Studies on the Sapogenins of Dioscorea tokoro Makino. III[1]. *Synthesis of Isorhodeasapogenin and Some Reactions of Tokorogenin*

By Katsura MORITA

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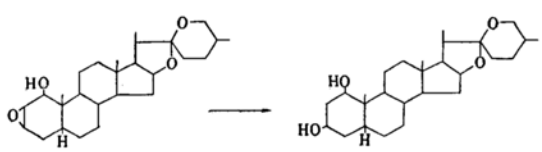
In order to obtain further information concerning the configuration of the hydroxyl groups in tokorogenin, the following reactions were investigated.

Reduction of anhydrotokorogenin (I) with lithium aluminum hydride in anhydrous ether caused ring-opening of the oxide giving a new dihydroxysapogenin, desoxytokorogenin. Since this dihydroxysapogenin neither formed an acetonide nor suffered oxidation by means of periodic acid and lead tetraacetate, the two hydroxyl groups in this substance have to be located at carbon atoms C_1 and C_3. As lithium aluminum hydride reduction of an oxide in the cyclohexane series is known to result in formation of an axial hydroxyl bond[2], the configuration of the hydroxyl group in position 3 of desoxytokorogenin must be β; that is, the structure of 25D-5β-spirostan-1β, 3β-diol (II) has to be assigned to this substance.

Chart 1

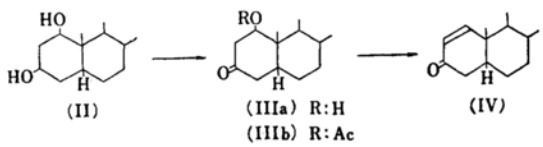


(I) (II)

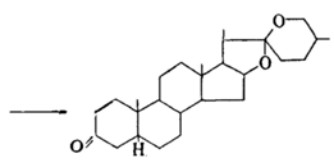
Further evidence for the structure of desoxytokorogenin (II) is given by the following reaction sequence:

Desoxytokorogenin (II) was oxidized with *N*-bromoacetamide to 25D-5β-spirostan-1β-ol-3-one (IIIa), which was acetylated and then treated with activated alumina giving 25D-5β-spirost-1-en-3-one (IV)[3]. The U.V.-absorption spectrum of this substance (IV) showed a good identity with those of known Δ^1-3-ketosteroids[4]. Upon catalytic hydrogenation over Pd/$CaCO_3$ IV yielded smilagenone (V)[5].

Chart 2



(II) (IIIa) R:H (IIIb) R:Ac (IV)



(V)

Some years ago, Nawa[6] isolated a new dihydroxysapogenin, rhodeasapogenin, from *Rhodea japonica* Roth. and suggested that it was 25L-5β-spirostan-2β, 3α-diol on the basis of various reactions, none of which led to a known steroid. Djerassi[7] attempted the oxidation of isorhodeasapogenin, an acid isomerization product of rhodeasapogenin, with chromic anhydride with the aim of obtaining a dibasic acid, isorhodeasapogenic acid, which he supposed to be identical with one of his four 2, 3- and 3, 4-seco-sapogenic acids. Unfortunately, the acid obtained by Djerassi was identical with none of his four sapogenic acids.

Quite recently, Takeda et al.[8] isolated a new dihydroxysapogenin, yonogenin, from *Dioscorea tokoro* Makino and defined its structure as 25D-5β-spirostan-2β, 3α-diol, which was the very one suggested

1) This is a full paper of the previous commuication (K. Morita, *Pharm. Bull.*, **5**, 496 (1957).), and constitutes Part X of Nishikawa's paper entitled "Studies in Steroids". Part IX: K. Morita, This Bulletin, **32**, 791 (1959).

2) M. S. Newman, "Steric Effects in Organic Chemistry", John Wiley & Sons, Inc., New York (1956), p. 130.

3) A similar reaction sequence was reported by W. Schlegel et al. (*Helv. Chim. Acta*, **38**, 1013 (1955).), who synthesized methyl 3-oxo-1-etienate from methyl 1β,3β-dihydroxyetianate.

4) See Table I in the foregoing paper (Part II) of this series.

5) The sample of smilagenone was synthesized from diosgenin by Oppenauer oxidation followed by catalytic hydrogenation.

6) H. Nawa, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **73**, 1192 (1953).

7) C. Djerassi and J. Fishman, *J. Am. Chem. Soc.*, **77**, 4291 (1955).

