N,N-Bis(2-Hydroxyethyl)trimethylenediamine——This and the following trimethylenediamine derivatives were obtained by a method similar to that for the corresponding ethylenediamine derivatives.

A solution of 3-bis(2-hydroxyethyl)aminopropionitrile (100 g.) in EtOH (100 cc.) containing NH_3 (5 g.) was placed in an autoclave with Raney Ni catalyst (10 g.) and the mixture was shaken with H_2 at 50 atm. and 60° for 1 hr. The catalyst was removed by filtration and EtOH was distilled off. The oily residue was fractionated *in vacuo*. N,N-Bis(2-hydroxyethyl)trimethylenediamine, b.$p_{0.5}$ 150～165°. (yield, 70%).

N,N-Bis(2-hydroxyethyl)-N′,N′-bis(carboxymethyl)trimethylenediamine——This was prepared by a method similar to that for the corresponding ethylenediamine derivative except that Amberlite IRC-120 was used instead of IRC-50. The free acid was not obtained as crystals. The corresponding Cu chelate with 1 mole of crystal water was obtained as fine blue crystals, m.p. 95～100°. The analytical data did not agree acurately with the theoretical values because of the difficulty in its complete combustion. *Anal.* Calcd. for $C_{11}H_{20}O_6N_2Cu \cdot H_2O$: N, 7.84. Found : N, 7.94.

N,N-Bis(2-chloroethyl)-N′,N′-bis(carboxymethyl)trimethylenediamine (No. 723)——This was obtained by the usual chlorination procedure.

Picrate : m.p. 87～90°. *Anal.* Calcd. for $C_{23}H_{26}O_{18}N_8Cl_2$: N, 14.49. Found : N, 15.05.

Copper Salt of N,N-Bis(2-chloroethyl)-N′,N′-bis(2-carboxymethyl)trimethylenediamine (No. 662)——The monohydrochloride of this compound was obtained by the similar method as in the case of No. 601; m.p. 155～160° (decomp.). *Anal.* Calcd. for $C_{11}H_{18}O_4N_2Cl_2Cu \cdot HCl$: Ionic Cl, 8.6; total Cl, 24.16. Found : Ionic Cl, 8.8; totat Cl, 24.57.

The authors wish to thank Prof. T. Yoshida for his kind advices through this investigation and Dr. H. Satoh and Dr. H. Imamura for their collaboration in animal experiments. The determination of constants of the chelates was carried out by Dr. A. Hanaki, to whom they are indebted. Expenses for this work were defrayed by the Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.

Summary

Derivatives of nitrogen mustard having metal chelate-forming activity and their copper chelates were prepared and their chemical properties and their antitumor effect on Yoshida sarcoma were discussed.

(Received December 20, 1960)

UDC 547.92 : 582.572.2

110. Kanzo Sasaki : Studies on the Steroidal Components of Domestic Plants. XXXII.[1] Constituents of *Reineckia carnea* KUNTH. (4). Structure of Kitigenin. (1).

(*Research Laboratory, Shionogi & Co., Ltd.*[*1])

Kitigenin is a sapogenin isolated from *Reineckia carnea* KUNTH, of which the following facts have been clarified previously[1,2] : 1) The empirical formula of the sapogenin (I) is $C_{27}H_{44}O_6$ and it belongs to the 25D-series. 2) Acetylation of the sapogenin yields a triacetate, $C_{33}H_{50}O_9$, which has still one free hydroxyl group. 3) By periodic acid oxidation followed by silver oxide oxidation, the sapogenin affords des-A-spirostan-5-one (V). These facts show that kitigenin is a tetrahydroxy-sapogenin and all the hydroxyl groups in kitigenin are located in ring A.

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*1 Fukushima-ku, Osaka (佐々木勘造).

1) Part XXXI : K. Takeda, T. Okanishi, K. Sasaki, A. Shimaoka : This Bulletin, **9**, 631 (1961).

2) K. Takeda, T. Okanishi, A. Shimaoka : Yakugaku Zasshi, **75**, 560 (1955).

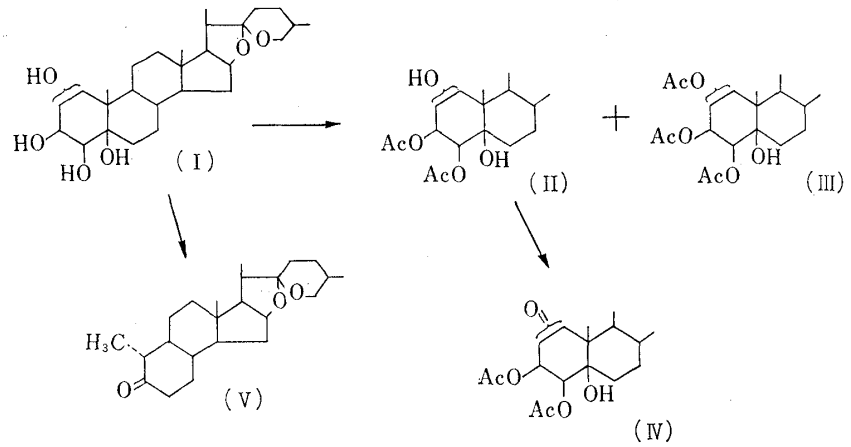
In the present paper, the study on the positions and the configurations of the three hydroxyl groups in kitigenin is described.

