UDC 547.92 : 582.572.2

111. Kanzo Sasaki : Studies on the Steroidal Components of Domestic Plants. XXXIII.[1] Constituents of *Reineckia carnea* KUNTH. (5). Structure of Kitigenin. (2).

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In the previous paper,[1] it was demonstrated that kitigenin (I) has four hydroxyl groups, three of them being located at C_3, C_4 and C_5. Furthermore, it was assumed that the one remaining hydroxyl group may be situated at C_1 from the following results : i) The easy formation of the 4-hydroxy-1,4-dien-3-one (III) from the keto-diol (II) by elimination of the hydroxyl group; ii) the ultraviolet spectrum of the ketone (V) (obtained from the diacetate (IV) by oxidation) showing a maximum at 291 mμ in ethanol solution.

The absorption of the ketone (V) exhibits the maximum at 6~11 mμ longer wave length than that of the C_3-ketone (280~285 mμ) and C_2-ketone (280 mμ) and this suggests the existence of C_1-ketone as shown in Table I. In order to obtain further rigid proof of the location of the hydroxyl group at C_1, the following experiments were carried out.

TABLE I. Ultraviolet Absorption Maximum of Ketones on Ring A

	Solvent	max (mμ)	log ε	Ref.
Cholestan-1-one	Et_2O	297	1.47	*a*)
Methyl 1-Oxo-5α-etianate	EtOH	296	1.51	*a*)
Methyl 1-Oxo-etianate	EtOH	293~294	1.66	*a*)
2β,3α,5-Trihydroxy-25D,5β-spirostan-1-one 2,3-diacetate	〃	289	1.76	*b*)
(VII)	〃	291	1.73	
Cholestan-2-one	〃	279.5	1.39	*c*)
	〃	280~286	1.20~1.70	*d*)
Cholestan-4-one	〃	285	1.70	*e*)

a) F. Sallmann, Ch. Tamm : Helv. Chim. Acta, **39**, 1340 (1956).
b) Author's experiment.
c) R.C. Cookson, S.H. Dangegaenker : J. Chem. Soc., **1955**, 352.
d) L. Dorfmann : Chem. Revs., **53**, 47 (1953).
e) C.W. Shoppee, *et al.* : J. Chem. Soc., **1959**, 630.

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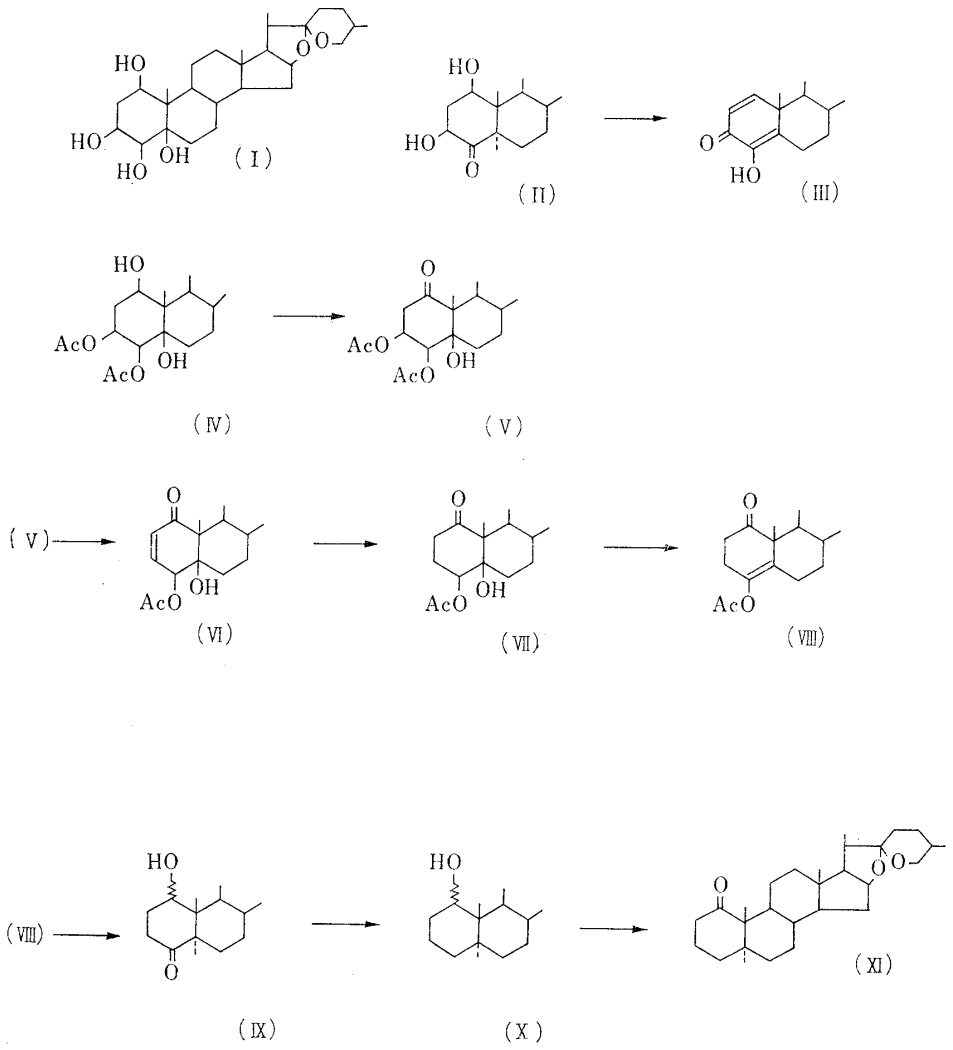
1) XXXII : This Bulletin, **9**, 684 (1961)

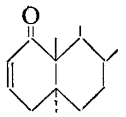
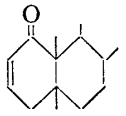
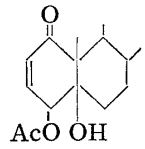
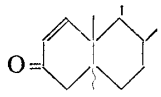
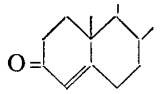
Treatment of (V) with alumina in benzene solution for one and half an hour at room temperature gave an unsaturated ketone (VI), m.p. 246~248°. Its empirical formula, $C_{29}H_{42}O_6$, indicated the elimination of one acetoxyl group and its infrared and ultraviolet spectra were consistent with the structure of (VI). Such an easy elimination of an acetoxyl group by alumina indicates that this acetoxyl group is situated in β-position to the ketone group. The ultraviolet spectrum of the above showed a maximum at 220 mμ (log ε 3.92), an extraordinarily short wave length for a conjugated ketone. Based on the comparison with calculated and measured values of various conjugated ketones as shown in Table II, the maximum of this α,β-unsaturated ketone is located at a somewhat shorter wave length than that of the normal 2-en-1-one type absorption, but it is most likely that this absorption maximum is due to the presence of the 2-en-1-one group. Such a hypsochromic shift is also observed in the 4ξ-bromo-2-en-1-one compound (A/B *cis*)[2] and may be attributed to the substituent (acetoxyl group in the case of (VI)) adjacent to the α,β-unsaturated ketone.

