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

THE STRUCTURE OF KOGAGENIN, A SAPOGENIN FROM *DIOSCOREA TOKORO*, MAKINO[2]

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Abstract—The structure of kogagenin, a steroidal sapogenin isolated from the epigeous part of *Dioscorea tokoro*, Makino, has been shown to be 25D-spirostane-1β,2β,3α,5β-tetrol. This sapogenin is the first example of a naturally occurring spirostane tetrol.

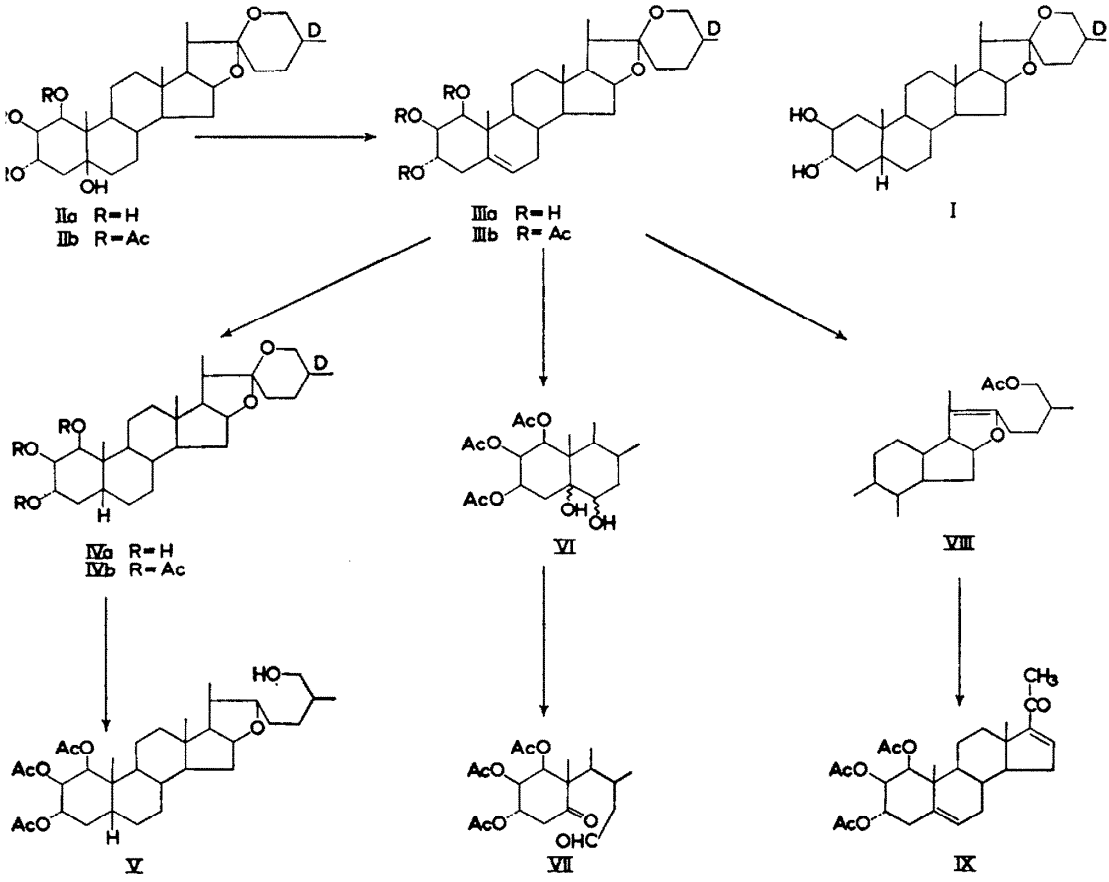
RECENTLY, we isolated two new sapogenins, yonogenin and kogagenin,[3,4] together with the known tokorogenin[5,6] from the epigeous part of *Dioscorea tokoro*, Makino. The structure of yonogenin was established as 25D,5β-spirostane-2β,3α-diol (I), and this sapogenin is the first example of a 3α-hydroxylated sapogenin from a plant source. After publication of the outline of the above results, the structure of tokorogenin was clarified as 25D,5β-spirostane-1β,2β,3α-triol (IVa), that is 1β-hydroxyyonogenin, by Morita.[6]

The elucidation of the structure of kogagenin, an uncharacterized sapogenin of this plant, is the subject of the present paper.