In a preceding paper,[2] only the triacetate, m.p. 219~220.5°,*2 had been obtained from kitigenin by crystallization of the acetylation product. However, it was now found that the acetylation products could be separated into a diacetate and a triacetate on the basis of the difference of their solubilities in carbon disulfide. The sparingly soluble fraction gave an acetate, m.p. 217~219°, which clearly showed melting point depression on admixture with the triacetate, m.p. 219~220.5°, obtained previously, and the infrared spectrum of each sample was different. The results of elemental analysis, water of crystallization and acetyl number measurement showed it to be the dihydroxy diacetate, $C_{31}H_{48}O_8 \cdot \frac{1}{2}H_2O$ (II). The carbon disulfide soluble fraction afforded the triacetate (III), previously obtained, by chromatographic separation.

The diacetate (II) was oxidized with chromium trioxide-sulfuric acid in acetone solution and gave (IV), m.p. 200~202°. As the ultraviolet and infrared spectra of this substance showed the presence of the hydroxyl, acetoxyl and ketone groups, it is clear that only one of the two free hydroxyl groups in (II) was attacked.

Since one of the four hydroxyl groups in kitigenin resists oxidation and acetylation, as mentioned above, this hydroxyl group is evidently a tertiary one, the position of which is limited to C_5.

Chart 1



The triacetate (III) afforded a dehydration product (VI), $C_{33}H_{48}O_8$, m.p. 218~221°, by the action of thionyl chloride-pyridine in 74% yield. The ultraviolet spectrum of this substance (Fig. 1) showed a maximum at 206 mμ in ethanol, indicating the existence of a double bond. The position of this double bond is not at C_5-C_6 on the basis of the negative tetranitromethane color test and of the ultraviolet absorption curve. The infrared spectrum showed absorption bands at 1762 cm^{-1} and 1730 cm^{-1} characteristic of the enol acetate[3] and of the normal acetoxyl function respectively, and no hydroxyl band. From this observation, it can be deduced that there is an acetoxyl group adjacent to the tertiary hydroxyl group participated on dehydration and that the acetoxyl group must be at C_4.

In the dehydration reaction of the C_5-hydroxyl group with thionyl chloride-pyridine, there is a possibility of resulting in 4-ene and 5-ene. In this case, only one product was obtained in a pure state and was assigned as a 4-ene compound from the above-mentioned results. This is probably due to stereochemical factors.

*2 m.p. 226° reported in Ref. (2) was determined in a capillary tube.

3) L. J. Bellamy : "The Infra-red Spectra of Complex Molecules," 178 (1958). John Wiley & Sons, Inc., New York.

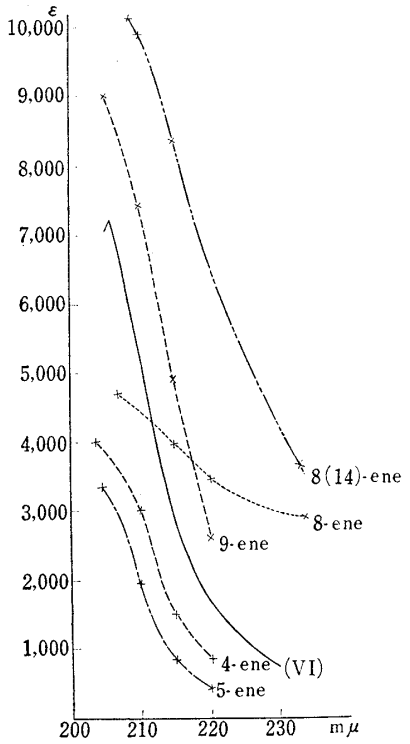


Fig. 1. Ultraviolet Absorption of the Isolated Double Bond in the Steroids

Treatment of (VI) with lithium aluminium hydride under mild conditions yielded the ketodiol (VII or VIIa), $C_{27}H_{42}O_5$, m.p. 192~196°. This substance gave a negative ferric chloride color test but a positive ketol test (2,3,5-triphenyltetrazolium chloride).

By oxidation with cupric acetate in acetic acid, the ketodiol (VII or VIIa) yielded a substance, which gave a negative ketol test (2,3,5-triphenyltetrazolium chloride) and a positive ferric chloride color test. From the infrared (ν_{max}^{Nujol} cm^{-1} : 3300~3500, 1643, 1600) and the ultraviolet spectrum (λ_{max}^{EtOH} mμ(log ε) : 212(3.95), 245(3.72), 302(3.53)), the product is not the expected diketone (VIIIa), which must have only one maximum at 270~280 mμ, but its dehydration product (VIII). The correctness of this assumption was also ascertained by the following experiments. Acetylation of (VIII) with acetic anhydride-pyridine afforded an acetate (IX), $C_{29}H_{40}O_5$, m.p. 212~215°, which showed an ultraviolet absorption maximum at 245 mμ(log ε 4.10) in ethanol corresponding to the 1,4-dien-3-one. Its infrared spectrum also showed absorption bands of enol ester (1760 cm^{-1}), of 1,4-dien-3-one (1675, 1648, 1613 cm^{-1}) but no normal acetoxyl band.

The product (VIII) was also obtained from (VII) merely by warming with sodium hydroxide in methanol. It is clear that the air-oxidation and the dehydration occurred in alkaline solution.