Chart 1



2) Unpublished data.

Table II. Ultraviolet Absorption Maximum of α,β-Unsaturated Ketones on Ring A.

	Solvent	max (mμ) Calcd.	max (mμ) Found	log ε	Ref.
 Cholest-2-en-1-one	EtOH	227	224	3.90	*a*)
 Methyl 1-Oxo-2-etienate	〃	227	225	3.93	*b*)
25D,5β-Spirost-2-en-1-one	〃		225	3.89	*c*)
 (XVI)	〃		220	3.92	
		227	229～232	4.0～4.10	*d*)
		244	241	4.22	*d*)

a) P. Striebel, Ch. Tamm : Helv., Chim. Acta, **37**, 1094 (1954).
b) T. Reichstein, *et al.* : *Ibid.*, **38**, 1013 (1955).
c) K. Morita : Bull. Chem. Soc. Japan, **32**, 791 (1959).
d) L. Dorfmann : Chem. Revs., **53**, 47 (1953).

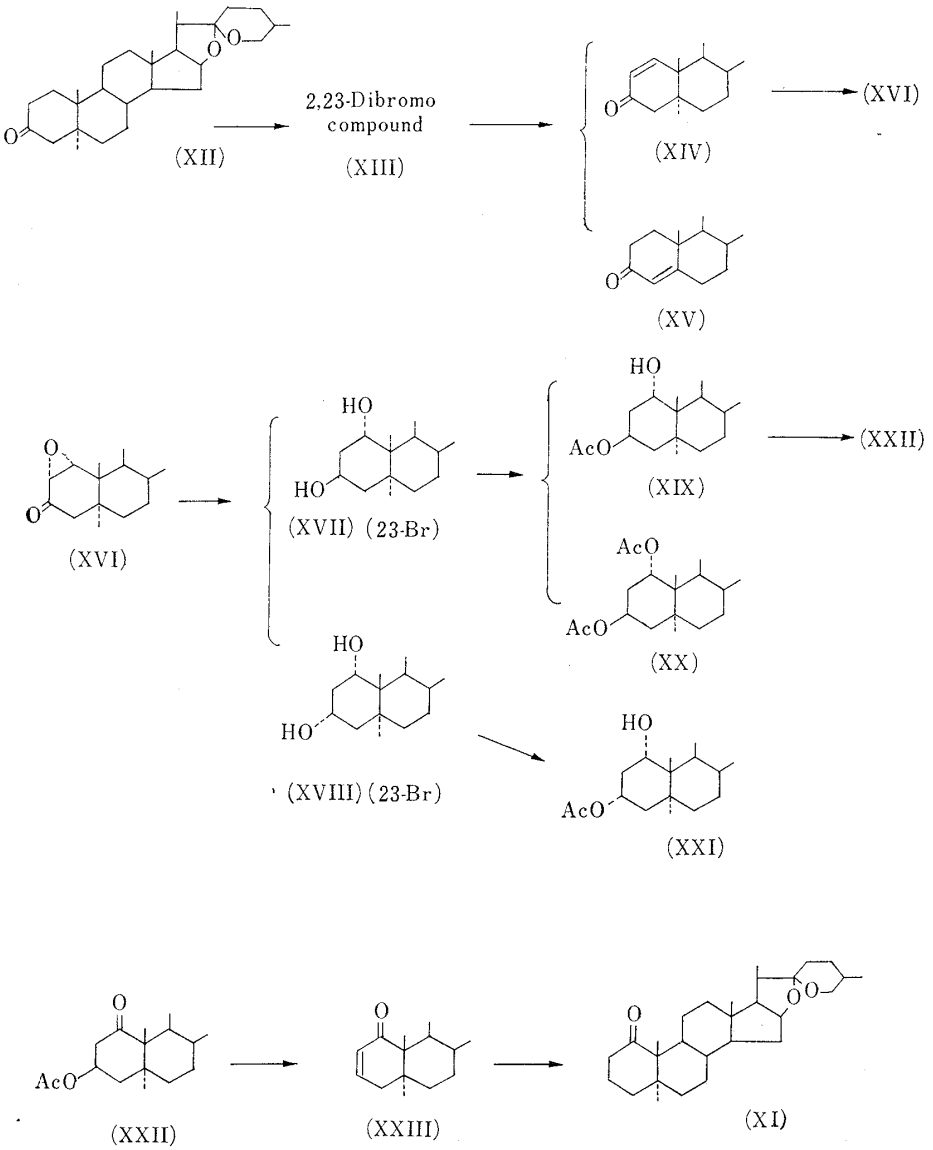
Catalytic hydrogenation of (VI) with palladium-carbon gave a saturated ketone (VII), m.p. 247～248°. Its ultraviolet spectrum showed a maximum at 292 mμ in ethanol, suggesting the possibility of having a C_1-ketone. By dehydration with pyridine-thionyl chloride at room temperature, (VII) gave a crystalline substance, m.p. 198°, and the latter is assigned to bo the diketo mono-enol acetate (VIII) from its infrared spectrum, showing bands at 1759 and 1714 cm^{-1}, and its analytical values. In this case, only one reaction product was obtained, in which dehydration occurred toward C_4 as in the case of the dehydration of kitigenin triacetate reported previously.

Lithium aluminium hydride reduction of (VIII) afforded a ketol (IX), m.p. 235～247°. Even after repeated recrystallization, this substance did not show a sharp melting point, but its infrared absorption bands agreed with the structure (IX). This ketol was used for the next Huang-Minlon reduction without subsequent purification. The reduction product was purified by repeated chromatography and a monohydroxy sapogenin (X), m.p. 222～225°, was obtained. This substance was oxidized with chromium trioxide-sulfuric acid in acetone to a ketone (XI), m.p. 165～170°, and its infrared spectrum showed only a ketone band at 1705 cm^{-1}. The ketone (XI) thus obtained was found to be identical with the authentic 25D,5α-spirostan-1-one prepared from tigogenone in the reaction sequences described below. It was determined, therefore, that the one hydroxyl group of kitigenin in question is situated at the C_1-position.