Kogagenin, $C_{27}H_{44}O_6$, $[\alpha]_D$ −27° (pyridine), melts at 318–322° (dec.) and gives a triacetate (IIb). The infra-red spectrum of this sapogenin shows neither a ketonic band nor an isolated double bond but hydroxyl group absorption. Comparison of the intensities of the characteristic absorption bands at 10·90 μ and 11·10 μ[7] shows that kogagenin is a 25D (iso) sapogenin. As the triacetate (IIb) still shows a hydroxyl absorption band in the infra-red spectrum, this sapogenin is assumed to be a 25D-tetrahydroxyspirostane. Kogagenin afforded an acetonide (XII). The triacetate (IIb) was not affected by chromium trioxide–pyridine oxidation and was very easily dehydrated to an anhydro derivative, $C_{33}H_{48}O_8$, (IIIb), with thionyl chloride and pyridine. Thus kogagenin has one *cis*-α-glycol (or less likely, a diaxial 1,3-glycol) group and a tertiary hydroxyl function.

The catalytic hydrogenation of the above-mentioned anhydrokogagenin triacetate (IIIb) gave important evidence for the structural elucidation of this sapogenin. Thus when the IIIb was hydrogenated catalytically with platinum oxide in acetic acid it gave two saturated compounds. One of them, having a melting point of 253–255°, was identical in all respects with tokorogenin triacetate (IVb). Based on the analytical

[1] Part XVIII; Ken'ichi Takeda and Tokuo Kubota, *Chem. Pharm. Bull.* **6**, 536 (1958).
[2] An outline of this paper was presented at the Symposium on *Organic Natural Products* of the Chemical Society of Japan in Osaka, Oct. 18 (1958).
[3] K. Takeda, T. Okanishi and A. Shimaoka, *Yakugaku Zassi* **77**, 822 (1957).
[4] K. Takeda, T. Okanishi and A. Shimaoka, *Chem. Pharm. Bull.* **6**, 532 (1958).
[5] M. Nishikawa, K. Morita, H. Hagiwara and M. Inoue, *Yakugaku Zassi* **74**, 1165 (1954).
[6] K. Morita, *Pharm. Bull.* **5**, 494 (1957).
[7] M. E. Wall, C. R. Eddy, M. L. McClennen and M. E. Klumpp, *Analyt. Chem.* **24**, 1337 (1952); R. N. Jones, E. Katzenellenbogen and K. Dobriner, *J. Amer. Chem. Soc.* **75**, 158 (1953).



values and the infra-red spectrum it is suggested that the structure of the other saturated compound, m.p. 167–169°, is that of dihydrotokorogenin triacetate where ring F is opened by catalytic reduction. This has now been proved by direct comparison with dihydrotokorogenin triacetate (V) which was prepared from tokorogenin acetate by the method of Marker *et al.*[8] We could not detect any isomer among the reduction products other than tokorogenin triacetate and its dihydro derivative. From these results the constitution of kogagenin is now established as x-hydroxytokorogenin.

In order to determine the position of the fourth, tertiary hydroxyl group, the location of the double bond in anhydrokogagenin acetate (IIIb) was examined. The possibility of the double bond being at C-7, C-8 or C-8(14) was excluded since the hydrogenation of IIIb with platinum oxide in acetic acid proceeded smoothly without migration of the double bond.[9] Osmium tetroxide oxidation of IIIb followed by cleavage of the resulting diol (VI) with lead tetracetate gave an amorphous product, which not only showed an absorption band at 5·85 μ due to the six-membered ring ketone in the infra-red spectrum but also indicated the presence of an aldehyde

[8] R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, P. J. Goldsmith and C. H. Ruof, *J. Amer. Chem. Soc.* **69**, 2167 (1947).
[9] L. F. Fieser and M. Fieser, *Natural Products related to Phenanthrene* (3rd Ed.) p. 240 (1949).

function by the positive triphenyltetrazolium test. From this observation the position of the double bond in III is limited to Δ^5 (or Δ^4) or Δ^{14}.

