UDC 547.918[547.92] : 582.572.7

167. Tokuo Kubota : Studies on the Steroidal Components of Domestic Plants. XX.[1] The Structure of Kogagenin. (2).

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

Kogagenin, isolated from the epigeous parts of *Dioscorea Tokoro* MAKINO together with yonogenin[2] (XIVa) and tokorogenin[3] (VII), is the sole example of a naturally occurring spirostanetetrol. The structure of this sapogenin was recently[1] shown as 25D-spirostane-1β,2β,3α,5β-tetrol (I).

As described in the preceding paper,[1] the three secondary hydroxyl groups were confirmed to be at 1β, 2β, and 3α by hydrogenation of anhydrokogagenin acetate, which was obtained by dehydration of kogagenin triacetate with thionyl chloride in pyridine, since the reduction product was identical with tokorogenin acetate. Also, the 3-ketone (III) derived from kogagenin acetonide (II) was easily dehydrated to form a 4-ene-3-ketone group and thus the position of the fourth hydroxyl group was proved ultimately to be at C-5. However, β-configuration for its hydroxyl group was assigned only on the following circumstantial evidences : (a) The rotatory dispersion curve of the above 3-ketone (III) closely resembles that of the authentic 5β-3-ketosteroids; (b) kogagenin acetonide (II) did not react with phosgene, suggesting the absence of a 3α,5α-glycol group; and (c) the acetylation of kogagenin under mild conditions gave the diacetate, considered to be (IV), and this was condensed with phosgene to yield a diacetate-carbonate expected to be (V). A preference of β-configuration for the C-5 hydroxyl group was based upon the assumption that the easily acetylated hydroxyl groups must be equatorial and that phosgene reacts with a diaxial 1,3-glycol to give a carbonate. Thus, for rigorous proof of the structure of kogagenin, it is necessary to confirm positively that the structure of the above-mentioned diacetate-carbonate is formulated as (V), as anticipated.