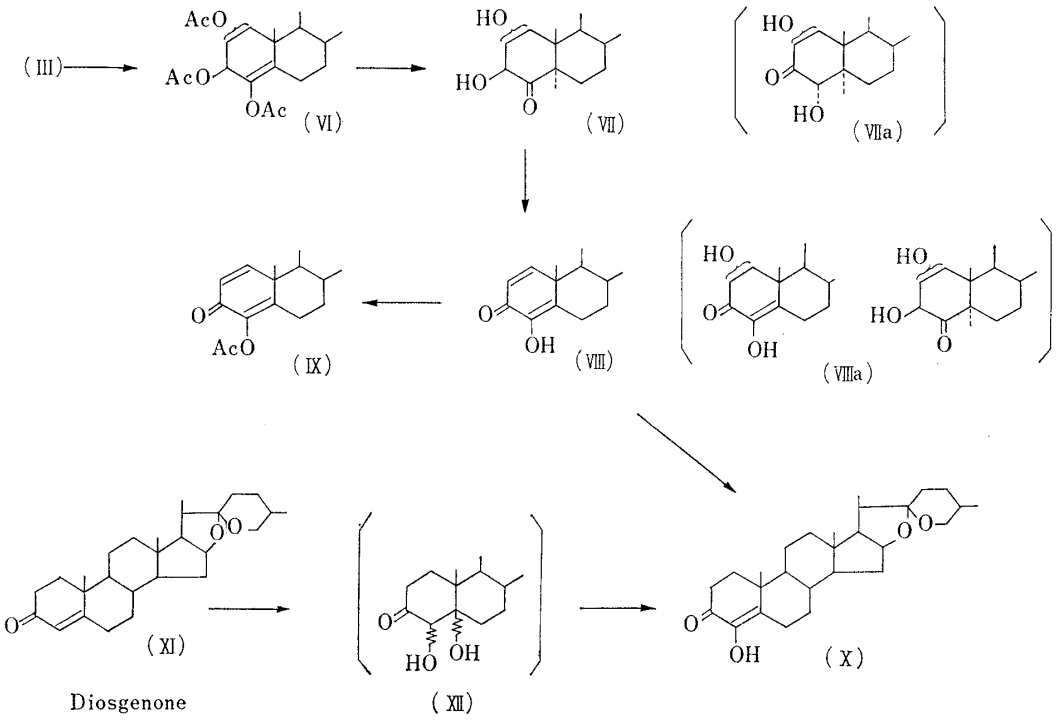
The fact that the dehydration reaction proceeds easily by either acetic acid or alkali indicates that the hydroxyl group participating in this reaction takes β-position to a ketone group. These results suggest that one hydroxyl group in kitigenin is located at C_1.

Catalytic hydrogenation of (VIII) with palladium-carbon gave a dihydro derivative (X), m.p. 215~219°. This substance showed a positive ferric chloride color test and also showed an ultraviolet absorption maximum at 279 mμ(log ε 4.08) in ethanol, corresponding to the enol form of α-diketone, and the analytical values were in good agreement with the formula $C_{27}H_{40}O_4$. It was proved that (X) was identical with 4-hydroxy-25D-spirost-4-en-3-one, prepared form diosgenone (XI), by mixed melting point determination and comparison of the infrared spectra.

The synthesis of (X) from diosgenone (XI) is as described below.

Oxidation of diosgenone (XI) was carried out with hydrogenperoxide in the presence

Chart 2



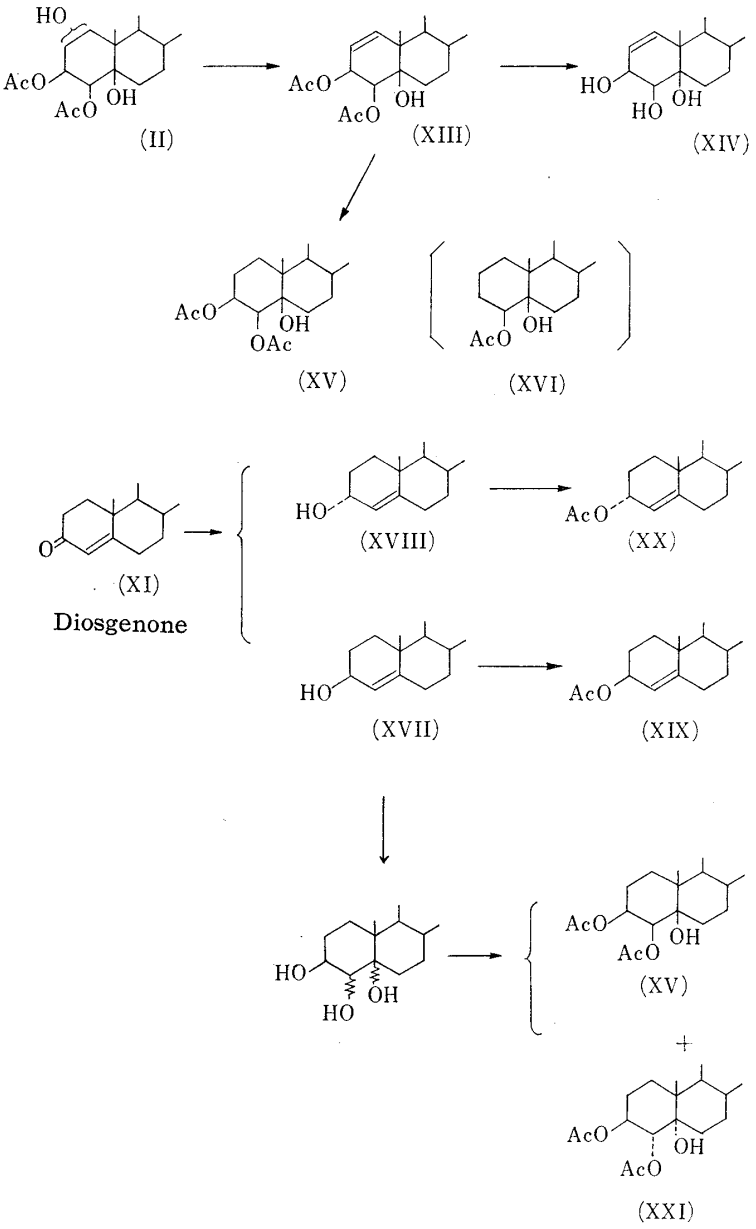
of osmium tetraoxide in ether.[4] When the reaction was stopped after 24 hours, about 75% of the starting material and a small amount of the oxidation product (5% yield) were obtained by chromatography on alumina. This oxidation product, m.p. 215~220°, showed a positive ferric chloride color test and its infrared spectrum suggested the presence of the hydroxyl group (3350~3510 cm^{-1}) and of the α,β-unsaturated ketone group (1661, 1630 cm^{-1}). The ultraviolet spectrum showed a maximum at 279 mμ in ethanol which shifted to 330 mμ by addition of one drop of 1*N*-sodium hydroxide solution. All these properties indicate the presence of an enolized α-diketone system. This substance is apparently 4-hydroxy-25D-spirost-4-en-3-one, which was produced by the addition of hydrogenperoxide to diosgenone (XI) followed by dehydration by alumina.