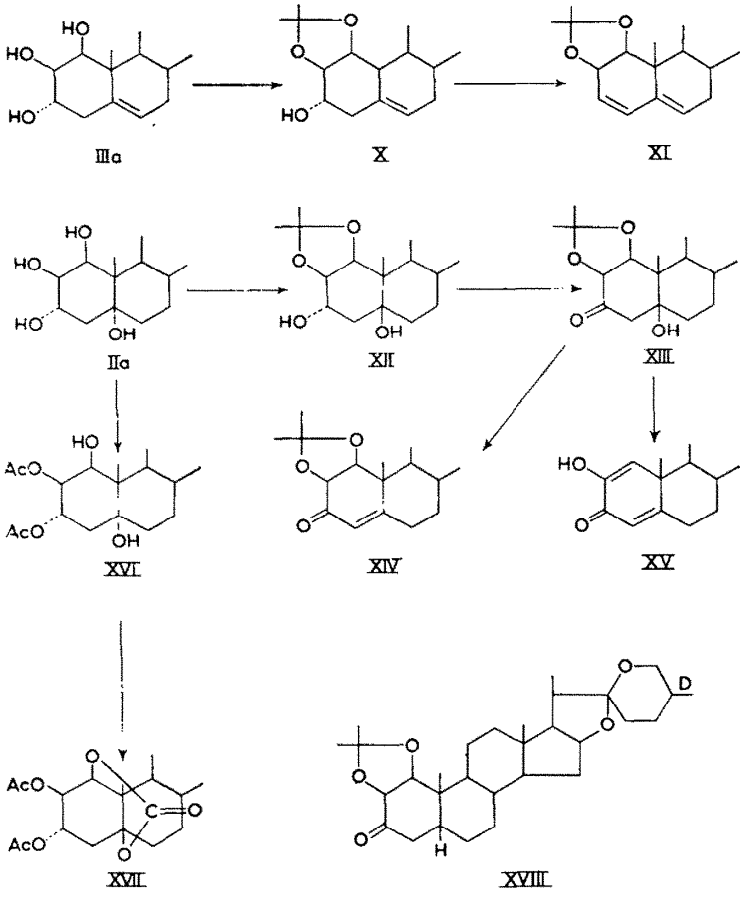
If the double bond in IIIb were located at C-14, the corresponding pregnene derivative (IX) of anhydrokogagenin would now be a $\Delta^{14,16}$-dien-20-one which should exhibit typical ultra-violet absorption at 307–309 mμ[10]. A Δ^{16}-pregnene derivative (IX), was obtained from anhydrokogagenin *via* the *pseudo*sapogenin (VIII) followed by the oxidation. This Δ^{16}-pregnene derivative showed an ultra-violet absorption at 239 mμ ($\log \varepsilon = 4{\cdot}00$) thus establishing the presence of Δ^{16}-20-ketone group[10] without additional conjugation.

The remaining position, C-5, for the tertiary hydroxyl group was proved by the following methods. Attempts to obtain a Δ^4-3-keto derivative of kogagenin by oxidation of anhydrokogagenin acetonide (X) with chromium trioxide–pyridine, chromium trioxide–acetone–sulphuric acid or by the Oppenauer oxidation were all unsuccessful, and in all cases the starting material was recovered almost quantitatively. When anhydrokogagenin (IIIa) was treated with acetone and *p*-toluenesulphonic acid in order to synthesize the acetonide (X), a small amount of by-product (XI) was obtained and this showed an ultra-violet maximum at 236 mμ ($\log \varepsilon = 4{\cdot}31$) in keeping with the chromophore of the $\Delta^{3,5}$-diene system. The dehydration of the above acetonide (X) with phosphorus oxychloride in pyridine also gave the same diene (XI) but the yield was very poor. These findings support the fact that the position of the fourth tertiary hydroxyl group is at C-5.

Anhydrokogagenin triacetate (IIIb) was saponified by methanolic potassium hydroxide to the free triol (IIIa), m.p. 240–243°, which does not contain an allylic hydroxyl function since this triol does not show any colouration by the Rosenheim test.[11] Acetylation of IIIa regenerated IIIb. It appeared reasonable, therefore, that the position of the double bond in anhydrokogagenin is at 5-6 rather than at 4-5. The fact that the hydrogenation of anhydrokogagenin acetate yielded only the normal (5β) steroid agrees with the results of the reduction of 3α-acetoxy-Δ^5-steroids.[12]