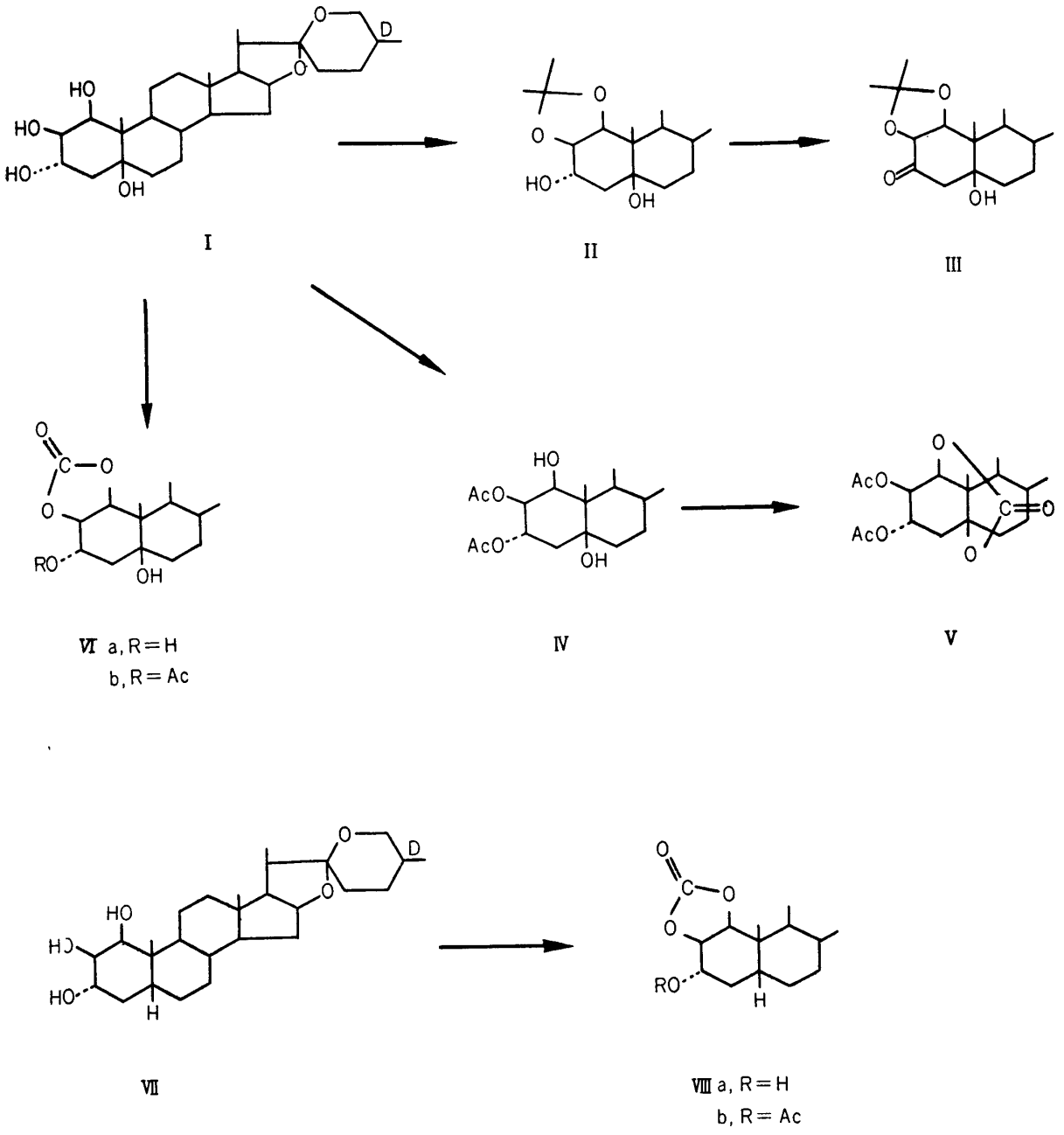
Free kogagenin was subjected at first to a reaction with phosgene and it gave kogagenin carbonate, $C_{28}H_{42}O_7$, m.p. 295~298° (decomp.), in a good yield. Next, examinations were made to see whether the diacetate-carbonate (V), identical with that derived from kogagenin diacetate and phosgene, can be obtained by acetylation of this kogagenin carbonate, but the results were contrary to expectations and only a monoacetate, $C_{30}H_{44}O_8$, m.p. 279~283°, was obtained even on refluxing with acetic anhydride-pyridine.

From this finding it was assumed that a tertiary hydroxyl group at C-5 in kogagenin was not concerned with the carbonyldioxy group formation, and phosgene may have reacted with a *cis*-α-glycol at 1β and 2β. In order to confirm this assumption, the reaction of tokorogenin (VII) with phosgene was examined. In this case, the same type of carbonate (VIIIa) as in the case of kogagenin was obtained and this was led to a carbonate-acetate (VIIIb) by acetylation. Consequently, it is almost certain that phosgene reacts predominantly with a *cis*-α-glycol in preference to a diaxial 1,3-glycol in kogagenin.

The position of acetoxyl groups in kogagenin diacetate assumed to be located at 2β- and 3α, as discussed in a recent paper,[1] had to be confirmed and this was accomplished by the following reaction sequence. The remaining secondary hydroxyl group in koga-

