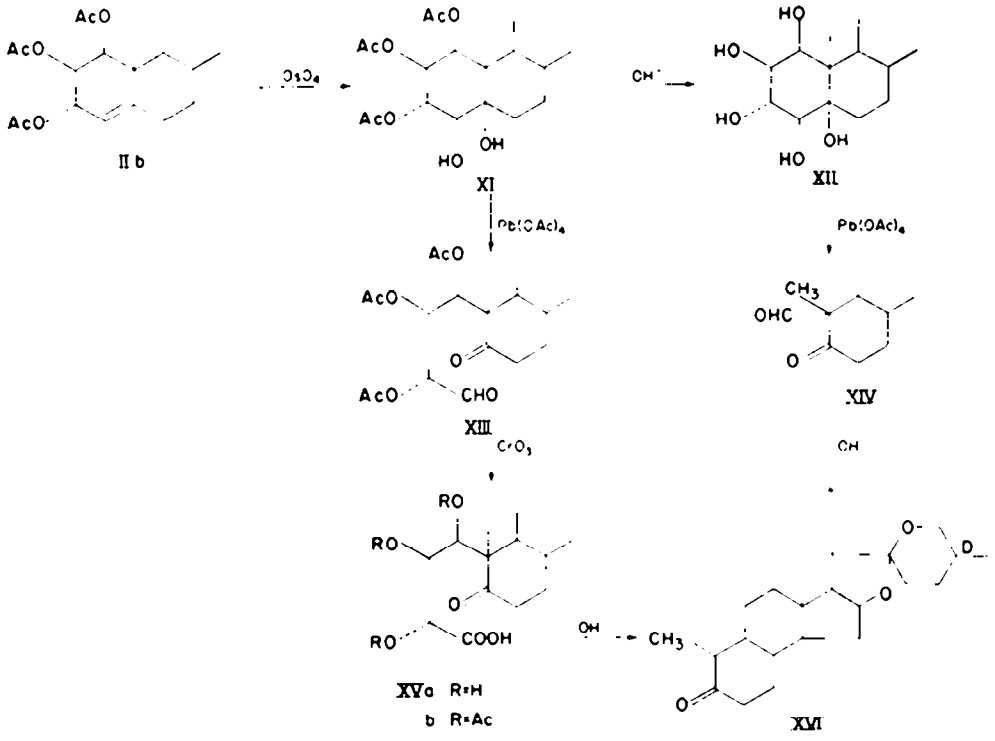


CHART 3

At present, with the constitution of anhydrokogagenin having been established, it is more difficult to understand why the so-called anhydrokogagenin acetonide, which was obtained on treatment of IIa with acetone and *p*-toluenesulphonic acid, was unaffected with chromium trioxide pyridine, chromium trioxide–acetone–sulphuric acid or by the Oppenauer oxidation, as described earlier.[2] Thus, in order to clarify this question, the reaction of acetonide formation was reinvestigated.

Anhydrokogagenin (IIa) was refluxed in acetone containing a small amount of *p*-toluenesulphonic acid. Chromatography of the product over alumina afforded, as described in the previous experiment,[2] a small amount of the acetonide diene (XVII) and a main product (A), m.p. 208–210°, previously assigned to be anhydrokogagenin acetonide (XVIIIa) on the basis of its analytical values and infra-red spectrum. Further elution of the above chromatography gave another isomer (B) having a melting point of 227–230°. The analytical values for the isomer (B) are in

good agreement with the formula $C_{30}H_{46}O_5$ corresponding to XVIIIa. Its infra-red spectrum showed the bands at 2·83 μ (hydroxyl), 6·00 μ (double bond) and at 7·98, 8·12 and 8·23 μ (acetonide) and satisfied the structure XVIIIa. The ultra-violet spectra of the two isomers, (A) and (B), were examined. The intensity of the absorption maximum at 206 mμ due to the double bond, for the isomer (A) was merely ε 880, in contrast with the ε 6050 and ε 5100 for anhydrokogagenin (IIa) and the isomer