Finally, we obtained the Δ^4-3-keto derivative by the direct oxidation of kogagenin acetonide (XII). Chromium trioxide–pyridine oxidation of kogagenin acetonide gave a ketone (XIII), but its infra-red spectrum showed the weak absorption bands at 5·96 μ and 6·16 μ corresponding to a Δ^4-3-ketone group, besides the absorption bands at 2·84 μ (hydroxyl) and 5·76 μ (ketone). The ultra-violet absorption spectrum also showed absorption at 246 mμ ($\varepsilon = 1670$), indicating that this ketone was contaminated by ca. 10 per cent of the Δ^4-3-keto derivative (XIV). Attempts to purify XIII without dehydration of the C-5 tertiary hydroxyl group were unsuccessful. The crude ketone was easily dehydrated by silica gel chromatography and afforded the Δ^4-3-keto acetonide (XIV) in a pure state. Treatment of XIII with potassium hydroxide in aqueous methanol at room temperature in order to obtain XIV gave a different compound, having an empirical formula $C_{27}H_{38}O_4$, m.p. 224–227°. The analytical values suggest the elimination of one mole of acetone with the loss of one mole of water from the starting material during the reaction. The postulated constitution XV for this compound was in good agreement with the following facts: (a) XV gave a quinoxaline derivative, m.p. 283–284°, with *o*-phenylenediamine;

[10] L. Dorfman, *Chem. Rev.* **53**, 47 (1953).
[11] R. Schoenheimer and E. A. Evans, Jr., *J. Biol. Chem.* **114**, 567 (1936).
[12] J. R. Lewis and C. W. Shoppee, *J. Chem. Soc.* 1365 (1955).



(b) the infra-red spectrum of this compound showed absorption bands at 2·96 μ (hydroxyl) and at 6·09 and 6·17 μ (conjugated unsaturated ketone) and the ultra-violet absorption maximum appeared at 254 mμ (log $\varepsilon = 4{\cdot}13$) with a small shoulder at 290 mμ.[13]

Consequently, kogagenin can now be defined as 25D-spirostane-1β,2β,3α,5ξ-tetrol and there remains only the determination of the configuration of the C-5 hydroxyl group for the complete elucidation of the structure. Closely related to kogagenin in *Dioscorea tokoro* are yonogenin (I) and tokorogenin (IVa), and it seems likely that kogagenin should have the same *cis* configuration of the A/B ring juncture as the other two. This assumption was supported by the fact that the rotatory dispersion curve[14] of the above-mentioned 3-ketone (XIII) closely resembles that of XVIII derived from tokorogenin acetonide (Fig. 1). The rotatory dispersion curves of other 5β-hydroxy-3-keto steroids also support our conclusion.[15]

[13] J. S. Baran, *J. Amer. Chem. Soc.* **80**, 1687 (1958).

[14] Rotatory dispersion curves were measured by Mrs. T. Nakano at Wayne State University through the kind offices of Dr. Carl Djerassi. The sample of XIII contained 12% of the Δ^4-3-ketone (XIV); however, by comparison with the curve of the pure Δ^4-3-ketone (XIV) it was confirmed that the Δ^4-3-ketone contaminant practically did not affect the rotatory dispersion curve of XIII. Rotatory dispersion curves of authentic Δ^4-3-keto and 5β-3-keto steroids can be found in the review article of C. Djerassi, *Bull. Soc. Chem. Fr.* 741 (1957).

[15] Private communication of Dr. C. Djerassi.

More convincing evidence for the A/B ring *cis*-fusion was achieved by experiments described below. Kogagenin acetonide (XII) did not condense with phosgene under the usual conditions, thus indicating the lack of a 3α,5α-glycol grouping. On the other hand, it was now found that the acetylation of kogagenin under mild conditions gave in excellent yield the corresponding diacetate (XVI). It can be assumed that orientation of these two hydroxyl groups is equatorial from the ease of acetylation and this requires the A/B ring *cis* juncture with the 5β-hydroxyl group. On the basis of these observations, the above diacetate (XVI) should be 25D-spirostane-1β,2β,3α,-5β-tetrol 2,3-diacetate and this could be condensed with phosgene to yield a

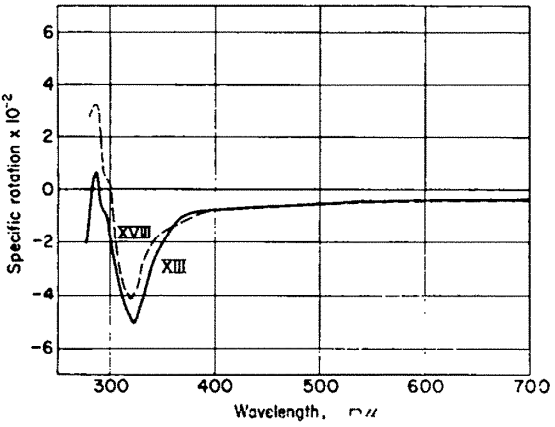


FIG. 1. Optical rotary dispersion curves (dioxane solution) of 25D-spirostane-1β-2β,5β-triol-3-one 1,2-acetonide (XIII) and 25D,5β-spirostane-1β,2β-diol-3-one acetonide (XVIII).