The 2,23-dibromo compound (XIII), obtained by bromination of tigogenone (XII) in chloroform, was refluxed in collidine, which yielded a mixture of the 1-en-3-one and the 4-en-3-one.*2 Each component could be separated by chromatography, since the former eluted

*2 It has been reported that the 4-en-3-one is obtained as a by-product in the dehydrobromination of the 2-bromo-3-one by refluxing collidine. cf. C. Djerassi, C.R. Scholz : J. Am. Chem. Soc., **69**, 2404 (1947); M. Rubin, H. Wishinsky, F. Bompard : *Ibid.*, **73**, 2338 (1951).

Chart 2.



faster than the latter. The structure of each compound (XIV, m.p. 220~225°, and XV)*3 was assigned from the results of the corresponding ultraviolet spectrum. The product (XIV) gave an epoxide by the action of sodium hydroxide-hydrogenperoxide in dioxane or methanol. It is reasonable to consider that the reagent attacked from the rear side (α) to give an α-epoxide (XVI), as in the case of methyl 3-oxo-5α-1-etienate[3] and cholest-1-en-3-one.[4] Lithium aluminium hydride reduction of the epoxide (XVI) gave two isomeric dihydroxy monobromo compounds, (XVII), m.p. 205~207° and (XVIII), m.p. 231~233° after chromatography on alumina. Since both products were derived from the same epoxide, they should have a hydroxyl group of the same configuration at C_1, and they can be considered isomeric at C_3. The compound (XVIII) eluted faster in alumina chromatography was not so easily acetylated, whereas the other (XVII) easily gave a monoacetoxyl compound. Therefore,

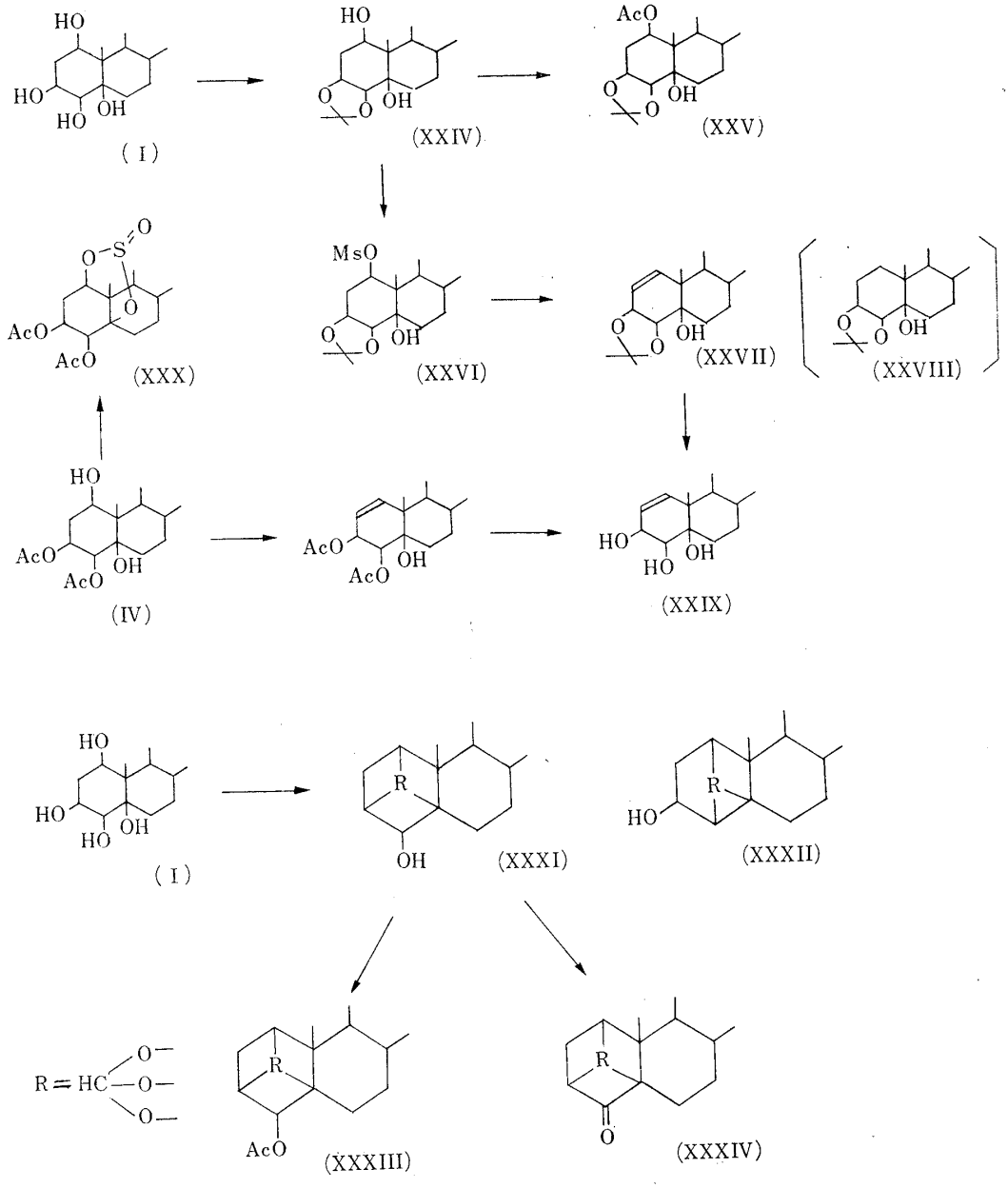
*3 (XV) is the fraction which shows a maximum at 240 mμ in ethanol solution and it is not isolated as a crystal.

3) F. Sallman, Ch. Tamm : Helv. Chim. Acta, **39**, 1340 (1956).

4) P. Striebel, Ch. Tamm : *Ibid.*, **37**, 1094 (1954).

it is reasonable to consider that (XVII) is the 1α,3β-compound and (XVIII) the 1α,3α-compound. Acetylation of (XVII) with acetic anhydride followed by debromination with zinc-acetic acid afforded a monoacetate (XIX), m.p. 276~278°. The monoacetate (XIX) gave a ketone (XXII), m.p. 215~217°, by oxidation with chromium trioxide-sulfuric acid. The ketone (XXII) was deacetylated to (XXIII), m.p. 170~173°, by alumina as in the case of (V). The structure of this substance is assigned as (XXIII) on the basis of its analytical values and infrared and ultraviolet spectra. The rate of this deacetylation is somewhat slower, when compared with that of (V) and this difference is probably due to the conformational factor of each acetoxyl group in (XXII) and (V). Catalytic hydrogenation of this α,β-unsaturated ketone (XXIII) with palladium-carbon in acetic ester gave a saturated ketone, m.p. 167~169°, which is the expected 25D,5α-spirostan-1-one from its infrared spectrum and analysis. As the location of the one hydroxyl group of kitigenin at C_1 was thus determined, it was established that the four hydroxyl groups in kitigenin are located at C_1, C_3, C_4, and C_5.