8) K. Takeda, T. Okanishi and A. Shimaoka, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **77**, 822 (1957).

for isorhodeasapogenin. The present author also isolated the sapogenin from the same plant and confirmed that yonogenin was not identical with isorhodeasapogenin[9].

In the course of the present investigations it was noticed that the melting points of desoxytokorogenin (II) and its diacetate were similar to those of isorhodeasapogenin and its diacetate, respectively. Similarly, the melting points of tokorogenic acid[10] and its dimethyl ester[10] were found to be in close proximity to those of isorhodeasapogenic acid and its dimethyl ester, respectively.

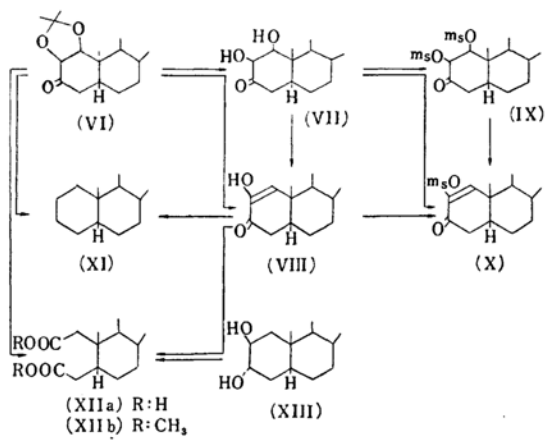
Most of the evidence for a glycol structure of rhodeasapogenin had been sought by Nawa in the fact that the sapogenin underwent ready oxidation to a dibasic acid, rhodeasapogenic acid, but it seemed probable that desoxytokorogenin (II) would be able to undergo oxidation with the same reagent to an intermediate 1,3-diketone, which would enolize and suffer further oxidation to a dibasic acid, namely, tokorogenic acid. Comparison of the I.R.-spectra and determination of the mixed melting points proved that desoxytokorogenin (II) is identical with isorhodeasapogenin, and tokorogenic acid with isorhodeasapogenic acid. Basing on these findings, Nawa has recently corrected all the structural assignments of rhodeasapogenin and its derivatives[11].

When tokorogenone acetonide (VI) was heated with acetic acid and water, tokorogenone (VII) was obtained. Tokorogenone (VII), on heating with alkali in methanol solution, lost 1 mol. of water to give a diosphenol (VIII), $C_{27}H_{40}O_4$, U. V. λ_{max} in EtOH: 269.5 mμ (ε: 6900). Direct conversion of tokorogenone acetonide (VI) into the diosphenol (VIII) could also be effected by the action of alkali at 100°C.

Stiller et al.[12] reported that selenium dioxide oxidation of cholestanone gave a mixture of cholest-1-en-2-ol-3-one (diosphenol A) and cholest-3-en-3-ol-2-one (diosphenol B). They also demonstrated that in equilibrium diosphenol A predominated in alkaline medium, while diosphenol B in acidic solution. The diosphenol (VIII) from tokorogenone (VII), however, was transformed in a mineral acid solution into an unidentified isomeric substance ($C_{27}H_{40}O_4$), from which the diosphenol (VIII) could no longer be recovered by any means. This discrepancy seems to be due to the fact that cholestanone and its diosphenols are 5α-steroids (allo-series) while tokorogenone (VII) and its diosphenol (VIII) are 5β-steroids (normal-series).

Tokorogenone (VII), on treatment with mesyl chloride and pyridine, gave a mixture of a dimesylate (IX) and an enol mesylate (X). The latter (X) was yielded also by heating the former (IX) with pyridine or with sodium iodide in acetone, and by treating the diosphenol (VIII) with mesyl chloride and pyridine. These reactions unambiguously show that the diosphenol (VIII) has to be defined as 25D-5β-spirost-1-en-2-ol-3-one.

Chart 3



It is interesting to note that the Huang-Minlon reduction of the diosphenol (VIII) as well as of tokorogenone acetonide (VI) gave 25D-5β-spirostan (XI), while the reduction of the acid isomerization product of the diosphenol (VIII) did not lead to XI.

The diosphenol (VIII) suffered oxidation with hydrogen peroxide and alkali at room temperature to give samogenic acid (XIIa)[7]. The same acid was prepared also from yonogenin by the known method, and the identity of both specimens was established by the mixed melting point determination and by the comparison of their I.R.-spectra.