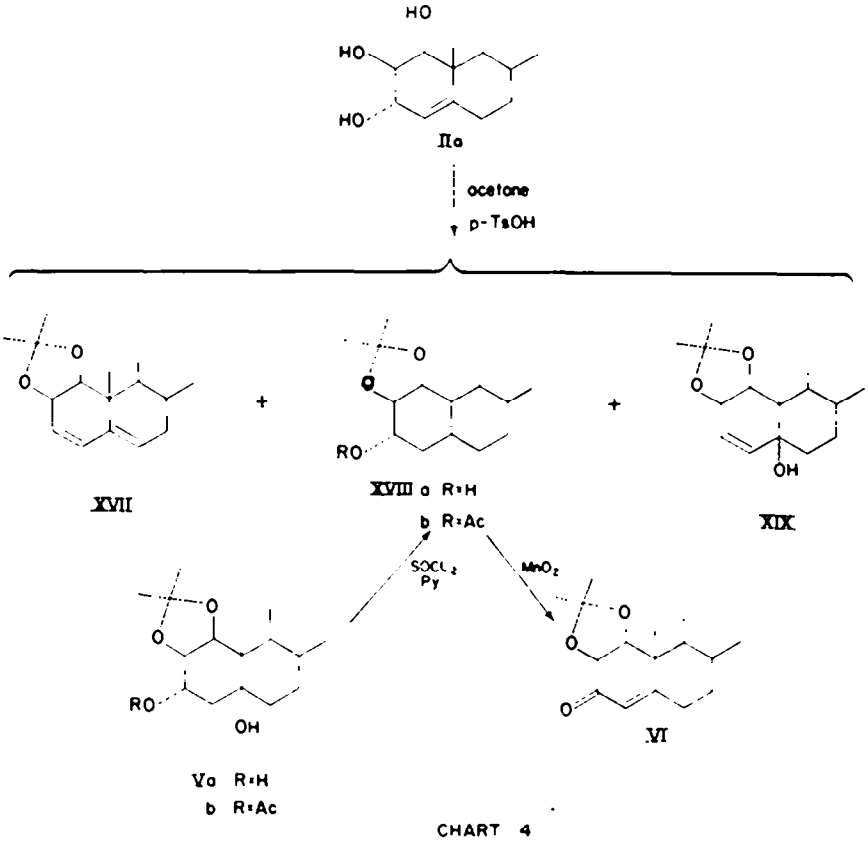


CHART 4

(B), respectively. This observation suggested that the double bond in the isomer (A) may be a disubstituted one.[11] When the infra-red spectrum of each isomer was minutely determined with KBr prism, the above assumption was supported by the facts that the isomer (B) showed the absorption band at 12·18 μ corresponding to a trisubstituted double bond while the isomer (A) exhibited the band at 14·41 μ due to a *cis*-disubstituted double bond. According to these facts it is deduced that the isomer (A) previously presumed to be XVIIIa has the structure XIX in which allylic rearrangement and acetonide formation occurred simultaneously by refluxing of IIa and *p*-toluenesulphonic acid in acetone. The newly isolated isomer (B) is concluded to have the structure XVIIIa expected for the normal acetonide of IIa. This has been proved by the identification with a sample prepared by the following route, i.e. acetylation of kogagenin acetonide (Va) followed by dehydration of the C_5-hydroxyl

[11] P. Bladon, H. B. Henbest and G. W. Wood, *J. Chem. Soc.* 2737 (1952).

group with thionyl chloride-pyridine and saponification. This compound was also oxidized smoothly with manganese dioxide to the acetonide-Δ^4-3-ketone (VI). Moreover, the isomer (A) was resistant to manganese dioxide oxidation or acetylation by refluxing acetic anhydride-pyridine and the starting material was recovered almost quantitative, as would be expected.

Although there are very few examples on the allylic rearrangement in the steroid field,[12] it is well-known that acidic treatment of Δ^4-sterols readily causes $\Delta^{3,5}$-diene formation.[13] It is interesting that the above-mentioned reaction of IIa resulted chiefly in allylic rearrangement than in dehydration.