In the case of reaction for 90 hours, crude products of m.p. 225~241° and m.p. 190~206° were obtained in 23% and 7% yields respectively by chromatographic separation. Since the ultraviolet spectrum of the former showed a ketone band at 280 mμ with very low intensity (log ε ca. 1.9) in ethanol solution and the ferric chloride color test of this substance was negative, this product was assumed to be a hydroxylation product (XII) of diosgenone. As the latter product showed a positive ferric chloride color test and had an absorption maximum at 280 mμ (log ε ca. 3.3) in ethanol solution, it seemed to be a mixture of (XII) and (X). When the crude mixture of these two substances (m.p. 225~241° and m.p. 190~206°) was treated with potassium hydroxide in methanol at room temperature, it gave 4-hydroxy-25D-spirost-4-en-3-one, m.p. 215~220°, which was identical with (X) obtained from kitigenin as described already.

It is concluded that three of the four hydroxyl groups in kitigenin are located at C_3, C_4, and C_5, from the identity of the diketone, a degradation product of kitigenin, with 4-hydroxy-25D-spirost-4-en-3-one (X). The determination of the configuration of the hydroxyl groups at C-3 was then attempted.

4) J.F. Eastham, G.B. Miles, C.A. Krauth : J. Am. Chem. Soc., 81, 3114 (1959); A. Butenandt, H. Wolz : Chem. Ber., **71**, 1483 (1938).

Chart 3



Treatment of (II) with methanesulfonyl chloride in pyridine gave a compound, m.p. 236~240°, which contained no sulfur atom contrary to expectation. The infrared spectrum of this substance showed a weak absorption band of the *cis*-disubstituted double bond at 3045 cm^{-1} in addition to the normal acetoxy band and the analytical values agreed with formula $C_{31}H_{46}O_9$. This substance is therefore assigned as the unsaturated triol diacetate (XIII). Hydrolysis of (XIII) gave an unsaturated triol (XIV), m.p. 235~239°. The product (XIII) resists catalytic hydrogenation using Adams platinium catalyst in neutral solvents, but it could be hydrogenated in acetic acid to give a saturated triol diacetate (XV).*[3]

On the other hand, lithium aluminium hydride reduction of diosgenone (XI) gave an epimeric mixture of 4-en-3-ols, from which the 3α-ol (XVIII), m.p. 182~184°, and 3β-ol (XVII), m.p. 155~157°, were separated in a ratio of 1:2.5 respectively by the digitonine precipita-

*[3] In this case, the diol monoacetate (XVI) seemed also to be formed, but it was not studied in details because of a small amount of the material.

Table I. $[M]_D$ Differences of the 4-En-3-ols

	$[M]_D$	$\Delta[M]_D$(OAc-OH)
Cholest-4-en-3α-ol	+445.0°	
Cholest-4-en-3α-ol Acetate	+759.0°	
		+314°
Cholest-4-en-3β-ol	+178°	
Cholest-4-en-3β-ol Acetate	+ 34.3°	
		−143.7°
(XVIII) (3α-OH)	− 21.2°	
(XX) (3α-OAc)	+408.0°	
		+429.2°
(XVII) (3β-OH)	−164.3°	
(XIX) (3β-OAc)	−374.7°	
		−210.4°

tion. The $[M]_D$ differences between the free alcohols and the acetates, when compared with those of cholest-4-en-3-ols and their acetates, support strongly the assigned structure (XVIII) and (XVII). (see Table I). *cis*-Hydroxylation of the 4-en-3β-ol (XVII) by osmium tetraoxide gave a mixture, which was found to contain two products besides the starting 4-en-3β-ol by paper chromatography. After Florisil chromatography of the mixture of free ols, the eluate was acetylated and purified by alumina chromatography to give two trihydroxydiacetates, m.p. 200~203° and m.p. 245.5~246°, in 3% and 50% yield respectively. Based on the β-configuration of the starting 4-en-3-ol and the order of elution of the trihydroxydiacetates in chromatography, the former may accordingly be assigned as 3β,4β,5β- and the latter as 3β,4α,5α-trihydroxy 3,4-diacetate, respectively. The substance, m.p. 200~203°, was identical with the above compound (XV).