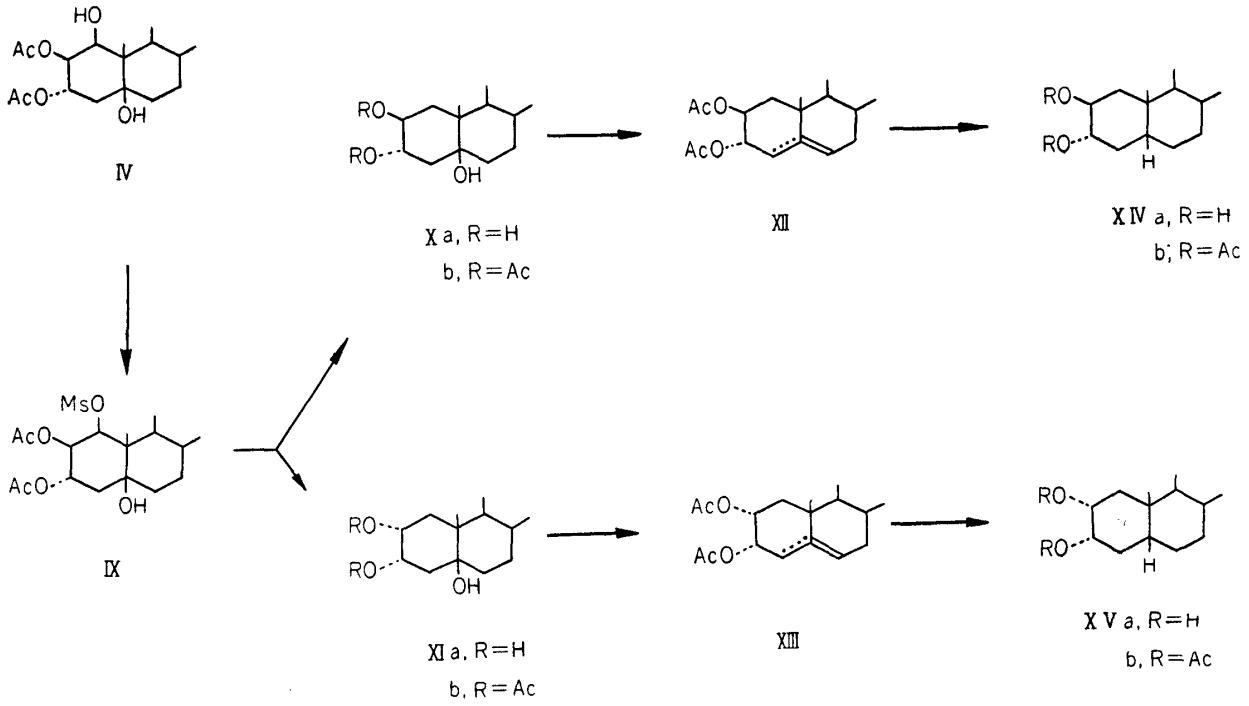
*1 Imafuku, Amagasaki, Hyogo-ken (久保田徳夫).

1) Part XIX : K. Takeda, T. Kubota, A. Shimaoka : Tetrahedron (in contribution).
2) K. Takeda, T. Okanishi, A. Shimaoka : Yakugaku Zasshi, **77**, 822(1957); This Bulletin, **6**, 532 (1958).
3) M. Nishikawa, K. Morita, H. Hagiwara, M. Inoue : Yakugaku Zasshi, **74**, 1165(1954); K. Morita: This Bulletin, **5**, 494(1957).



genin diacetate was converted with methanesulfonyl chloride and pyridine to a mesyloxyl group which was eliminated reductively with lithium aluminum hydride. By subsequent reacetylation, the product gave two isomeric triol-diacetates, (A) m.p. 259~262°, $[\alpha]_D$ −14°, and (B) m.p. 168~170°, $[\alpha]_D$ −62°. These triol-diacetates will give the respective corresponding 25D,5β-spirostanediol diacetates with the procedure — dehydration followed by catalytic hydrogenation — which was used in the case of kogagenin triacetate to tokorogenin triacetate reported in the preceding paper.[1]

One of the isomers (A) was dehydrated with thionyl chloride and pyridine to an oily anhydro derivative, which was hydrogenated catalytically over platinum oxide in acetic acid. The product, m.p. 212°, was identified with the known yonogenin diacetate[2] (XIVb), as expected, by mixed melting point determination and by comparison of the infrared spectrum.