EXPERIMENTAL

All melting points are uncorrected. Ultra-violet absorption spectra were taken with a Beckman Model DU Spectrophotometer. Infra-red spectra were measured using a Koken Infra-red Spectrophotometer Model DS 301. Rotations were determined with a Roudolf Photoelectric Polarimeter Model 200.

Epoxidation of anhydrokogagenin triacetate (IIb)

To a solution of IIb[8] (1·658 g) in chloroform (3 ml) was added a 0·46 M solution (12 ml) of perbenzoic acid in chloroform. After standing at room temp (15–21°) for 48 hr, the mixture was diluted with ether, washed with NaOH solution and water, and dried. The solvent was removed under reduced pressure leaving an oil (1·7 g) which was chromatographed on alumina (30 g). The fractions (1·294 g), eluted with a mixture of petroleum ether and benzene (1:1), were crystallized from aqueous methanol to prisms (1·00 g) of 1β,2β,3α-*triacetoxy*-4β,5β-*epoxy*-25D-*spirostane* (IIIb), m.p. 149–152°, $[\alpha]_D$ −3° (c 0·60, chloroform). (Found: C, 67·18; H, 8·22. $C_{33}H_{48}O_9$ requires: C, 67·32; H, 8·22%).

The further elution of the above chromatography with benzene and with chloroform gave an oil (390 mg) showing a hydroxyl band in the infra-red spectrum. Saponification with refluxing methanolic KOH solution gave 1β,2β,3α-*trihydroxy*-4β,5β-*epoxy*-25D-*spirostane* (IIIa, described below) in crystalline form.

Reduction of 1β,2β,3α-*triacetoxy*-4β,5β-*epoxy*-25D-*spirostane* (IIIb) *with lithium aluminium hydride*

To a suspension of lithium aluminium hydride (200 mg) in dry ether (30 ml), a solution of IIIb (215 mg) in dry benzene (30 ml) was added dropwise under stirring. The mixture was heated under reflux (52°) for 3 hr. After cooling, a small portion of water was added carefully to decompose the complex and then the mixture was acidified with dil HCl to dissolve an amorphous metal hydroxide. The precipitated crystals were collected by filtration, washed with water and dried yielding scales (168 mg), m.p. 298–303° (decomp). The organic layer of the filtrate gave only 4 mg of the organic material. The combined product (172 mg) was acetylated by refluxing with acetic anhydride and pyridine. The crude acetate was chromatographed over alumina (7 g). The fractions (15 mg) eluted with petroleum ether benzene (1:3) were crystallized from methanol to scales, m.p. 167–170°, which was identical with anhydrokogagenin triacetate (IIb). The eluates with benzene and with benzene-chloroform (19:1 to 4:1) were combined and recrystallization of the fraction (190 mg) from methanol furnished prisms (144 mg), m.p. 248–251°, which showed no depression on admixture with an authentic sample of kogagenin triacetate (Ib).[8] Infra-red spectra of these samples were identical.

Further elution of the above chromatography with chloroform and chloroform–methanol (1:1) afforded only an impure material (13 mg).

1β,2β,3α-*Trihydroxy*-4β,5β-*epoxy*-25D-*spirostane* (IIIa)

(a) *By saponification of* 1β,2β,3α-*triacetoxy*-4β,5β-*epoxy*-25D-*spirostane* (IIIb). The aforementioned IIIb (100 mg) prepared by epoxidation of IIb was refluxed in 10% methanolic KOH

[12] B. Pelc, *Coll. Czech. Chem. Comm.* **22**, 1457 (1957); R. E. Ireland, T. I. Wrigley and W. G. Young, *J. Amer. Chem. Soc.* **80**, 4604 (1958).
[13] H. McKennis, Jr. and G. W. Gaffney, *J. Biol. Chem.* **175**, 217 (1948); W. G. Danben and L. F. Eastham, *J. Amer. Chem. Soc.* **73**, 3260 (1951).

solution (10 ml) for 2 hr. The cooled reaction mixture was diluted with water and the precipitate was filtered and recrystallized from chloroform–methanol giving small plates of IIIa, m.p. 273–275°, $[\alpha]_D$ 63° (*c* 0·99, 1:1 chloroform methanol mixture). (Found: C, 70·31; H, 9·11. $C_{27}H_{42}O_6$ requires: C, 70·10; H, 9·15%).