These facts, not only support the above-mentioned assumption but also indicate that the configuration of the 3-hydroxyl group is β oriented and the two hydroxyl groups at C-4 and C-5 in kitigenin are in *cis*-relation. The position of the one remaining hydroxyl group and the definite configuration of all hydroxyl groups will be discussed in a subsequent paper.

Experimental*4

Acetylation of Kitigenin (I)——Kitigenin (588 mg.) was dissolved in a warm mixture of 10 cc. of Ac_2O and 10 cc. of pyridine and allowed to stand for 3 days at room temperature. After concentrating under a reduced pressure, the solution was diluted with water and extracted with Et_2O. The Et_2O extract was washed successively with 5% HCl, water, 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (724 mg.) was treated with CS_2 to separate an insoluble (370 mg.) and a soluble fraction (354 mg.). Recrystallization of the former from $CHCl_3$-MeOH gave kitigenin diacetate (II), m.p. 217~219°, $[\alpha]_D^{29.5}$ −45.6°±2° (c=0.868, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_8 \cdot \frac{1}{2}H_2O$: C, 66.76; H, 8.68; H_2O, 1.62. Found: C, 66.89; H, 8.76; H_2O, 1.64. Calcd. for $C_{31}H_{48}O_8$*5: Ac, 15.69. Found: Ac, 15.81. IR ν_{max}^{Nujol} cm^{-1}: 3630, 3520~3300 (OH), 1745, 1730 (OAc), 1662 (H_2O).

The latter fraction was chromatographed on alumina (Brockmann, 11 g.). The benzene fraction gave a crude triacetate (III) (238 mg.). Recrystallization from MeOH gave a pure sample, m.p. 219~220.5°. $[\alpha]_D^{30}$ −53.6°±2° (c=1.023, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{50}O_9$: C, 67.02; H, 8.51. Found: C, 67.09; H, 8.53. IR ν_{max}^{Nujol} cm^{-1}: 3610 (OH, sharp), 1740, 1746, 1754 (OAc).

This sample was identical with that of the triacetate reported previously*2 in mixed melting point and comparison of the infrared spectra.

Oxidation of the Diacetate (II)——To a stirred solution of 200 mg. of the diacetate (II) in 24 cc. of Me_2CO was added dropwise 0.14 cc. of CrO_3-H_2SO_4 solution*6 (containing 24.8 mg. of CrO_3, 1.1 equiv.)

*4 All melting points were determined by the Monoscop IV. Infrared spectra were measured with the Koken Infrared Spectrophotometer, Model DS-301.

*5 An anhydrous sample was used for acetyl number determination.

*6 A solution of 26.72 g. of CrO_3 in 23 cc. of conc. H_2SO_4 was diluted with water to a volume of 100 cc. and it was used as a standard solution. *cf.* C. Djerassi, R. R. Engle, A. Bowers: J. Org. Chem., **21**, 1547 (1956).

at 15°. After 3 min., the reaction mixture was diluted with water and extracted with Et_2O. The Et_2O extract was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. Recrystallization of the residue (225 mg.) from MeOH gave (IV) as plates, m.p. 200~202°, $[\alpha]_D^{24}$ −64.7°±2° (c=1.002, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{46}O_8$: C, 68.10; H, 8.48. Found: C, 68.07; H, 8.54. UV λ_{max}^{EtOH}: 291 mμ (log ε 1.73). IR ν_{max}^{Nujol} cm^{-1}: 3568 (OH), 1743 (OAc), 1708 (>C=O).

Dehydration of the Triacetate (III)——To a solution of 200 mg. of the triacetate (III) in 3 cc. of pyridine 0.2 cc. of $SOCl_2$ was added with ice cooling. After 50 min. at room temperature, the reaction mixture was diluted with ice-cold water and Et_2O. The Et_2O layer was washed successively with 5% HCl, water, 5% Na_2CO_3, and water, dried over Na_2SO_4, and evaporated. The product was recrystallized from MeOH to give the crystals of (VI), m.p. 218~221°. A mixed melting point with the starting material (III) showed a clear depression, $[\alpha]_D^{24}$ −129° ±2° (c=1.105, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{48}O_8$: C, 69.20; H, 8.45. Found: C, 69.05; H, 8.49. UV λ_{max}^{EtOH}: 206 mμ(ε 7200). IR ν_{max}^{Nujol} cm^{-1}: 1762 (enol acetate), 1730 (OAc). IR $\nu_{max}^{CS_2}$ cm^{-1}: 1766 (enol acetate), 1746 (OAc).

Reductive Hydrolysis of the 4-Ene-triacetate (VI) with $LiAlH_4$——To an ice-cooled suspension of 100 mg. of $LiAlH_4$ in 10 cc. of dehyd. Et_2O was added dropwise a solution of 100 mg. of (VI) in 5 cc. of dehyd tetrahydrofuran with stirring. After 20 min., the reaction mixture was decomposed with ice cautiously, acidified, and extracted with Et_2O. The Et_2O extract was washed with water, 5% Na_2CO_3, and water, dried over Na_2SO_4, and evaporated. The residue (81 mg.) was recrystallized from Me_2CO-MeOH to give a pure sample of the ketodiol (VII), m.p. 192~196°. This showed a negative ferric chloride color test and a positive ketol test (2,3,5-triphenyltetrazolium chloride). *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.61; H, 9.52. IR ν_{max}^{Nujol} cm^{-1}: 3360~3540 (OH, broad), 1707 (>C=O).