Chart 3



Finally, the stereochemical relationship of these four hydroxyl groups in kitigenin was further examined.

Kitigenin gave a monoacetonide (XXIV), m.p. 227~228°, by refluxing in anhydrous acetone in the presence of *p*-toluenesulfonic acid. This acetonide gave only a monoacetate (XXV), m.p. 180.5~182.5°, under usual conditions. By the action of methanesulfonyl chloride in pyridine the acetonide (XXIV) gave a mesylate (XXVI), m.p. 163~164.5°. An attempt to obtain a saturated trihydroxyl compound (XXVIII) from the mesylate (XXVI) by reductive removal of the mesyl group with lithium aluminium hydride was unsuccessful. The reaction product, m.p. 172~176°, showed absorption bands corresponding to *cis*-disubstituted double bond at 3050 and 1650 cm^{-1} and its analytical values are also in better agreement with the formula $C_{30}H_{46}O_5$ rather than with $C_{30}H_{48}O_5$. The reaction product was therefore, considered to be (XXVII) and not (XXVIII). Hydrolysis of (XXVII) in 80% acetic acid afforded smoothly an unsaturated triol (XXIX), m.p. 236.5~239.5°, which also showed absorption bands of the *cis*-disubstituted double bond at 3020 and 1656 cm^{-1} in its infrared spectrum.

This substance was identical with the unsaturated 3β,4,5-triol*4 prepared from kitigenin diacetate (IV). As the C_5-hydroxyl group is considered not a participant in the formation of the acetonide, the structure of the acetonide is assumed to be a C_3,C_4-acetonide and the C_3- and C_4-hydroxyl groups are in *cis* relation. Based on this fact and the previously obtained results of the *cis* relation of C_4- and C_5-hydroxyl groups, it wsa regarded that the C_3,C_4- and C_5-hydroxyl groups were all *cis* and β.

Treatment of kitigenin diacetate (IV) with thionylchloride-pyridine afforded a substance, m.p. 284.5~286°, containing sulfur atom. This showed an absorption band of R-O-S(=O)-O-R type[5] at 1199 cm^{-1} in addition to that of the acetoxyl group. From this observation, this substance is shown to have a sulfite group formed between C_1- and C_5-hydroxyl groups as represented by the formula (XXX) which is also supported by its analytical values. Such examples are often found in the cases of 1,3-diaxial alcohols.[6] Consequently, it was confirmed that the hydroxyl groups at C_1 and C_5 are *cis*.

The reaction of kitigenin with orthoformic ester in 1% methanolic hydrogen chloride gave an orthoester (XXXI),*5 m.p. 235~237°. This substance gave an acetate (XXXIII), m.p. 280~282°, and a ketone (XXXIV), m.p. 264.5~266.5°. From these facts, it was assumed that the orthoester is the 1,3,5- (XXXI) or 1,4,5-ester (XXXII), of which the former is more probable.

From the results obtained till now all the hydroxyl groups in kitigenin are considered to have β-configuration and so kitigenin is assigned as 25D,5β-spirostane-1β,3β,4β,5-tetrol.

Experimental*6

Elimination of Acetic Acid from 3,4-Diacetoxy-5-hydroxy-1-one (V)——A solution of 3,4-diacetoxy-5-hydroxy-1-one (V) (50 mg.) in benzene (4 cc.) was stirred with Al_2O_3 (Brockmann, 500 mg.) for 1.5 hr. After filtration and evaporation, 42 mg. of the product (VI), m.p. 241~247°, was obtained.

*4 The preparation of this compound from kitigenin diacetate was reported in Part XXXII.

*5 The infrared spectrum of this substance showed a sharp hydroxyl band at 3540 cm^{-1} and a strong orthoester band at 986, 991 and 1146 cm^{-1}. cf. R. Tschesche, G. Snatzke: Chem. Ber., **80**, 1558 (1955).

*6 All melting points are uncorrected. The infrared spectrum was measured with the Koken Infrared Spectrophotometer, Model DS-301 and the ultraviolet spectrum was taken with the Hitachi Spectrophotometer EPS-2.

5) L. J. Bellamy: "The Infra-red Spectra of Complex Molecules," 350 (1958). John Wiley & Sons, Inc., New York.

6) Pl. A. Plattner, *et al.*: Helv. Chim. Acta, **30**, 1432 (1947); G. Volpp, Ch. Tamm: *Ibid.*, **40**, 1860 (1957).

Recrystallization from Me_2CO gave a pure sample, m.p. 246~248°. $[\alpha]_D^{22}$ −12.4°±3° (c=0.868, $CHCl_3$). UV λ_{max}^{EtOH} : 220 mμ (log ε 3.92). IR ν_{max}^{Nujol} cm^{-1} : 3566 (OH, sharp), 1724 (OAc), 1679, 1587 (α,β-unsaturated ketone). *Anal.* Calcd. for $C_{29}H_{42}O_6$: C, 71.57; H, 8.70. Found : C, 71.61; H, 9.10.

Catalytic Hydrogenation of the Unsaturated Ketone (VI)——A solution of the unsaturated ketone (VI) (50 mg.) in AcOEt (10 cc.) was shaken with 10% Pd-C (50 mg.) in an atmosphere of hydrogen until the absorption of hydrogen stopped. The catalyst was filtered off and the filtrate was concentrated in a reduced pressure. Recrystallization of the product (51 mg.) from MeOH and then from Me_2CO gave a pure saturated ketone (VII) as needles, m.p. 247~248°. *Anal.* Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found; C, 71.54; H, 9.16. UV λ_{max}^{EtOH} : 292 mμ (log ε 1.74). IR ν_{max}^{Nujol} cm^{-1} : 3535, 3466 (OH), 1738 (OAc), 1716, 1704 (>C=O).

Dehydration of the Saturated Ketone (VII)——Thionyl chloride (0.3 cc.) was added to a solution of (VII) (283 mg.) in pyridine (4 cc.) under ice-cooling. After 1 hr. at 15~25°, the reaction mixture was diluted with ice-water and extracted with Et_2O. The extract was washed successively with 5% HCl, water, 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (275 mg.) was crystallized from Me_2CO to give the crude anhydro derivative (VIII) (155 mg.), m.p. 185~195°. The analytical sample was obtained by recrystallization from MeOH or MeOH-Me_2CO, m.p. 195~198°. $[\alpha]_D^{31}$ −191.7° ±2° (c=1.070, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found : C, 74.08; H, 8.82. IR ν_{max}^{Nujol} cm^{-1} : 1759 (enol acetate), 1714 (>C=O).