(b) *By epoxidation of anhydrokogagenin* (IIa). To a 0·21 M solution (2·5 ml) of perbenzoic acid in chloroform, IIa (200 mg) was added. When the solution was stored in a refrigerator for 48 hr, a precipitate appeared. Filtration and washing with chloroform furnished needles (114 mg), m.p. 266–270°, which was recrystallized from ethyl acetate methanol giving small plates of IIIa, m.p. 272–274°, $[\alpha]_D$ 61° (*c* 1·00, 1:1 chloroform methanol mixture). The m.p. was not depressed on admixture with a specimen prepared by the method (a) and the infra-red absorption spectra of the two samples were identical. (Found: C, 70·18; H, 9·05. Calc. for $C_{27}H_{42}O_6$: C, 70·10; H, 9·15%).

From the filtrate the additional IIIa (67 mg) was obtained.

TABLE 1

Min	log ε	
	$\lambda_{224\ m\mu}$	$\lambda_{282\ m\mu}$
10	3·84	2·73
20	3·84	3·02
30	3·82	3·15
60	3·79	3·36
120	3·76	3·50
240	3·57	3·72
360	3·45	3·74
480	3·45	3·76

Manganese dioxide oxidation of anhydrokogagenin (IIa)

A solution of IIa (100 mg) in chloroform (10 ml) was shaken at room temp (30 : 1) with manganese dioxide (1·0 g) prepared by Rosenkranz's method.[14] Small quantities of the reaction mixture were withdrawn at intervals and freed from the manganese dioxide and solvent. These samples were determined by ultra-violet spectrum in a 95% ethanolic solution with the result as shown in Table 1.

Δ^4-25D-*Spirostene*-1β,2β-*diol*-3-*one* (IV)

(a) *By manganese dioxide oxidation of anhydrokogagenin* (IIa). A solution of IIa (110 mg) in chloroform (11 ml) was shaken with manganese dioxide (1·1 g) for 45 min under cooling in an ice-bath. After removal of the manganese dioxide and the solvent, the crystalline residue (85 mg) was chromatographed on silica gel (2 g). Elution with benzene–chloroform (9:1 to 4:1) gave yellow crystals (8 mg) of the further oxidized product (VII) described below. The next fractions (50 mg) eluted with chloroform, on crystallization from aqueous acetone, furnished long, silky needles, m.p. 202–204°, λ_{max}^{EtOH} 244 mμ (log ε 4·10). No depression in m.p. was observed on admixture with an authentic sample of the dihydroxy-Δ^4-3-ketone (IV) prepared by the method (b) described below and the infra-red spectra of these two substances were identical.

The 1:1 chloroform–methanol eluate (25 mg) of the above chromatography gave the starting material (IIa), m.p. 240–243° (decomp).

(b) *By acidic hydrolysis of* Δ^4-25D-*spirostene*-1β,2β-*diol*-3-*one acetonide* (VI). The acetonide (VI, 135 mg), prepared by oxidation of kogagenin acetonide followed by dehydration as described previously,[2] was refluxed for 30 min in methanol (10 ml) containing conc HCl (1 drop). The mixture was diluted with ether, washed 3 times with water and dried. After removal of the solvent, the crystalline residue was chromatographed on silica gel (3 g). The fractions (15 mg) eluted with benzene–chloroform (4:1) furnished, on recrystallization from acetone, $\Delta^{1,4}$-25D-spirostadien-2-ol-3-one[2] as scales, m.p. 234–237°. The eluate (111 mg) with benzene chloroform and with chloroform

[14] O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.* 2189 (1953).

was crystallized from aqueous acetone to long needles of IV, m.p. 202–204°. For analysis, the substance was recrystallized once more from the same solvent and dried *in vacuo* for 5 hr at 110°: $[\alpha]_D$ −141° (*c* 0·66, chloroform), λ_{max}^{EtOH} 244 mμ (log ε 4·10). (Found: C, 73·11; H, 9·04. $C_{31}H_{46}O_6$ requires: C, 72·94; H, 9·07%.)