In the same way, the other isomer (B) furnished via an anhydro derivative, m.p. 202°, a diol-diacetate melting at 154°. This substance was found by mixed melting point determination and infrared spectra to be identical with 25D,5β-spirostane-2α,3α-diol diacetate (XVb) which has recently been synthesized by another method in this laboratory.[4]

Thus, two isomers of the above-mentioned triol-diacetate, (A) and (B), can be formulated as (Xb) and (XIb), respectively. The formation of the C-2 epimer (XI) by reduction of the diacetate-mesylate (IX) with lithium aluminum hydride is assumed to arise from demesylation followed by reduction of the C-2 ketone or enol acetate produced.

The above results proved, at all events, that the acetoxyl groups in kogagenin diacetate are located at C-2 and C-3. Therefore, it is clear that a carbonyldioxy group in the above-mentioned diacetate-carbonate (V) derived from kogagenin diacetate consists of the diaxial 1,3-glycol at C-1 and C-5, as expected. The hydroxyl group at C-5 must have a β-configuration and the structure of kogagenin has been established unequivocally as 25D-spirostane-1β,2β,3α,5β-tetrol (I), as proposed earlier.[1]

The author expresses his deep gratitude to Dr. K. Takeda, Director of this Laboratory, for his helpful guidance thoughout the course of this work. Thanks are also due to the members of the analysis room of this Laboratory for the microanalyses and to Mr. Y. Matsui for the measurement of the infrared spectra.

Experimental*2

25D-Spirostane-1β,2β,3α,5β-tetrol 1,2-Carbonate (VIa)—Kogagenin (I) (88 mg.) was suspended in alcohol-free $CHCl_3$ (6 cc.) and about 1 cc. of the $CHCl_3$ was removed by distillation. To this slurry, pyridine (4 cc.) was added and the sapogenin was dissolved completely by warming. The mixture

*2 All melting points are uncorrected. Infrared spectra were measured with Koken Infrared Spectrophotometer Model DS 301. Rotations were determined with Rudolf Photoelectric Polarimeter Model 200.

4) K. Takeda, T. Okanishi, A. Shimaoka : This Bulletin, 7, 942(1959).

was cooled to −18° and a 10% $COCl_2$-toluene solution (12 cc.) was added dropwise. The temperature of the reaction mixture was allowed to rise to +13° during 1 hr. and kept at 13~18° for an additional 1 hr. After destroying excess $COCl_2$ with ice, water and Et_2O were added. The solvent layer was washed successively with dilute HCl, $NaHCO_3$ solution, and water, dried over anhyd. Na_2SO_4, and concentrated to dryness *in vacuo*. Recrystallization of the crystalline residue (90 mg.), m.p. 289~295° (decomp.), from MeOH and then from $CHCl_3$-MeOH afforded needles, m.p. 295~298° (decomp.), $[\alpha]_D$ −37° (c=0.93, 1:1 $CHCl_3$-MeOH). I. R. λ_{max}^{Nujol} μ: 2.80, 2.85 (OH); 5.54, 5.67, 8.29, 8.39. *Anal.* Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.56; H, 8.67.

25D-Spirostane-1β,2β,3α,5β-tetrol 1,2-Carbonate 3-Acetate (VIb)—A mixture of the above kogagenin carbonate (VIa) (60 mg.), Ac_2O (1 cc.), and pyridine (1cc.) was allowed to stand at room temperature overnight. The product, isolated with Et_2O in the usual manner, was crystallized from acetone-hexane and then from acetone to prisms, m.p. 279~283°. I. R. λ_{max}^{Nujol} μ: 2.83 (OH); 5.63, 5.67, 8.18, 8.42. *Anal.* Calcd. for $C_{30}H_{44}O_8$: C, 67.64; H, 8.33. Found: C, 67.69; H, 8.28.

Treatment of this carbonate-acetate with Ac_2O-pyridine under refluxing for 1.5 hr. resulted in the recovery of unchanged starting material.