$LiAlH_4$ Reduction of the Anhydro Compound (VIII)——To a suspension of $LiAlH_4$ (220 mg.) in dehyd. Et_2O (15 cc.) was added dropwise a solution of (VIII) (190 mg.) in dehyd. tetrahydrofuran (8 cc.) at room temperature. After refluxing for 5 hr., the mixture was allowed to stand overnight at room temperature. The excess reagent was decomposed with ice-water and the mixture was treated with 5% HCl and Et_2O. The Et_2O solution was washed with water, 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (188 mg.) was treated with a mixture of benzene and petr. ether (2:1) to separate an insoluble product (10.3 mg.), m.p. 260~290° (decomp.), and the filtrate was chromatographed on alumina (Brockmann, 5.5 g.). Elution with benzene furnished a crude substance (IX) (123.1 mg.), m.p. 200~240°. In spite of repeated recrystallization from MeOH or Me_2CO, a substance having a sharp melting point could not be obtained. The sample having a melting point of 235~247° was used for the infrared measurement. IR ν_{max}^{Nujol} cm^{-1} : 3550 (OH), 1685~1690 (>C=O).

Huang-Minlon Reduction of the Ketol (IX)——The above crude substance (IX) (58 mg.) was dissolved in triethylene glycol (6.5 cc.) by warming. After addition of 100% hydrazine (2 cc.), the mixture was heated at 125~135° (bath temperature) for 3.5 hr. and then allowed to stand overnight at room temperature. A portion (0.8 cc.) of the reaction mixture was extracted with Et_2O. The infrared spectrum of the extract showed no ketone band. After heating for an additional 1 hr., NaOH (0.8 g.) was added to the reaction mixture, and the temperature was raised gradually to 190° over a period of 1 hr. After refluxing at 190~210° (bath temperature) for 2 hr., the mixture was diluted with water and extracted with Et_2O. The Et_2O solution was washed with water, dried over Na_2SO_4, and evaporated. The residue (49 mg.) was chromatographed on alumina (Woelm II, 1.4 g.). The petr. ether-benzene (9:1) fraction gave a mixture of oil and crystals (23.9 mg.). The petr. ether-benzene (4:1, 2:1) fractions gave a crystalline substance (10.3 mg.), m.p. 220~223°. Elution with benzene gave crystals (5.1 mg.). The first (petr. ether-benzene=9:1) and the third (benzene) fractions were combined and again chromatographed on alumina (Woelm II, 0.75 g.). The petr. ether fraction gave an oil (15.1 mg.). The petr. ether-benzene (4:1, 2:1) fractions gave a crystalline substance (12.1 mg.), m.p. 218~221°. The substances of m.p. 220~223° and m.p. 218~221° were combined and recrystallized from Me_2CO to give a pure sample of (X), m.p. 222~225°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found : C, 78.10; H, 10.75. IR ν_{max}^{Nujol} : 3520 cm^{-1} (OH, sharp).

Oxidation of the Monohydroxy Compound (X)——To a stirred solution of (X) (18 mg.) in Me_2CO (10 cc.) was added a CrO_3-H_2SO_4 solution*7 (0.015 cc.) (containing 3.9 mg. of CrO_3, 1.36 equiv.) at ca. 15°. After stirring for 5 min., the reaction mixture was diluted with water and extracted with Et_2O. The extract was washed with water, 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (20 mg.), m.p. 150~167°, was recrystallized from MeOH and then from Me_2CO-MeOH to give a pure sample of the ketone (XI), m.p. 165~170°. IR λ_{max}^{Nujol} : 1705 cm^{-1} (>C=O). This sample was identical with 25D, 5α-spirostan-1-one prepared from tigogenone (XII) as described below by a mixed melting point determination and the comparison of infrared spectra.

Bromination of Tigogenone (XII)——A HBr-AcOH solution (3 drops) was added to a solution of tigogenone (XII) (1 g.) in $CHCl_3$ (15 cc.). To this solution was added dropwise a solution of Br_2 (870 mg.) in $CHCl_3$ (6 cc.) at ca. 20°. Br_2 was immediately consumed. After all Br_2 solution was added, the mixture was allowed to stand for about 10 min. The solution was washed successively with water,

*7 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, *et al.* : J. Org Chem., **21**, 1547 (1956).

$Na_2S_2O_3$ solution, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated under a reduced pressure. The solution of the residue in $CHCl_3$ was decolorized by filtration through alumina (Woelm II, 10 g.). The filtrate was crystallized from Et_2O, yielding 740 mg. of the 2,23-dibromo (XIII), m.p. compound (XIII). Recrystallization from $CHCl_3$-MeOH and then from Me_2CO-CH_2Cl_2 gave a sample of 208° (decomp.). *Anal.* Calcd. for $C_{27}H_{40}O_3Br_2$: C, 56.65; H, 7.04; Br, 27.92. Found: C, 56.26; H, 7.02; Br, 28.35. IR ν_{max}^{Nujol}: 1723 cm^{-1} (>C=O).

Dehydrobromination of the 2,23-Dibromo Compound (XIII)——A solution of the 2,23-dibromo compound (XIII) (1.436 g.) in collidine (12 cc.) was refluxed for 3 hr. After the precipitated collidine hydrobromide was filtered off, the filtrate was diluted with Et_2O. The Et_2O solution was washed with 5% HCl, water, 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated. The residue (1.16 g.) was chromatographed on alumina (Woelm II, 30 g.). The petr. ether-benzene (2:1) fraction gave a crystalline substance (340 mg.) which showed an absorption maximum at 230 mμ in EtOH. Recrystallization from $CHCl_3$-MeOH and then from CH_2Cl_2-Me_2CO gave a pure sample of (XIV), m.p. 220~225° (decomp.). $[\alpha]_D^{30}$ −55.3°±2° (c=0.602, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{39}O_3Br$: C, 65.97; H, 8.00; Br, 16.26. Found: C, 65.71; H, 8.04; Br, 16.11. UV λ_{max}^{EtOH}: 230.5 mμ (log ε 4.03). IR ν_{max}^{Nujol} cm^{-1}: 1682, 1610 (α,β-unsaturated ketone). The next fraction eluted with the same solvent (54 mg.) showed a maximum at 235 mμ in EtOH and petr. ether-benzene (3:2) fraction (89 mg.) showed a maximum at 240 mμ in EtOH, which is presumed to be the 4-en-3-one (XV).