Experimental

25D-5β-Spirostan-1β,3β-diol (Desoxytokorogenin) (II).—A solution of 400 mg. of 2β,3β-oxido-25D-5β-spirostan-1β-ol (anhydrotokorogenin) (I) in 50 ml. of anhydrous ether was added to a solution of 1.5 g. of lithium aluminum hydride in 60 ml. of the same solvent through a dropping

9) A sample of yonogenin was kindly given by Dr. Takeda[8] and that of isorhodeasapogenin by Dr. Nawa[6] in Takeda Research Laboratories. The mixture of these specimens melted at a temperature lower than that of either.

10) See previous paper (Part I) in this series.

11) H. Nawa, *Chem. & Pharm. Bull.*, **6**, 255 (1958).

12) E. T. Stiller and O. Rosenheim, *J. Chem. Soc.*, **1938**, 353.

funnel with stirring. The reaction mixture was stirred for two hours at room temperature and poured into a large volume of ice-water, 10% sulfuric acid was added, and the mixture was extracted with ether. The ether solution was thoroughly washed, dried, and evaporated to give colorless needles, m. p. 236~238°C. Yield, 310 mg. Recrystallization from methanol raised the m. p. to 240~242°C.

Anal. Found: C, 74.82; H, 10.46. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25%.

On admixture with a sample of isorhodeasapogenin the melting point of the diol (II) was not depressed. The infrared spectra also established the identity of the two specimens.

Treatment of the diol (II) with acetic anhydride and pyridine at room temperature for twenty-four hours furnished the diacetate, m. p. 207~209°C.

Anal. Found: C, 72.12; H, 9.64. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36%.

The substance thus obtained showed a good identity with isorhodeasapogenin diacetate in all respects.

25D-5β-Spirostan-1β-ol-3-one (IIIa).—25D-5β-Spirostan-1β, 3β-diol (II) (200 mg.) was added to a solution of 139 mg. of *N*-bromoacetamide in 20 ml. of 90% acetone and the mixture was warmed on a steam bath for a short time to clear the solution. After one hour, a portion of the solution was titrated with 1/10-N sodium thiosulfate solution and potassium iodide and it was indicated that the reaction proceeded stoichiometrically. A large volume of water was added and the mixture was extracted with ether. The ethereal solution was washed, dried and evaporated to dryness. Recrystallization of the residue from methanol afforded colorless needles melting at 232~233°C (decomp.).

Anal. Found: C, 74.96; H, 9.63. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83%.

25D-5β-Spirostan-1β-ol-3-one Acetate (IIIb).—25D-5β-Spirostan-1β-ol-3-one (IIIa) (70 mg.) was acetylated with acetic anhydride and pyridine to give colorless needles of the acetate (IIIb), m. p. 200~201°C.

Anal. Found: C, 73.66; H, 9.31. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38%.

25D-5β-Spirost-1-en-3-one (IV).—A solution of 70 mg. of IIIb was passed through unwashed alumina to furnish 50 mg. of platelets melting at 191~192°C. U. V. λ_{max} in EtOH: 232.5 mμ (ε: 9700).

Anal. Found: C, 78.55; H, 9.63. Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77%.

25D-5β-Spirostan-3-one (Smilagenone) (V).—A solution of 35 mg. of 25D-5β-spirost-1-en-3-one (IV) in 30 ml. of methanol was subjected to catalytic hydrogenation over 5% palladium-charcoal catalyst in an atmospheric pressure. The solution rapidly took up 1.6 ml. of hydrogen and the reaction almost stopped. The catalyst was removed and the filtrate was concentrated under diminished pressure to give colorless platelets. Recrystallization from methanol afforded the pure material melting at 183~184°C.

The sample here obtained showed no depression of the melting point on admixture with an authentic specimen prepared from diosgenone by the known method[5].

25D-5β-Spirostan-1β, 2β-diol-3-one (Tokorogenone) (VII).—25D-5β-Spirostan-1β, 2β-diol-3-one acetonide (VI) (100 mg.) in 3 ml. of 80% acetic acid was heated on a steam bath for forty minutes. The resulting solution was evaporated under diminished pressure to give a crystalline solid (VII), which upon recrystallization from methanol afforded thin plates melting at 224~227°C. Yield, 60 mg.

Anal. Found: C, 72.54; H, 9.57. Calcd. for $C_{27}H_{42}O_5$: C, 72.65; H, 9.42%.