The by-product (A-*nor*-Δ$^{3(5)}$-25D-*spirostene*-1,2-*dione*, VII) *of manganese dioxide oxidation of anhydrokogagenin* (IIa)

(a) *Isolation of the product* (VII). A mixture of IIa (300 mg) and manganese dioxide (3·0 g) in chloroform (30 ml) was stirred for 6 hr at room temp. The dioxide was filtered and the filtrate was evaporated to dryness and chromatographed over silica gel (3 g). Elution with benzene and recrystallization of the eluate (188 mg) from chloroform–methanol mixture gave yellow plates (140 mg) of VII, m.p. 228–230° (decomp), $[\alpha]_D$ −118° (*c* 0·75, chloroform), negative ferric chloride reaction; λ_{max}^{EtOH} 230, 282 mμ (log ε 3·60, 3·75); λ_{max}^{EtOH} 345 mμ (log ε 3·70). λ_{max}^{Nujol} 5·69, 5·80, 6·22 μ, no hydroxyl absorption. (Found: C, 75·71, 75·62; H, 8·81, 8·72. $C_{31}H_{44}O_4$ requires: C, 75·69; H, 8·80%.)

(b) *Acetylation of* VII. A mixture of the foregoing VII (100 mg), acetic anhydride (2 ml) and pyridine (2 ml) was heated under reflux for 2 hr. The product, extracted with ether in the usual manner, was recrystallized from ethyl acetate–alcohol mixture giving colourless prisms of the *enol acetate* (VIII), m.p. 222–224°, $[\alpha]_D$ −95° (*c* 1·01, chloroform); λ_{max}^{EtOH} 299 mμ (log ε 3·97). λ_{max}^{Nujol} 5·62, 8·37–8·53 μ (enol acetate), 5·84, 5·97, 6·25 μ (five-membered conj. dienone). (Found: C, 73·77; H, 8·49. $C_{33}H_{46}O_5$ requires: C, 73·98; H, 8·43%.)

(c) *Treatment of* VII *with o-phenylenediamine*. The above VII (100 mg) in alcohol (20 ml) was refluxed with *o*-phenylenediamine (100 mg) for 2 hr and then concentrated until crystals started separating. After cooling the crystals (87 mg), m.p. 260–265°, were collected and recrystallized twice from chloroform–alcohol to give pale yellow needles of the *quinoxaline derivative* (IX), m.p. 272–274° (decomp), $[\alpha]_D$ −191° (*c* 1·03, chloroform); λ_{max}^{EtOH} 223, 260, 266, 338, 354 mμ (log ε 4·39, 4·35, 4·34, 4·13, 4·11). λ_{max}^{Nujol} 6·17, 6·22, 6·30, 6·36, 13·12 μ. (Found: C, 79·24; H, 8·30; N, 5·51. $C_{37}H_{46}O_3N_2$ requires: C, 79·30; H, 8·32; N, 5·78%.)

(d) *Treatment of* VII *with zinc and acetic acid*. A yellow solution of VII (100 mg) in acetic acid (20 ml) was decolourized as soon as zinc dust (1·0 g) was added. After refluxing for 3 hr, zinc was removed by decantation and ether and water were added to the solution. The ether layer was washed, dried and evaporated to dryness *in vacuo*. The residue was recrystallized twice from methanol yielding needles (30 mg) of the *unsaturated ketol* (X), m.p. 233–235°, $[\alpha]_D$ −104° (*c* 0·96, chloroform); λ_{max}^{EtOH} 237 mμ (log ε 4·16). λ_{max}^{Nujol} 2·86 μ (OH), 5·85, 6·17 μ (five-membered conj. unsat. ketone). (Found: C, 75·67; H, 9·47. $C_{31}H_{48}O_4$ requires: C, 75·32; H, 9·24%.)