Epoxidation of the 1-En-3-one (XIV)——To a solution of the 1-en-3-one (XIV) (305 mg.) in MeOH (100 cc.) were added 10% KOH-MeOH (8 cc.) and then 30% H_2O_2 (7 cc.) at 9~11°. After allowing to stand overnight in an ice-box, the solution was diluted with water and Et_2O. The Et_2O solution was washed with water, dried over Na_2SO_4, and evaporated. The residue (232 mg.), m.p. 200~204°, was recrystallized from Me_2CO and then from $CHCl_3$-MeOH to give an epoxide (XVI) as prisms, m.p. 208~211°. *Anal.* Calcd. for $C_{27}H_{39}O_4Br$: C, 63.89; H, 7.75; Br; 15.75. Found: C, 63.91; H, 7.77; Br, 15.33. UV λ_{max}^{EtOH}: 300 mμ (log ε 1.52). IR ν_{max}^{Nujol}: 1715 cm^{-1} (>C=O).

$LiAlH_4$ Reduction of the Epoxide (XVI)——To a stirred suspension of $LiAlH_4$ (740 mg.) in dehyd. Et_2O (30 cc.) was added dropwise a solution of the epoxide (XVI) (731 mg.) in dehyd. tetrahydrofuran (50 cc.) and the mixture was refluxed for 3 hr. The excess $LiAlH_4$ was decomposed with ice-water and treated with 5% HCl and Et_2O. The Et_2O solution was washed with water, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated. The residue (742 mg.) was chromatographed on alumina (Brockmann, 22 g.). The benzene-$CHCl_3$ (1:1) fraction gave a crystalline substance (327 mg.). Recrystallization from $CHCl_3$-MeOH gave a pure dihydroxymonobromo compound (XVIII), m.p. 231~233°. Its infrared spectrum showed no carbonyl band. The benzene-$CHCl_3$ (1:1,1:2) fractions gave a mixture of (XVII) and (XVIII) (33 mg.). The $CHCl_3$ and $CHCl_3$-MeOH (20:1) fractions gave a crystalline substance (253 mg.). Recrystallization from $CHCl_3$-MeOH gave a pure dihydroxymonobromo compound (XVII), m.p. 205~207°. Its infrared spectrum showed no carbonyl band.

Preparation of the 1α-Hydroxy-3β-acetate (XIX) from the Dihydroxymonobromo Compound (XVII)——A solution of the dihydroxymonobromo compound (XVII) (63 mg.) in Ac_2O (2 cc.) was heated at 90° for 30 min. The reaction mixture was treated in the usual way. The product (64 mg.) was recrystallized from $CHCl_3$-MeOH, giving a substance, m.p. 225~228°, which is regarded as a monoacetate on the basis of its infrared spectrum. IR ν_{max}^{Nujol} cm^{-1}: 3495 (OH), 1737 (OAc).

The monoacetate (306 mg.) obtained by the above method was dissolved in AcOH (14 cc.) and refluxed with Zn dust (3.6 g.). After 1.5 hr., Zn dust (1 g.) was again added and refluxing was continued for 2.5 hr. The precipitate was filtered and washed with Et_2O. Filtrate and washings were combined and diluted with Et_2O. The Et_2O solution was washed with water, 5% Na_2CO_3, and water. The product (250 mg.) was negative to Beilstein test and was recrystallized from $CHCl_3$-MeOH to yield the 1-hydroxy-3-acetate (XIX) (127 mg.) as needles, m.p. 276~278°. $[\alpha]_D^{27}$ −47.9°±3° (c=0.877, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.19; H, 9.71. IR ν_{max}^{Nujol} cm^{-1}: 3490 (OH), 1740 (OAc).

The residue obtained by evaporation of the mother solution was dissolved in Ac_2O (3 cc.) and heated at 90° for 30 min. The reaction mixture was treated in the usual way. The residue (122 mg.) was chromatographed on alumina (Woelm II 3.6 g.). The petr. ether-benzene (5:1, 3:1) fractions gave a crystalline substance (49.5 mg.). Recrystallization from $CHCl_3$-MeOH and then from CH_2Cl_2-Me_2CO gave a diacetate (XX), m.p. 226~228°. $[\alpha]_D^{29}$ −54.1°±3° (c=0.483, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.20; H, 9.45. IR ν_{max}^{Nujol}: 1743 cm^{-1} (OAc).

The benzene-$CHCl_3$ (10:1, 5:1) fraction gave a crude monoacetate (XIX) (23.5 mg.), m.p. 260~271°.

Debromination of the Dihydroxymonobromo Compound (XVIII)——The dihydroxymonobromo compound (XVIII) (84 mg.) was dissolved in warm Ac_2O (6 cc.) and heated at 90° for 30 min. After treatment in the usual way, 89 mg. of the product, m.p. 183~194°, was obtained. IR ν_{max}^{Nujol} cm^{-1}: 3290 (OH), 1733 (OAc). This product could not be purified by either recrystallization or chromatography on alumina (Woelm II).

A mixture (318 mg.) of the above crude product and (XVIII) was dissolved in AcOH (15 cc.) and

refluxed with Zn dust (3 g.). After 2 hr. and 45 min., an additional Zn dust (1 g.) was added and refluxing was continued for 75 min. Filtrate and Et_2O washings of the residue were combined and diluted with Et_2O. The product (269 mg.) obtained from the Et_2O solution was negative to Beilstein test.

This debrominated product (269 mg.) was refluxed with 4% NaOH-MeOH (15 cc.) for 40 min. After concentration in a reduced pressure, the reaction mixture was diluted with water to give a precipitate. After washing with water and drying, it was recrystallized from $CHCl_3$-MeOH and then from CH_2Cl_2-Me_2CO, yielding 25D,5α-spirostane-1α,3α-diol, m.p. 223~224.5°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.86; H, 10.42. IR ν_{max}^{Nujol}: 3285 cm^{-1} (OH, broad).

Acetylation of 25D,5α-Spirostane-1α,3α-diol——The 1α,3α-diol (202 mg.) was dissolved in Ac_2O (9 cc.) and heated at 90° for 2 hr. After treatment in the usual way, the residue (215 mg.) was chromatographed on alumina (Woelm II, 6 g.). The petr. ether-benzene (5:1-1:1) fractions gave a crystalline substance (72 mg.), m.p. 195~209°. Recrystallization from $CHCl_3$-MeOH and then from CH_2Cl_2-Me_2CO gave a monoacetate (XXI), m.p. 210~213°. *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.17; H, 9.74. IR ν_{max}^{Nujol} cm^{-1}: 3518 (OH), 1734, 1727 (OAc). The benzene, benzene-$CHCl_3$ and $CHCl_3$ fractions gave the diol (74 mg.).