1β,5β-carbonyldioxy derivative (XVII), as expected. It seems appropriate, therefore, to conclude that the hydroxyl group at C-5 in kogagenin is β-oriented and this sapogenin is described as 25D-spirostane-1β,2β,3α,5β-tetrol (IIa). Kogagenin is the first example of a naturally occurring spirostane tetrol.

Thus, the structures of the four sapogenins isolated until now from *Dioscorea tokoro* have been made clear. Yonogenin, tokorogenin and kogagenin are respectively dihydroxy-, trihydroxy- and tetrahydroxy-sapogenins, and at the same time all 3α-hydroxylated sapogenins, while the remaining one, diosgenin, is a 3β-hydroxylated sapogenin. It is of further interest to note that kogagenin has not been isolated from the rhizome of this plant,[16] while diogenin has not been isolated from the epigeous part of the plant. Also, according to Morita,[17] quantitative analysis of sapogenins in the rhizome of *Dioscorea tokoro* showed that this plant could be divided into two varieties; in one variety the sapogenin component being mainly yonogenin and tokorogenin, while in the other being diosgenin.

It should be noted, however, that the biogenetic relationship between 3α-hydroxylated sapogenins and 3β-hydroxylated sapogenins, and the reason for the particular location of diosgenin and kogagenin in plant material is unknown.

[16] M. Yamagishi and I. Nakamura, *Chem. Pharm. Bull.* 6, 421 (1958).
[17] K. Morita, Abstract of the Symposium on *Organic Natural Products* of the Chemical Society of Japan, p. 101, (1958).

EXPERIMENTAL

All melting points are uncorrected. Ultra-violet absorption spectra were measured in 95% ethanolic solutions with a Beckman DU spectrophotometer. Infra-red spectra were determined with a Perkin–Elmer single-beam instrument, Model 12C, in Nujol mull (unless otherwise stated).

Kogagenin triacetate (IIb). A mixture of kogagenin (600 mg), acetic anhydride (6 ml) and pyridine (3 ml) was refluxed for 2 hr. The product, isolated with benzene in the usual manner, was recrystallized from methanol to give IIb, m.p. 249–252°, $[\alpha]_D$ -26° (*c* 1·0, chloroform). (Found: C, 67·09; H, 8·50. $C_{33}H_{50}O_9$ requires: C, 67·09; H, 8·53%).

Kogagenin acetonide (XII). A mixture of kogagenin (500 mg), acetone (500 ml), benzene (200 ml) and *p*-toluenesulphonic acid (500 mg) was heated under reflux for 22 hr. After neutralizing with sodium bicarbonate, the solution was concentrated under reduced pressure and extracted with benzene. The extract was washed with water, dried and evaporated. The resulting crystalline residue was chromatographed on alumina (15 g), and elution with benzene–chloroform (1 : 1) and with chloroform furnished crude acetonide (316 mg), which was recrystallized from chloroform–methanol to give prisms (260 mg), m.p. 273–275°, $[\alpha]_D$ −23° (*c* 1·15, chloroform). (Found: C, 71·59; H, 9·75. $C_{30}H_{48}O_6$ requires: C, 71·39; H, 9·59%).

The chloroform–methanol (1 : 1) eluate from the above chromatogram gave unchanged kogagenin (230 mg).

Dehydration of kogagenin triacetate (IIb). The triacetate (500 mg) was dissolved in pyridine (5 ml), cooled to 0° and a mixture of thionyl chloride (0·50 g) in pyridine (2 ml) was added dropwise. After 1 hr at 0°, ice was added to the mixture and the product was extracted with ether. The extract was washed with dilute HCl, sodium bicarbonate solution and water, dried and evaporated leaving an oil (490 mg). Crystallization from methanol gave scales (395 mg) of *anhydrokogagenin triacetate* (IIIb), m.p. 171–173°, $[\alpha]_D$ +33° (*c* 1·0, chloroform). (Found: C, 69·05; H, 8·61. $C_{33}H_{48}O_8$ requires: C, 69·20; H, 8·45%).