Treatment of 25D-*spirostane*-1β,2β,3α,4β,5β-*pentol* 1β,2β,3α-*triacetate* (XI) *with lead tetracetate*

The pentol triacetate (XI), m.p. 252–254°, was prepared on treatment of IIb with osmium tetroxide as described previously.[3] To a solution of XI (430 mg) in acetic acid (10 ml) was added lead tetracetate–acetic acid (29·68 mg/ml) solution (21 ml). After standing overnight at room temp., iodometric determination of the reaction mixture showed that one equivalent of the reagent was consumed. The reaction mixture was diluted with water and extracted with ether. The extract was washed with sodium carbonate solution and water, dried and evaporated. Crystallization of the glassy residue (420 mg) from ether–*n*-pentane mixture gave the 4,5-*secoaldehyde-ketone* (XIII) as needles (389 mg), m.p. 184–187°. The pure sample showed the following constants: m.p. 187–190°, $[\alpha]_D$ −90° (*c* 0·98, chloroform). λ_{max}^{Nujol} 5·68, 5·73 μ (acetate and aldehyde), 5·87 μ (ketone), no hydroxyl band. (Found: C, 65·88; H, 8·13. $C_{33}H_{48}O_{10}$ requires: C, 65·54; H, 8·00%.)

Oxidation of the 4,5-*secoaldehyde-ketone* (XIII)

To a solution of XIII (300 mg) in 90% acetic acid (10 ml), a solution (1·94 ml) of chromium trioxide in 90% acetic acid (20 mg/ml) was added. After standing for 1 hr at room temp., the reaction mixture was diluted with water and extracted with chloroform–ether (4:1) mixture. The solvent layer was washed with water and the acidic substances extracted with sodium carbonate solution. The alkaline solution was acidified with dil. HCl and extracted with ether. The ether solution was washed, dried and evaporated leaving an oil (297 mg) of the *triacetoxy-keto-acid* (XVb) which was not obtained in crystalline form.

Alkaline treatment of the triacetoxy-keto-acid (XVb)

(a) *With 1% methanolic potassium hydroxide solution.* When the foregoing XVb (297 mg) was dissolved in a 1% methanolic KOH solution (20 ml), the solution turned to yellow. After refluxing 1 hr, the mixture was diluted with water and the methanol was removed *in vacuo*. The alkaline solution was extracted with ether and the extract was washed with water, dried with sodium sulphate and evaporated to dryness. Crystallization of the oily residue (72 mg) from methanol gave needles (27 mg), m.p. 145–148°, which was purified by chromatographed over alumina followed by recrystallization from methanol yielding scales of *des*-A-25D-*spirostan*-5-*one* (XVI), m.p. 156–158°, $[\alpha]_D$ −84° (*c* 0.90, chloroform); λ_{max}^{Nujol} 5.84 μ (C=O), no hydroxyl absorption. (Found: C, 76.82; H, 10.08. $C_{23}H_{36}O_3$ requires: C, 76.62; H, 10.07%.) Treatment of this XVI (48 mg) with hydroxylamine by the usual manner gave needles (from alcohol) of the *oxime* (34 mg), m.p. 217°. (Found: C, 73.87; H, 10.03; N, 3.68. $C_{23}H_{37}O_3N$ requires: C, 73.56; H, 9.93; N, 3.73%.)

The alkaline solution after separation of the neutral fraction was acidified with dil HCl and extracted with ether. The ether extract was washed with water, dried and evaporated leaving a gummy residue (177 mg) presumed to be the *trihydroxy-keto-acid* (XVa). This acid was esterified with ethereal diazomethane to the oily *methyl ester*. The infra-red spectrum satisfied the given structure as follows: λ_{max}^{Nujol} 2.86–2.96 μ (OH), 5.75 μ (ester), 5.85 μ (ketone). However this trihydroxy-keto-ester was recovered unchanged after treatment with excess lead tetracetate in acetic acid or with periodic acid in 85% methanol for 20 hr at room temp.