Preparation of the 1-Oxo-3β-acetate (XXII) from the 1-Hydroxy-3β-acetate (XIX)——A CrO_3-H_2SO_4 solution (0.097 cc.) (containing 25.3 mg. of CrO_3) was added dropwise to a stirred solution of the 1-hydroxy-3β-acetate (XIX) (120 mg.) in Me_2CO (80 cc.) at 15~19°. After stirring for 8 min., the mixture was diluted with water. The precipitate was dissolved in Et_2O and the solution was washed, dried, and evaporated. Recrystallization of the crude product (114 mg.), m.p. 200~211°, from $CHCl_3$-MeOH, gave a pure sample of (XXII), m.p. 215~217°. $[\alpha]_D^{30}$ −19.7°±2° (c=0.934, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.73; H, 9.50. UV λ_{max}^{EtOH}: 292 mμ (log ε 1.78). IR ν_{max}^{Nujol} cm^{-1}: 1736 (OAc), 1711 (>C=O).

Preparation of the 2-En-1-one (XXIII) from the 1-Oxo-3β-acetate (XXII)——A solution of (XXII) (95 mg.) in benzene (6 cc.) was stirred with alumina (Brockmann, 1.4 g.) at room temperature for 2.5 hr. Filtration and concentration of the reaction mixture afforded a substance which showed a maximum at 225 mμ (ε ca. 6000) in EtOH. In view of the fact that 2-en-1-one compounds have generally an absorption intensity of about 8000~9000, it was judged that the reaction proceeded only 60~70%. Then, this reaction was repeated.

A solution of 83 mg. of the above product in benzene (9 cc.) was stirred with alumina (1.5 g.) at room temperature for 5 hr. Treatment as above afforded a crude 2-en-1-one (XXIII) (69 mg.), m.p. 163~168°. Recrystallization from Me_2CO gave a pure sample, as plates, m.p. 170~173°. $[\alpha]_D^{27}$ +102.4° ±5° (c=0.462, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 78.59; H, 9.77. Found: C, 78.67; H, 10.11.

UV λ_{max}^{EtOH}: 225.5 mμ (log ε 3.93). IR ν_{max}^{Nujol} cm^{-1}: 3043 (-C(H)=C(H)-), 1682, 1627 (α,β-unsaturated ketone).

Catalytic Hydrogenation of the 2-En-1-one (XXIII)——A solution of (XXIII) (56 mg.) in AcOEt (9 cc.) was shaken with 10% Pd-C (56 mg.) in an atmosphere of hydrogen, until the absorption of hydrogen stopped. The product (50 mg.) was chromatographed on alumina (Woelm I, 1 g.).

The petr. ether and petr. ether-benzene (10:1) fractions afforded 10.5 mg. of a ketone, m.p. 160~170°. Recrystallization from CH_2Cl_2-Me_2CO gave a pure sample of 25D, 5α-spirostan-1-one (XI), m.p. 167~169°. *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.26; H, 10.43. IR ν_{max}^{Nujol}: 1708 cm^{-1} (>C=O).

Acetonide of Kitigenin——A mixture of 100 mg. of kitigenin in dehyd. Me_2CO (40 cc.) and *p*-toluenesulfonic acid (100 mg.) was refluxed for 6 hr. After basifying with Na_2CO_3 solution, it was concentrated in a reduced pressure and was diluted with water to give a precipitate. The precipitate was dissolved in $CHCl_3$ and the solution was washed, dried, and evaporated. The residue (103 mg.) was treated with Me_2CO to remove a sparingly soluble substance (25 mg.), m.p. >280°. The residue was chromatographed on alumina (Brockmann, 1.2 g.). The benzene fraction (60 mg.), m.p.215~230°, was recrystallized from MeOH to yield an acetonide (XXIV), m.p. 227~228°. *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.27; H, 9.65. IR ν_{max}^{Nujol}: 3480 cm^{-1} (OH).

Acetylation of Acetonide (XXIV)——A solution of the acetonide (114 mg.) in a mixture of pyridine (2 cc.) and Ac_2O (2 cc.) was heated at 90° for 2 hr. After treatment in the usual way, the product (122 mg.) was chromatographed on alumina (Woelm II). The petr. ether-benzene fraction (61 mg.) was recrystallized from MeOH to give the acetonide acetate (XXV), m.p. 180.5~182.5°. *Anal.* Calcd. for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found: C, 70.58; H, 9.13. IR ν_{max}^{Nujol} cm^{-1}: 3508 (OH, sharp), 1717 (OAc).

Mesylation of the Acetonide (XXIV)——To a solution of the acetonide (XXIV) (53 mg.) in pyridine (2 cc.) CH_3SO_2Cl (0.5 cc.) was added under ice-cooling. The mixture was allowed to stand for 40 hr. in an ice-box. It was treated with ice-water and extracted with Et_2O. After treatment in the usual way, the product (62.5 mg.) was recrystallized from $CHCl_3$-MeOH, giving a pure sample of the

mesylate (XXVI), as plates, m.p. 163~164.5°. *Anal.* Calcd. for $C_{31}H_{50}O_8S$: C, 63.89; H, 8.65; S, 5.50. Found : C, 63.53; H, 8.64; S, 5.92. IR ν_{max}^{Nujol} cm^{-1} : 3600 (OH, sharp), 1359, 1167 (-O-SO_2-).

Elimination of Methanesulfonic Acid from the Mesylate (XXVI)——To a stirred suspension of $LiAlH_4$ (110 mg.) in dehyd. Et_2O (8 cc.) was added dropwise a solution of the mesylate (XXVI) (42.5 mg.) in dehyd. tetrahydrofuran (4 cc.). The mixture was refluxed for 3 hr. After decomposition of the excess reagent with ice-water, the mixture was acidified and extracted with Et_2O. The Et_2O solution was washed with 5% Na_2CO_3 and water, dried, and evaporated. The product (XXVII) (32 mg.) was recrystallized from MeOH to yield a pure sample, m.p. 172~176°. *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found : C, 74.28; H, 9.61. IR ν_{max}^{Nujol} cm^{-1} : 3580 (OH, sharp), 3050 (-$\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}$-), 1650 (>C=C<).

Hydrolysis of the Unsaturated Acetonide (XXVII)——A solution of the unsaturated acetonide (XXVII) (147 mg.) in 80% AcOH (5 cc.) was heated on a steam-bath for 1.5 hr. After evaporation under a reduced pressure, the residue was dissolved in $CHCl_3$ and the solution was washed with 5% Na_2CO_3 and water. The product (132 mg.) was recrystallized from Me_2CO to give a pure sample of the unsaturated triol (XXIX), m.p. 236.5~239.5°. *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found : C, 72.40; H, 9.39. IR ν_{max}^{Nujol} cm^{-1} : 3586, 3530 (OH), 3020 (-$\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}$-), 1656 (-$\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}$-). This sample was identical with the unsaturated triol (XXIX) obtained from kitigenin diacetate (IV) in a mixed melting point and comparison of the infrared spectra.