IIIb (500 mg) was saponified by refluxing for 1 hr with 1·5% methanolic KOH solution (20 ml). The cooled reaction mixture was diluted with water, and the precipitate was filtered and recrystallized from methanol to give *anhydrokogagenin* (IIIa) as needles, m.p. 240–243°, $[\alpha]_D$ −70° (*c* 1·0, chloroform), negative Rosenheim test. (Found: C, 72·64; H, 9·41. $C_{27}H_{42}O_5$ requires: C,72·61;H,9·48%)

Catalytic reduction of IIIb. A solution of IIIb (100 mg) in glacial acetic acid (7 ml) was shaken with pre-reduced platinum oxide (100 mg) in an atmosphere of hydrogen. The absorption of hydrogen was rapid and 1·5 equivalent was taken up within 30 min when hydrogenation was stopped. The catalyst was filtered, the solvent was removed *in vacuo* and the crystalline residue was recrystallized from methanol to yield tokorogenin acetate (IVb) as prisms (45 mg), m.p. 253–255°, $[\alpha]_D$ −20° (*c* 1·0, chloroform). Identity with an authentic sample was confirmed by a mixed melting point determination and infra-red comparison. (Found: C, 69·27; H, 8·98. Calc. for $C_{33}H_{50}O_8$: C, 68·96; H, 8·77%).

Saponification of IVb with 3% methanolic KOH and recrystallization from methanol gave needles of tokorogenin, m.p. 266–268°, which was identical with an authentic sample.

After concentrating the mother liquor of the above acetate, the residue was chromatographed on alumina (3 g). Elution with petroleum ether–benzene (1 : 1) afforded an additional 5 mg of IVb, while elution with benzene and with benzene–ether (19 : 1) yielded *dihydrotokorogenin triacetate* (V) (35 mg), which was recrystallized from methanol giving needles, m.p. 167–169°, $[\alpha]_D$ +40° (*c* 1·0, chloroform). Identity with the specimen (described below) prepared from authentic tokorogenin acetate was established by a mixed m.p. and the respective infra-red spectra. (Found: C, 68·77; H, 9·30. $C_{33}H_{52}O_8$ requires: C, 68·72; H, 9·09%).

Dihydrotokorogenin triacetate (V, *furostane-1β,2β,3α,26-tetrol 1β,2β,3α-triacetate*). IVb (50 mg) in glacial acetic acid (6 ml) was shaken with Adams' catalyst (50 mg) in an atmosphere of hydrogen for 4·5 hr at 70–80°. The catalyst was filtered, the solvent removed *in vacuo* and the product recrystallized from methanol, m.p. 167–169°, $[\alpha]_D$ +37° (*c* 0·65, chloroform), λ_{max}^{Nujol} 2·83 μ (hydroxyl band) but no spiroketal bands at 10·19, 10·90, 11·10 and 11·58 μ. (Found: C, 68·38; H, 9·17. $C_{33}H_{52}O_8$ requires: C, 68·72; H, 9·09%).

Treatment of IIIb *with osmium tetroxide.* Osmium tetroxide (350 mg) in benzene (10 ml) was added to a solution of IIIb (300 mg) in pyridine (5 ml) and stored in the dark for 53 hr at room temp. The mixture was saturated with hydrogen sulphide, the precipitate was filtered and the filtrate

evaporated to dryness *in vacuo*. Crystallization from ethanol yielded the *triacetate-diol* (VI) (150 mg), m.p. 252–254°, $[\alpha]_D$ −44° (*c* 1·0, chloroform). (Found: C, 65·34; H, 8·40. $C_{33}H_{50}O_{10}$ requires: C, 65·32; H, 8·31%).

To a solution of VI (100 mg) in glacial acetic acid (10 ml) lead tetraacetate (0·7 g) in glacial acetic acid (20 ml) was added. After standing overnight at room temp, the mixture was diluted with water (60 ml) and extracted with ether. The extract was washed with 10% sodium carbonate solution and water, dried and evaporated. Attempts to crystallize the resulting gum were unsuccessful. This product gave a positive triphenyltetrazolium test and showed a strong infra-red ketonic band at 5·85 μ.