(b) *With 5% alcoholic potassium hydroxide solution.* The oily triacetoxy-keto-acid (XVb, 153 mg) derived from XIII (150 mg) was heated on a steam bath for 1 hr with a 5% alcoholic KOH solution (20 ml). The reaction mixture was diluted with water and extracted with ether. The ether layer was treated in the usual manner and the crude substance (77 mg) was recrystallized from methanol to scales (50 mg) of m.p. 156–158°. Identity with a sample of the des-A-ketone (XVI) was established by the infra-red and mixed m.p. determination.

25D-*Spirostane*-1β,2β,3α,4β,5β-*pentol* (XII)

The pentol triacetate (XI, 450 mg) was dissolved in methanol (30 ml) by warming. When KOH (450 mg) in water (1 ml) was added to this solution, precipitation immediately occurred. The mixture was refluxed for 4 hr with this precipitate remaining. After cooling the crystals were filtered, washed with methanol and water, and recrystallized from chloroform–methanol to give the pentol (XII) decomposing gradually above 330°, yield 326 mg. (Found: C, 67.77; H, 9.28. $C_{33}H_{44}O_5$ requires: C, 67.47; H, 9.23%.)

Treatment of 25D-*spirostane*-1β,2β,3α,4β,5β-*pentol* (XII) *with lead tetracetate*

To a solution of lead tetracetate (1.6 g), acetic acid (40 ml) and chloroform (40 ml), XII (220 mg) was added. After the solution was allowed to stand at room temp. overnight, water and ether were added. The ether extract was washed with sodium carbonate solution and water, dried and evaporated under reduced pressure. The crystalline residue (189 mg) was recrystallized from chloroform–methanol yielding prisms (117 mg) of the A-*trisnor*-1,5-*secoaldehyde-ketone* (XIV), m.p. 220–224° (decomp). After further recrystallization the pure sample showed the following constants: m.p. 222–225° (decomp), $[\alpha]_D$ −99° (*c* 0.97, chloroform), λ_{max}^{Nujol} 5.78 μ (aldehyde), 5.91 μ (ketone), no hydroxyl absorption. (Found: C, 73.98; H, 9.35. $C_{31}H_{46}O_4$ requires: C, 74.19; H, 9.34%.)

Alkaline treatment of the A-trisnor-1,5-*secoaldehyde-ketone* (XIV)

A mixture of XIV (40 mg) and KOH (100 mg) in alcohol (5 ml) was refluxed for 30 min in an atmosphere of nitrogen. The solution was diluted with ether, washed with water and dried. After removal of the solvent, the crystalline residue (24 mg) was recrystallized from methanol to give scales (20 mg) of the des-A-ketone (XVI), m.p. 156–158°.

Treatment of anhydrokogagenin (IIa) *with acetone and p-toluenesulphonic acid*

A solution of IIa (139 mg) and *p*-toluenesulphonic acid dihydrate (15 mg) in acetone (30 ml) was refluxed for 5 hr and then allowed to stand at room temp. overnight. The reaction mixture was neutralized with sodium bicarbonate solution and concentrated to about one third of the initial volume under reduced pressure. The concentrated solution was diluted with water and extracted

with ether. The extract was washed with water and dried. After removal of the solvent, the crystalline residue (156 mg) was chromatographed on alumina (5 g). The fraction (14 mg) eluted with petroleum ether benzene (9:1) gave, on crystallization from methanol, the acetonide-$\Delta^{3,5}$-diene (XVII),[2] m.p. 162–165°.