The 1,5-Sulfite (XXX) of Kitigenin Diacetate (IV)——To a solution of kitigenin diacetate (IV) (50 mg.) in pyridine (1 cc.) $SOCl_2$ (3 drops) was added under ice-cooling and then it was allowed to stand at room temperature for 40 min. The mixture was diluted with ice-water and Et_2O. The Et_2O solution was treated in the usual way. The crude product (57 mg.) was recrystallized from $CHCl_3$-MeOH to give a substance, needles, m.p. 278~282°. This substance was purified by alumina chromatography (Woelm III, 1.5 g.). The benzene-petr. ether (1:2) fraction (18 mg.) was recrystallized from Me_2CO to give a pure sample of the 1,5-sulfite (XXX), m.p. 284.5~286°. *Anal.* Calcd. for $C_{31}H_{46}O_9S$: C, 62.60; H, 7.80; S, 5.39. Found : C, 62.81; H, 7.82; S, 5.52. IR ν_{max}^{Nujol} cm^{-1} : 1744 (OAc), 1199 (R-O-$\overset{O}{\underset{||}{S}}$-O-R).

The Orthoester (XXXI) of Kitigenin——Kitigenin (50 mg.) was dissolved in warm MeOH (28 cc.). After cooling, 21.6% MeOH-HCl (0.66 cc.) and H·$C(OEt)_3$ (2 cc.) was added and the mixture was allowed to stand at room temperature for 16 hr. Concentration in a reduced pressure without warming and dilution with water separated a precipitate which was recrystallized from $CHCl_3$-MeOH to yield the orthoester (XXXI) (40 mg.) as plates, m.p. 235~237°. $[\alpha]_D^{24}$ −44.3° ±3° (c=0.815, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found : C, 70.99; H, 8.94. IR ν_{max}^{Nujol} cm^{-1} : 3540 (OH, sharp), 986, 991, 1146 ($(RO)_3C$-).

Acetylation of the Orthoester (XXXI)——The orthoester (XXXI) (33 mg.) was treated with a mixture of pyridine (2 cc.) and Ac_2O (2 cc.) at room temperature for 16 hr. After treatment in the usual way, the crude product (33 mg.), m.p. 270~279°, was obtained. Recrystallization from Me_2CO afforded a pure sample of the acetoxyorthoester (XXXIII) as prisms, m.p. 280~282°. $[\alpha]_D^{23}$ −41.1° ±3° (c=0.734, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found : C, 69.89; H, 8.54. IR ν_{max}^{Nujol} cm^{-1}: 1730 (OAc), 1148, 998, 989 ($(RO)_3C$-).

Oxidation of the Orthoester (XXXI)——To a stirred solution of the orthoester (XXXI) (85 mg.) in Me_2CO (12 cc.) was added dropwise a CrO_3-H_2SO_4 solution (0.069 cc.) (containing 17.7 mg. of CrO_3, 1.5 equiv.) at 14~15°. After stirring for 20 min. and diluting with water, the mixture was extracted with Et_2O and washed with 5% Na_2CO_3 and water, dried, and evaporated. The residue (88 mg.) was chromatographed on alumina (Brockmann, 2.5 g.). The petr. ether-benzene (1:1) fraction (60 mg.), m.p. 255~260°, was recrystallized from $CHCl_3$-MeOH and then CH_2Cl_2-Me_2CO to give a pure sample of the keto-orthoester (XXXIV), m.p. 264.5~266.5°. *Anal.* Calcd. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found : C, 71.34; H, 8.72. IR ν_{max}^{Nujol} cm^{-1} : 1742 (>C=O), 1135, 1002, 993, 982 ($(RO)_3C$-). UV λ_{max}^{EtOH}: 325 mμ (log ε 1.58).

The author wishes to express his deep gratitude to Dr. K. Takeda, Director of this Laboratory, for his helpful guidance and to Mr. A. Murabayashi for his technical assistance throughout the course of this work. Thanks are also due to the members of the Analysis Room of this Laboratory for microanalyses and to the members of the Section of Physical Chemistry for the measurement of infrared, ultraviolet spectra and optical rotation.

Summary

It has been already established that the three hydroxyl groups in kitigenin, a tetrahydroxy sapogenin, are located at C_3, C_4 and C_5, and that the C_3-hydroxyl group is β and the C_4- and C_5-hydroxyl groups are in *cis* relation. It is now, clarified that the one remaining hydroxyl group is situated at C_1, from the result of the conversion of kitigenin to 25D,5α-spirostan-1-one synthesized independently from tigogenone. And also, from the results of the C_3,C_4-acetonide formation and C_1,C_5-sulfite formation, it was clarified that the four hydroxyl groups are all β. Consequently, it was determined that kitigenin is 25D,5β-spirostane-1β,3β,4β,5-tetrol.

(Received December 23, 1960)

UDC 612.398.145

112. Takeo Naito, Miyoshi Hirata, Tomoyoshi Kawakami, and Mitsuji Sano :
Studies on Nucleosides and Nucleotides. I. Synthesis of Glycosylthymines from Glycosylureas.

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The general method for synthesis of pyrimidine nucleosides includes those of Hilbert-Johnson[1] and of Fox,[2] and a new different route of synthesis was recently devised by Shaw.[3] A new synthetic process for pyrimidine nucleoside was attempted, starting with glycosylurea, by pyrimidine ring cyclization.

Goodman[4] reported the synthesis of 1-(tetra-O-acetyl-D-glucosyl)-6-aminouracil from tetra-O-acetyl-D-glucosylurea and cyanoacetic acid for the same purpose but he failed to give any details of this reaction. As a preliminary experiment for the present series of work, examinations were made on the mode of condensation of N-methylurea (I) and N-methylthiourea (II) with methyl 3-methoxy-2-methylacrylate (A), 3-methoxy-2-methylacryloyl chloride (B), and ethyl 2-formylpropionate (C) to form N-methylthymine and N-methylthiothymine.

Syntheses of N-methylthymine and N-methylthiothymine from methacrylic acid derivatives have been reported by Shaw[5] and Smith.[6] In the present series of work, condensation of (I) and (II) with (A) and (C) was attempted in the presence of sodium alkoxide and it was found that the reaction of (I) and (A) did not proceed but that of (II) and (A) afforded N-methyl-2-thiothymine (III), m.p. 230~232°, which, on treatment with monochloroacetic acid, was derived to N-methylthymine (IV), m.p. 282~284°. While the reaction of (I) and (C) afforded (IV), though in a minute amount, condensation of (II) and (C) afforded N-methylthiothymine (V) of m.p. 207~209°, different from (III). Treatment of (V) with

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