25D,5β-Spirostane-1β,2β,3α-triol 1,2-Carbonate (VIIIa) and its Acetate (VIIIb)—The reaction of tokorogenin (VII) (100 mg.) with $COCl_2$ was carried out in the manner described above for that of kogagenin. Recrystallization of the product from MeOH and then from $CHCl_3$-MeOH afforded (VIIIa) as needles, m.p. 305~309° (decomp.), $[\alpha]_D$ −46° (c=1.0, 1:1 $CHCl_3$-MeOH). I. R. λ_{max}^{Nujol} μ: 2.83 (OH); 5.57, 5.64, 8.44. *Anal.* Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found: C, 70.51; H, 8.94.

This tokorogenin carbonate (VIIIa) (50 mg.) was acetylated by standing at room temperature with a mixture of Ac_2O (1 cc.) and pyridine (1 cc.) overnight. The crude product, m.p. 230~239°, was recrystallized from MeOH to the carbonate-acetate (VIIIb) as needles, m.p. 236~239°. I. R. λ_{max}^{Nujol} μ: 5.55, 5.74, 8.00, 8.06, 8.49; no hydroxyl band. *Anal.* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found: C, 69.93; H, 8.49.

25D-Spirostane-1β,2β,3α,5β-tetrol 1-Mesylate 2,3-Diacetate (IX)—To a solution of kogagenin diacetate (IV) (500 mg.) in pyridine (2.5 cc.), methanesulfonyl chloride (0.5 cc.) was added dropwise under cooling in an ice bath, and the reaction mixture was stored in a refrigerator overnight. The excess reagent was decomposed with ice and the product was extracted with Et_2O-$CHCl_3$ (5:1). The organic layer was washed with diluted HCl, Na_2CO_3 solution, and water, and dried over anhyd. Na_2SO_4. After removal of the solvent, the vitreous residue was crystallized from Et_2O to needles (515 mg.), m.p. 202~204°, $[\alpha]_D$ −6° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{50}O_{10}S$: C, 61.32; H, 8.04; S, 5.12. Found: C, 61.59; H, 7.97; S, 4.88.

Reaction of 25D,5β-Spirostane-1β,2β,3α,5β-tetrol 1-Mesylate 2,3-Diacetate (IX) with $LiAlH_4$—A solution of the mesylate diacetate (IX) (480 mg.) in dehyd. benzene (50 cc.) was added dropwise into a suspension of $LiAlH_4$ (480 mg.) in anhyd. Et_2O (50 cc.) with stirring. The mixture was heated under reflux (51°) for 5 hr. After cool, a small portion of water was added carefully to decompose the complex and then 5% HCl was added. The product was extracted several times with $CHCl_3$ and the combined $CHCl_3$ solution was washed, dried, and evaporated under a reduced pressure. The crystalline residue (374 mg.), m.p. 256~264° (decomp.), was recrystallized twice from $CHCl_3$-MeOH to the 2β,3α,5β-triol (Xa) as small plates (113 mg.), m.p. 292~295° (decomp.). *Anal.* Calcd. for $C_{27}H_{44}O_5 \cdot \frac{1}{4}H_2O$: C, 71.58; H, 9.90. Found: C, 71.44, 71.58; H, 9.85, 9.99.

Acetylation of this triol (Xa) with Ac_2O-pyridine and recrystallization of the product from MeOH afforded 2β,3α,5β-triol diacetate (Xb) as needles, m.p. 258~262° (decomp.), $[\alpha]_D$ −14° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.49; H, 8.92.

The mother liquor left after separation of (Xa) was evaporated to dryness *in vacuo* and the residue (245 mg.) was acetylated by heating with Ac_2O-pyridine on a steam bath for 1 hr. The crude acetate, separated with Et_2O in the usual way, was chromatographed over alumina (9 g.). The fraction (56 mg.), m.p. 170~210°, eluted with benzene-$CHCl_3$ (4:1 to 1:1) was recrystallized twice from MeOH and an additional 19 mg. of the above 2β,3α,5β-triol diacetate (Xb), m.p. 257~261° (decomp.), was obtained.