Elution with petroleum ether benzene (4:1) and with benzene and recrystallization of the eluate (79 mg) from methanol furnished needles (66 mg), m.p. 208–210°, $[\alpha]_D$ −58° (*c* 1·00, chloroform), corresponding to a specimen reported to be anhydrokogagenin acetonide in the previous paper.[2] The structure of this compound was assigned Δ^3-25D-*spirostene*-1β,2β,5ξ-*triol* 1,2-*acetonide* (XIX) based on the following constants: λ_{max}^{EtOH} 206 mμ (log ϵ 2·94); λ_{max}^{Nujol} 2·84 μ (OH), 8·10 μ (—O—), 6·03, 14·41 μ (*cis* CH=CH).

This acetonide-Δ^3-5-ol (XIX) was recovered unchanged after treatment with manganese dioxide in chloroform for 6 hr and it was not acetylated by refluxing for 1 hr with acetic anhydride in pryidine.

Further elution of the above chromatography with benzene-chloroform and with chloroform gave the crystalline eluate (51 mg). Recrystallization from methanol gave needles (34 mg), m.p. 202–213°, which was recrystallized twice from the same solvent to yield the pure sample, m.p. 226–230°, $[\alpha]_D$ −7° (*c* 0·88, chloroform); λ_{max}^{EtOH} 206 mμ (log ϵ 3·73); λ_{max}^{Nujol} 2·83 μ (OH), 7·98, 8·12, 8·23 μ (—O—), 6·00, 12·18 μ (—C=CH—). (Found: C, 74·03; H, 9·81. $C_{30}H_{46}O_4$ requires: C, 74·03; H, 9·53%). No depression was observed on mixed m.p. with a sample of Δ^4-25D-*spirostene*-1β,2β,3α-*triol* 1,2-*acetonide* (XVIIIa), m.p. 227–230°, prepared by the following method, and the infra-red absorption spectra of the two specimens were identical.

Preparation of anhydrokogagenin acetonide (XVIIIa) *from kogagenin acetonide* (Va)

A mixture of kogagenin acetonide (Va,[2] 230 mg), acetic anhydride (2·5 ml) and pyridine (5 ml) was allowed to stand at room temp for 48 hr. The product, extracted with ether in the usual way, was recrystallized from methanol to give *kogagenin acetonide* 3-*acetate* (Vb) as prisms (225 mg), m.p. 234–237°, $[\alpha]_D$ −13° (*c* 0·98, chloroform). (Found: C, 70·68; H, 9·40. $C_{32}H_{48}O_7$ requires: C, 70·30; H, 9·22%).

To a solution of the foregoing Vb (200 mg) in pyridine (2 ml), thionyl chloride (0·3 g) in pyridine (3 ml) was added dropwise under cooling in an ice-bath. After standing for 45 min at 0° the excess reagent was destroyed with ice and the product was extracted with ether. The extract was washed with dil HCl, sodium bicarbonate solution and water, dried and evaporated leaving the crystalline residue (194 mg), m.p. 184–187°, which was recrystallized twice from methanol to needles of Δ^4-25D-*spirostene*-1β,2β,3α-*triol* 1,2-*acetonide* 3-*acetate* (XVIIIb), m.p. 212–214°, $[\alpha]_D$ −21° (*c* 1·04, chloroform). (Found: C, 72·89; H, 9·27. $C_{32}H_{48}O_6$ requires: C, 72·69; H, 9·15%).

This XVIIIb (60 mg) was saponified with 1% methanolic KOH solution (5 ml) under reflux for 1 hr. Recrystallization of the product from methanol gave needles of XVIIIa, m.p. 227–230° (Found: C, 74·06; H, 9·54. $C_{30}H_{46}O_5$ requires: 74·03; H, 9·53%). This was identical with the above-mentioned sample of XVIIIa in all respects.

Oxidation of Δ^4-25D-*spirostene*-1β,2β,3α-*triol* 1,2-*acetonide* (XVIIIa) *with manganese dioxide*

To a solution of the foregoing XVIIIa (37 mg) in chloroform (4 ml) was added manganese dioxide (0·3 g) and the slurry was stirred for 2·5 hr at room temp. After working up in the usual manner it yielded Δ^4-25D-spirostene-1β,2β-diol-3-one acetonide (VI)[2], as needles from methanol, m.p. 205–208°.