Preparation of the 4-Acetoxy-1,4-dien-3-one (IX) from the Ketodiol (VII)——$(AcO)_2Cu$ (30 mg.) was added to a solution of 48 mg. of the ketodiol (VII) in a mixture of 2.5 cc. of AcOH, 0.5 cc. of MeOH, and a little water. The mixture was heated on a steam bath for 3.5 hr. and then refluxed for 2 hr. A small amount of a reddish brown precipitate was formed. The reaction mixture was filtered, evaporated *in vacuo*, and extracted with Et_2O. The Et_2O extract was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The product (VIII) (45 mg.), m.p. 175~195°, clearly showed a positive ferric chloride color test and negative ketol test (2,3,5-triphenyltetrazolium chloride). In spite of repeated recrystallizations, a sample having a sharp melting point could not be obtained. UV λ_{max}^{EtOH} mμ (log ε): 212 (3.95), 245 (3.72), 302 (3.53). IR ν_{max}^{Nujol} cm^{-1}: 3300~3510 (OH, broad), 1643, 1600 (α,β-unsaturated ketone).

The above product (41 mg.) was acetylated with 1 cc. of pyridine and 0.5 cc. of Ac_2O by heating on a steam bath for 1 hr. After treatment in the usual manner, the crude acetate was chromatographed on alumina (Woelm III, 1.2 g.). The petr. ether-benzene (3:1, 1:1) fractions gave 15 mg. of (IX), which was recrystallized from Et_2O-MeOH to give an analytical sample, m.p. 212~215°. *Anal.* Calcd. for $C_{29}H_{40}O_5$: C, 74.32; H, 8.60. Found: C, 74.20; H, 8.72. UV λ_{max}^{EtOH}: 245 mμ (log ε 4.10). IR ν_{max}^{Nujol} cm^{-1}: 1766 (enol acetate), 1675, 1648, 1613 (1,4-dien-3-one).

Preparation of 4-Hydroxy-25D-spirost-4-en-3-one (X) from the Ketodiol (VII)——The ketodiol (VII) (62 mg.) was refluxed with 3% NaOH-MeOH (5 cc.) for 30 min. The reaction mixture was acidified, extracted with Et_2O, washed with water, dried over Na_2SO_4, and evaporated. The residue (46 mg.), m.p. 180~192°, clearly showed a positive ferric chloride color test. The comparison of the infrared spectrum of this sample and that of the product, m.p. 178~195°, obtained by $(AcO)_2Cu$ oxidation of (VII) showed that both products consist of the same principal component.

A solution of this product in 4 cc. of AcOEt was shaken with 10% Pd-C in an atmosphere of H_2. 1.5 equivalent of H_2 was absorbed. The catalyst was filtered off and the solvent removed. The residue, m.p. 206~214°, was recrystallized from Et_2O-MeOH to give a pure sample of (X), m.p. 215~219°. $[\alpha]_D^{29}$ +10.3°±6° (c=0.361, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.70; H, 9.46. UV λ_{max}^{EtOH}: 279 mμ (log ε 4.08). IR ν_{max}^{Nujol} cm^{-1}: 3355~3530 (OH), 1668, 1635 (4-en-3-one).

This substance was identified with 4-hydroxy-25D-spirost-4-en-3-one prepared from diosgenone as described below by a mixed melting point determination and comparison of the infrared spectra.

Preparation of 4-Hydroxy-25D-spirost-4-en-3-one (X) from Diosgenone (XI)——a) A mixture of 400 mg. diosgenone (XI), 20 mg. of OsO_4 and 0.7 cc. of 30% H_2O_2 in 40 cc. of Et_2O was allowed to stand, with occasional shaking, for 24 hr. at room temperature and then evaporated *in vacuo*. In cases when the mixture became dark during evaporation, it was treated with a few drops of 30% H_2O_2 to decolorize and evaporated to dryness. The residue was again dissolved in Et_2O, washed with 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The gummy residue was crystallized from MeOH to recover 180 mg. of the starting material, m.p. 179~180°. The residue (218 mg.) of the mother liquor was chromatographed on alumina (Brockmann, 4 g.). The benzene-petr. ether (1:2, 1:1) and benzene fractions gave 107 mg. of the starting material, m.p. 180~182°. The benzene-$CHCl_3$ (4:1, 2:1) and $CHCl_3$ fractions gave 20 mg. of (X), m.p. 215~220°. This showed a positive

ferric chloride color test. UV λ_{max}^{EtOH} : 279 mμ (log ε 4.06). λ_{max} 330mμ : (in EtOH, added one drop of 1*N* NaOH). IR ν_{max}^{Nujol} cm^{-1} : 3510～3350 (OH), 1661, 1630 (4-en-3-one). The infrared spectrum of this sample was identical with that of the product obtained in (b).