Degradation of anhydrokogagenin acetate (IIIb) *to* $\Delta^{5,16}$*-pregnadien-1β,2β,3α-triol-20-one triacetate* (IX). IIIb (450 mg) in pyridine (2 ml) and acetic anhydride (2 ml) was heated under reflux with ethylamine hydrochloride (0·3 g) for 5·5 hr. The cooled reaction mixture was poured on crushed ice and the product was extracted with ether. Chromium trioxide (0·3 g) in 80% acetic acid (3 ml) was added dropwise to a solution of the furosten (VIII) in acetic acid (9 ml). After stirring for 2 hr at room temp, the mixture was diluted with water and extracted with ether. The ether solution was washed with sodium carbonate solution and water and evaporated leaving a gummy solid which was saponified with 1% ethanolic KOH. After re-acetylation with acetic anhydride–pyridine (refluxing), the product was purified by chromatography, followed by recrystallization from aqueous ethanol; yield 95 mg, m.p. 150–152°, $[\alpha]_D$ +168° (*c* 1·0, chloroform), λ_{max}^{EtOH} 239 mμ, log ε 4·00. (Found: C, 68·45, H, 7·65. $C_{27}H_{36}O_7$ requires: C, 68·62; H, 7·68%).

Δ^5*-25D-Spirostene-1β,2β,3α-triol* 1,2*-acetonide* (X) *and by-product* (XI) *from the anhydro-triol* (IIIa). IIIa (160 mg) was refluxed for 6 hr in acetone (30 ml) containing *p*-toluenesulphonic acid (10 mg). The solution was neutralized with sodium bicarbonate solution and concentrated under reduced pressure. The product was extracted with ether and the extract washed with water and dried. After removal of the solvent, the crystalline residue was chromatographed on alumina. The fractions (7 mg), m.p. 157–161°, eluted with petroleum ether–benzene (9 : 1) yielded the diene-acetonide (XI) (see below). The next fractions, eluted with petroleum ether–benzene (4 : 1 to 1 : 1), on recrystallization from methanol furnished the acetonide (X) (93 mg), m.p. 208–210°, $[\alpha]_D$ −61° (*c* 1·0, chloroform). (Found: C, 74·26; H, 9·51. $C_{30}H_{46}O_5$ requires: C, 74·07; H, 9·46%).

The diene-acetonide (XI) on crystallization from methanol showed m.p. 162–164°, $[\alpha]_D$ −115° (*c* 1·0, chloroform), λ_{max}^{EtOH} 236 mμ, log ε 4·31. (Found: C, 76·82; H, 9·65. $C_{30}H_{44}O_4$ requires: C, 76·88; H, 9·46%).

Dehydration of Δ^5*-25D-spirostene-1β,2β,3α-triol* 1,2*-acetonide* (X). A mixture of the Δ^5-en-3-ol (X) (80 mg), pyridine (4 ml) and phosphorus oxychloride (0·5 g) was heated (steam bath) for 45 min. The solution was poured on crushed ice and extracted with ether. The extract was washed with dilute HCl, sodium bicarbonate solution and water, dried and evaporated yielding an oily residue (50 mg). Crystallization from methanol gave needles (15 mg), m.p. 150–154°, identical with a sample of the diene (XI), by-product in acetonization described above, by admixture and infra-red comparison.

Attempted oxidation of X. The Δ^5-en-3-ol (X) was recovered unchanged after treatment with the chromium trioxide–pyridine complex for 20 hr at 37° or with chromium trioxide–sulphuric acid–acetone solution for 10 min at 15°, and it was not oxidized by refluxing for 6 hr with aluminium isopropoxide and cyclohexanone in toluene.

Oxidation of kogagenin acetonide (XII). XII (120 mg) in pyridine (3 ml) was added at 0° to the complex prepared from chromium trioxide (150 mg) and pyridine (1·5 ml) and the mixture was allowed to stand at room temp for 16 hr. The product was extracted with ether and crystallized from methanol to give the crude ketone (XIII) as needles, m.p. 189–191° (dec.). The structure of XIII was supported by the infra-red spectrum having strong bands at 2·84 and 5·76 μ, but the co-existence of a small amount of the α,β-unsaturated ketone (XIV) was also indicated by bands at 5·93 and 6·16 μ. This was also shown by the ultra-violet absorption at 246 mμ (ε 1,670). This sample was used for the determination of the rotatory dispersion curve (Fig. 1), since further recrystallization brought no alteration in this ultra-violet spectrum.