The next fractions (245 mg.), m.p. 136~143°, eluted with benzene-$CHCl_3$ (1:1) and with $CHCl_3$ were recrystallized 3 times from MeOH to 2α,3α,5β-triol diacetate (XIb) as prisms (32 mg.), m.p. 168~170°, $[\alpha]_D$ −62° (c=1.1, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 70.00; H, 9.10.

The mother liquor was concentrated to dryness and the residue (204 mg.) was also used for the next step described below.

Yonogenin Diacetate (25D,5β-Spirostane-2β,3α-diol Diacetate) (XIVb)—The 2β,3α,5β-triol diacetate (Xb) (45 mg.) was dissolved in pyridine (1 cc.), cooled to 0°, and $SOCl_2$ (0.1 cc.) was added. After standing at 0° for 40 min., ice was added to the reaction mixture, and the product was extracted with Et_2O. The Et_2O solution was washed with dil. HCl, $NaHCO_3$ solution, and water, and dried over anhyd. Na_2SO_4. Removal of the solvent left a vitreous oil (44 mg.) of the anhydro derivative (XII) which was not obtained in crystalline form. I. R. λ_{max}^{Nujol} μ: 5.73, 8.07, 8.17 (AcO); 6.02 (double bond);

no band for hydroxyl group.

This substance was dissolved in glacial AcOH (5 cc.) and shaken with Adams' catalyst (20 mg.) in an atmosphere of H_2 for 20 min. The catalyst was filtered off and the solvent was removed *in vacuo*. The product was crystallized from MeOH to plates of m.p. 195~210°. Further recrystallization from the same solvent raised the melting point to 210~212°. No depression of the melting point was observed on admixture with yonogenin diacetate[2] (XIVb) and infrared spectra of these two substances were identical.

25D,5β-Spirostane-2α,3α-diol Diacetate (XVb)—The pure 2α,3α,5β-triol diacetate (XIb) (20 mg.) was dehydrated with $SOCl_2$-pyridine in the manner described above for (Xb). Crystallization of the product from MeOH afforded the anhydro derivative (XIII) as needles, m.p. 199~202°.

The mother liquor left after isolation of (XIb) was similarly dehydrated with $SOCl_2$ (0.2 cc.) and pyridine (2 cc.) to form the anhydro derivative (XIII) (67 mg.), m.p. 198~201°, which was identical with the above sample prepared from pure (XIb). For analysis the material was recrystallized once from MeOH: m.p. 199~202°, $[\alpha]_D$ +71° (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.80; H, 8.90.

A solution of the above anhydro derivative (XIII) (40 mg.) in glacial AcOH (5 cc.) was shaken with Adams' catalyst (20 mg.) in H_2 for 25 min. After removal of the catalyst, the filtrate was evaporated under a reduced pressure. The residue was crystallized from MeOH to needles (18 mg.), m.p. 110~140°. Further recrystallization of this substance from MeOH and drying *in vacuo* at 60° furnished a pure sample of m.p. 152~154°, which showed no depression on admixture with a sample of 25D, 5β-spirostane-2α,3α-diol diacetate (XVb) prepared by Takeda, *et al.*[4] Infrared spectra of these samples were identical.

Summary

Kogagenin diacetate (IV) has been correlated to the known yonogenin diacetate (XIVb) and the acetoxyl groups in the former were proved to be located at C-2 and C-3. The structure of the carbonate of kogagenin diacetate was also established. Consequently, the structure of kogagenin has been confirmed as 25D-spirostane-1β,2β,3α,5β-tetrol (I) as proposed earlier.[1]

Also, the reaction of kogagenin with phosgene was investigated and it was found that phosgene rather predominantly reacts with a *cis*-α-glycol than a diaxial 1,3-glycol in kogagenin.

(Received May 27, 1959)