b) A mixture of diosgenone (1 g.), OsO_4 (50 mg.) and 30% H_2O_2 (1.75 cc.) in Et_2O (55 cc.) was allowed to stand with occasional shaking at room temperature in dark for 90 hr. The reaction mixture was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (1.09 g.) was chromatographed on alumina (Brockmann, 30 g.). The benzene and benzene-$CHCl_3$ (10:1～1:1) fractions yielded 440 mg. of the starting material. The $CHCl_3$ fraction gave 230 mg. of crystals, m.p. 225～241°, UV λ_{max}^{EtOH} : 280 mμ (log ε ca. 1.9), which showed a negative ferric chloride color test. The $CHCl_3$-MeOH (10:1) fraction gave 69 mg. of crystals, m.p. 187～206°, UV λ_{max}^{EtOH} : 280 mμ (log ε ca. 3.3), which showed a positive ferric chloride color test. These fractions of m.p. 225～241° and m.p. 187～206° were combined, dissolved in 50 cc. of 2.5% KOH-MeOH and kept at room temperature overnight. The needle-like crystals separated. Both crystals and filtrate were treated separately with Et_2O and 5% HCl. The Et_2O solution was washed with water, dried over Na_2SO_4, and evaporated. Each residue (145 mg. and 142 mg. respectively) showed a positive ferric chloride color test, thus, were combined. Recrystallization from Me_2CO gave a pure sample of 4-hydroxy-25D-spirost-4-en-3-one, m.p. 215～220°. *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found : C, 75.82; H, 9.54. IR ν_{max}^{Nujol} cm^{-1} : 3355～3525 (OH), 1664, 1632 (4-en-3-one).

Dehydration of Kitigenin Diacetate by CH_3SO_2Cl-pyridine——To a solution of 200 mg. of kitigenin diacetate (II) in 8 cc. of pyridine 2 cc. of CH_3SO_2Cl was added with ice cooling and the mixture was kept overnight at 0°. After treatment in the usual way, the product (225 mg.) was crystallized from MeOH to yield a crude crystalline substance (98 mg.), m.p. 190～215°, which was negative to sulfur test. Recrystallization from Me_2CO gave a pure sample of (XIII), m.p. 236～240°. *Anal.* Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found : C, 69.95; H, 8.84. IR ν_{max}^{Nujol} cm^{-1} : 3580 (OH, sharp), 3045 (-$\overset{H}{\underset{|}{C}}$=$\overset{H}{\underset{|}{C}}$-), 1737 (shoulder), 1728 (OAc), 1643 (-$\overset{|}{C}$=$\overset{|}{C}$-).

Hydrolysis of the Unsaturated Triol Diacetate (XIII)——The unsaturated triol diacetate (XIII) (22 mg.) was hydrolyzed with 1.5% NaOH-MeOH (10 cc.) at room temperature for 2 hr. After concentration under a reduced pressure at room temperature, the mixture was diluted with water and extracted with Et_2O. Recrystallization of the crude product (20 mg.), m.p. 220～240°, from Me_2CO afforded a pure sample of the unsaturated triol (XIV), m.p. 235～239°. $[\alpha]_D^{24}$ +18.7°±4° (c=0.503, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found : C, 72.94; H, 9.67. IR ν_{max}^{Nujol} cm^{-1}: 3590, 3546 (OH), 3030 (-$\overset{H}{\underset{|}{C}}$=$\overset{H}{\underset{|}{C}}$-), 1658 (-$\overset{|}{C}$=$\overset{|}{C}$-).

Catalytic Hydrogenation of the Unsaturated Triol Diacetate——A solution of the unsaturated triol diacetate (XIII) (200 mg.) in AcOH (22 cc.) was shaken with Adams Pt (prepared from 200 mg. of $PtO_2 \cdot 2H_2O$) in an atmosphere of H_2 until the absorption of H_2 stopped. After filtration and evaporation *in vacuo*, the residue was dissolved in Et_2O, and the Et_2O, solution was washed with 5% Na_2CO_3, and water, dried over Na_2SO_4, and evaporated. The product (196 mg.) was chromatographed on alumina (Woelm II, 6.2 g.). The petr. ether-benzene (8:1～3:1) fractions gave a mixture, which was rechromatographed to give a small amount of the substance,*7 m.p. 195～212°, and 50 mg. of (XV). The benzene fraction (90 mg.) was recrystallized from Et_2O-MeOH to give a pure sample of (XV), m.p. 204～206°. $[\alpha]_D^{23}$ −21.1°±2° (c=0.848, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.72; H, 9.20. IR ν_{max}^{Nujol} cm^{-1} : 3580 (OH), 1744, 1732 (OAc).

$LiAlH_4$ Reduction of Diosgenone (XI)——To a stirred suspension of $LiAlH_4$ (5 g.) in dehyd. Et_2O (80 cc.)was added dropwise a solution of 5 g. of diosgenone in 100 cc. of dehyd. tetrahydofuran over a period of 25 min. After refluxing for 1 hr., the excess reagent was decomposed with ice-water. The mixture was acidified with 5% HCl and extracted with Et_2O. After treatment in the usual way, 5.02 g. of the crude product was obtained.

To a solution of 1.08 g. of this product in 110 cc. of 99% EtOH was added a solution of 3 g. of digitonine in 200 cc. of 80% EtOH. After allowing to stand at room temperature for 40 min., the precipitate was filtered, washed with 99% EtOH, and treated with 200 cc. of boiling Et_2O. The digitonide (3.6 g.) thus obtained was dissolved in 30 cc. of pyridine and the solution was heated at 90° for 2.5 hr. After evaporation *in vacuo*, the residue was extracted with boiling Et_2O. The Et_2O extract was washed with 5% HCl, water, 5% Na_2CO_3, and water, dried over Na_2SO_4, and evaporated. The crude 4-en-3β-ol (XVII) thus obtained was recrystallized from $CHCl_3$-MeOH to give a pure sample of (XVII), m.p. 155～157°. $[\alpha]_D^{23}$ −39.6°±3°(c=0.727, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found : C, 78.42; H, 10.36. IR ν_{max}^{Nujol} cm^{-} : 3400～3500 (OH, broad).