A solution of the crude ketone above (80 mg) in petroleum ether–benzene (1 : 1) was absorbed on a silica gel column. Elution with benzene and with benzene–chloroform (9 : 1) and recrystallization of the eluate from acetone–hexane furnished Δ^4*-25D-spirostene-1β,2β-diol-3-one acetonide* (XIV) (54 mg) as needles, m.p. 193–198°, λ_{max}^{EtOH} 246 mμ, log ε 4·10. Further recrystallization from methanol

gave the analytical sample with the following constants: m.p. 197–200°, $[\alpha]_D$ −100° (*c* 1·0, chloroform), λ_{max}^{EtOH} 246 mμ, log ε 4·15: λ_{max}^{Nujol} 5·93 and 6·16 μ, no hydroxyl absorption. (Found: C, 74·74; H, 9·04. $C_{30}H_{44}O_5$ requires: C, 74·34; H, 9·15%).

To the crude ketone above (20 mg) in methanol (5 ml), was added 10% aqueous KOH (0·5 ml) and the mixture was kept at room temp overnight. The mixture was diluted with water, neutralized with dilute HCl and extracted with ether. The ether extract was washed with water, dried and evaporated. The crystalline residue (15 mg) was recrystallized from acetone giving leaflets of $\Delta^{1,4}$-25D-*spirostadien-2-ol-3-one* (XV) showing the following properties; m.p. 224–227°, $[\alpha]_D$ −102° (*c* 0·36, chloroform); λ_{max}^{Nujol} 2·96, 6·09 and 6·17 μ; λ_{max}^{EtOH} 254 mμ, log ε 4·13 with a weak shoulder at 290 mμ; reddish-purple colour with alcoholic ferric chloride solution. (Found: C, 75·81; H, 9·06. $C_{27}H_{38}O_4$ requires: C, 76·02; H, 8·98%).

Refluxing of $\Delta^{1,4}$-3-on-2-ol (XV) with *o*-phenylenediamine in ethanol gave a quinoxaline derivative, orange-yellow needles, m.p. 283–284°.

Kogagenin diacetate (XVI). Kogagenin (98 mg) was added to a mixture of chloroform (4 ml), acetic anhydride (1 ml) and pyridine (4 ml). On standing overnight at room temp, the sapogenin had dissolved completely. Water and ether were then added, the ether layer was washed with dilute HCl, sodium bicarbonate solution and water and evaporated to leave a crystalline residue (123 mg). Crystallization from chloroform–methanol gave scales of the diacetate (XVI) (87 mg), m.p. 275–277°, $[\alpha]_D$ −13° (*c* 1·1, chloroform). (Found: C, 67·79; H, 8·85. $C_{31}H_{48}O_8$ requires: C, 67·85; H, 8·82%).

Reaction of XVI *with phosgene.* XVI (80 mg) was dissolved in alcohol-free chloroform (6 ml), about 1 ml of the chloroform was removed by distillation and then pyridine (4 ml) was added. The mixture was cooled to −15° and a 10% phosgene–toluene solution (12 ml) added dropwise. The temperature of the reaction mixture was slowly raised to 15° during 1 hr and was maintained at 15–20° overnight. After destruction of the excess phosgene with ice, water and ether were added, and the ether layer was washed successively with dilute HCl, sodium bicarbonate solution and water, dried and evaporated. The gummy residue was chromatographed on alumina (2 g) and the benzene eluate (56 mg) was crystallized from acetone–hexane and then from methanol yielding prisms (37 mg) of 25D-*spirostane-1β,2β,3α,5β-tetrol* 1,5-*carbonate* 2,3-*diacetate* (XVII), m.p. 169–172°, $[\alpha]_D$ +27° (*c* 1·1, chloroform); $\lambda_{max}^{CS_2}$ 5·66, 5.72, 8·08, 8·19 and 8·40 μ, no hydroxyl band. (Found: C, 66·57; H, 8·35. $C_{32}H_{46}O_9$ requires: C, 66·87; H, 8·07%).

The 1 : 1 chloroform–methanol eluate (34 mg) of the above chromatography furnished, after recrystallization from methanol, crystals (15 mg) with m.p. 257–266° probably representing impure starting material.

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