*7 Since this substance shows an acetoxyl band and hydroxyl band in its infrared spectrum, it appears to be a diol monoacetate (XVI).

The filtrate of the digitonide was evaporated *in vacuo* and extracted with Et_2O. After treatment of the Et_2O extract in the usual way, the crude 4-en-3α-ol (XVIII) (274 mg.) obtained, m.p. 171~176°, was recrystallized from $CHCl_3$-MeOH and then from CH_2Cl_2-Me_2CO to give a pure sample of (XVIII), m.p. 182~184°. $[\alpha]_D^{20}$ −5.1°±3° (c=0.831, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H 10.21. Found: C, 77.82; H, 10.25. IR ν_{max}^{Nujol} cm^{-1} : 3380~3440 (OH, broad).

Acetylation of the 4-En-3β-ol (XVII)——A solution of 159 mg. of 4-en-3β-ol in a mixture of pyridine (4 cc.) and Ac_2O (4 cc.) was heated at 90° for 30 min. After treatment in the usual way, the product (160 mg.), m.p. 160~165°, was recrystallized from Et_2O-Me_2CO to give a pure sample of (XIX) as plates, m.p. 167~169°. $[\alpha]_D^{22}$ −82.0°±2° (c=1.019, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found : C, 76.55; H, 9.95. IR ν_{max}^{Nujol} cm^{-1} : 1744 (OAc), 1669 (>C=C<).

Acetylation of the 4-En-3α-ol (XVIII)——A solution of 4-en-3α-ol (XVIII) (259 mg.) in a mixture of pyridine (3 cc.) and Ac_2O (3 cc.) was heated at 90° for 30 min. After treatment in the usual way, the product (267 mg.), m.p. 150~161°, was recrystallized from $CHCl_3$-MeOH and then from Me_2CO to give a pure sample of (XX) as plates, m.p. 170~172°. $[\alpha]_D^{18}$ +89.3°±2° (c=1.035, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found : C, 76.12; H, 9.72. IR ν_{max}^{Nujol} cm^{-1} : 1732 (OAc), 1662 (>C=C<).

Preparation of the Triol Diacetates (XV and XXI) from the 4-En-3β-ol (XVII)——To a solution of 4-en-3β-ol (1.01 g.) in 80 cc. of dehyd. Et_2O was added pyridine (1 cc.) and then a solution of OsO_4 (665 mg.) in dehyd. Et_2O (5 cc.). After allowing to stand at room temperature for 140 hr., the Et_2O solution was separated from the precipitation (osmate ester) by decantation. This solution was then washed with 5% HCl, water, 5% Na_2CO_3 and water dried, and evaporated. To a combined solution of the residue and the above precipitation in 250 cc. of EtOH, 100 cc. of 10% Na_2SO_3 was added and the mixture was refluxed for 3.5 hr. After filtration and concentration, the mixture was diluted with water to give a precipitate. This precipitate was dissolved in $CHCl_3$ containing a small amount of MeOH and the solution was washed with water, dried, and evaporated. The residue (860 mg.) was treated with benzene to separate a soluble fraction (508 mg.) and an insoluble fraction (376 mg.). The soluble fraction was chromatographed on Florisil (15 g.). The benzene-$CHCl_3$ (5:1~1:1) fractions gave the starting 4-en-3β-ol (332 mg.). The $CHCl_3$-MeOH (60:1~5:1) fractions gave a mixture of triols (97 mg.).

The $CHCl_3$-MeOH fractions and the above benzene-insoluble fraction (total, 473 mg.) were acetylated with a mixture of pyridine (8 cc.) and Ac_2O (8 cc.) at 90° for 45 min. The reaction mixture was treated in the usual way and gave a mixture of acetates (540 mg.), which was chromatographed on alumina (Woelm II, 16 g.). The petr. ether-benzene (3:1) fraction (27 mg.) was recrystallized from Et_2O-MeOH and then from MeOH to give the 3β,4β,5β-triol 3,4-diacetate, m.p. 200~203°. IR ν_{max}^{Nujol} cm^{-1} : 3576 (OH), 1733, 1745 (OAc). This sample was identical with the triol diacetate (XV) obtained from kitigenin in a mixed melting point and the comparison of the infrared spectra.
The benzene and $CHCl_3$ fractions (425mg.), m.p. 240~242°, were recrystallized from Et_2O-MeOH to give the 3β,4α,5α-triol 3,4-diacetate (XXI), m.p. 245.5~246°. $[\alpha]_D^{23}$ −23.4° ±2° (c=0.603, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found : C, 69.85; H, 9.22. IR ν_{max}^{Nujol} cm^{-1} : 3560 (OH), 1740 (OAc).

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Summary

Kitigenin has its four hydroxyl groups all on ring A. One of them is a tertiary, C_5-hydroxyl group, since it resists oxidation and acetylation. Subsequently, kitigenin was converted to 4-hydroxy-25D-spirost-4-en-3-one and 25D-spirostan-3β,4ξ,5ξ-triol, synthesized from diosgenone. Therefore, it was determined that the three hydroxyl groups are located at C_3, C_4, and C_5 and that the configuration of the C_3-hydroxyl group is β and the C_4- and C_5- hydroxyl groups are in *cis* relation.

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