A small portion of the diol (VII) was acetylated with acetic anhydride and pyridine by the usual means. Recrystallization of the crude acetate from methanol furnished colorless needles, m. p. 223~226°C.

Anal. Found: C, 70.10; H, 8.59. Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74%.

25D-5β-Spirostan-1β,2β-diol-3-one Dimesylate (IX) and 25D-5β-Spirost-1-en-2-ol-3-one Mesylate (X).—a) Mesyl chloride (1 ml.) was added to a solution of 500 mg. of 25D-5β-spirostan-1β, 2β-diol-3-one (VII) in 10 ml. of pyridine at 0°C and the solution was allowed to stand at room temperature in a dark place for three days. A large volume of water was added and the mixture was extracted with ether. The ether solution was thoroughly washed with water, dried and evaporated to furnish almost colorless needles of the dimesylate (IX), m. p. 223°C (decomp.). Yield, 340 mg.

Anal. Found: C, 57.71; H, 7.66. Calcd. for $C_{29}H_{46}O_9S_2$: C, 57.78; H, 7.69%.

The mother liquor from the above dimesylate (IX) was further concentrated and left overnight to give different crystals, m. p. 222~223°C. Yield, 120 mg. The substance here obtained showed a marked depression of the melting point on admixture with the dimesylate (IX). On the basis of the ultraviolet absorption spectrum and elementary analyses structure X was assigned to this substance. U. V. λ_{max} in EtOH: 238.5 mμ (ε: 7100).

Anal. Found: C, 66.10; H, 8.20. Calcd. for $C_{28}H_{42}O_6S$: C, 66.38; H, 8.36%.

b) A solution of 200 mg. of 25D-5β-spirostan-1β, 2β-diol-3-one dimesylate (IX) in 8 ml. of acetone containing 500 mg. of sodium iodide was heated in a sealed tube at 120°C for twenty-four hours. After cooling, the reaction mixture was diluted with water and extracted with ether. The ethereal solution was washed with water and then with sodium thiosulfate solution, dried, and evaporated to furnish colorless crystals, m. p. 222~223°C.

c) A portion of 25D-5β-spirost-1-en-2-ol-3-one (VIII) dissolved in pyridine was treated with mesyl chloride by the usual means. Dilution of the reaction mixture with water followed by washing, drying and evaporation afforded the same mesylate, m. p. 222~223°C.

25D-5β-Spirost-1-en-2-ol-3-one (Diosphenol) (VIII).—a) A solution of 25D-5β-spirostan-1β,2β-

diol-3-one (VII) (70 mg.) in 5 ml. of methanol containing 500 mg. of potassium hydroxide was boiled on a steam bath for thirty minutes. After cooling and diluting with a large volume of water, the mixture was extracted with methylene chloride. The organic layer was then washed, dried, and evaporated to give colorless needles. Recrystallization from methanol afforded the analytical sample, m. p. 227~228°C. Yield, 40 mg. U. V. λ_{max} in EtOH: 269.5 mμ (ε: 6900).

Anal. Found: C, 75.49; H, 9.37. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41%.

b) A portion of 25D-5β-spirostan-1β, 2β-diol-3-one acetonide (VI) was treated with potassium hydroxide as described and the resulting solution was worked up as above to give the same diosphenol (VIII); mixed melting point with the specimen from a, 227~228°C.

Acid Isomerization of 25D-5β-Spirostan-1β, 2β-diol-3-one (VII), 25D-5β-Spirostan-1β, 2β-diol-3-one Acetonide (VI) and 25D-5β-Spirost-1-en-2-ol-3-one (VIII).—a) A solution of 70 mg. of 25D-5β-spirostan-1β, 2β-diol-3-one (VII) in 5 ml. of methanol containing 0.2 ml. of concentrated hydrochloric acid was boiled under reflux for thirty minutes. After concentration under reduced pressure, water was added and the mixture was extracted with methylene chloride. Thorough washing with water followed by drying and evaporation furnished a crystalline solid, m. p. 245~248°C. Recrystallization from methanol raised the m. p. to 254~255°C. The ultraviolet absorption spectrum of this substance showed a slight shift to a lower wavelength than that of the alkali isomerization product VIII, i. e., U. V. λ_{max} in EtOH: 267 mμ (ε: 5700), no hydroxyl absorption bands being noticed.

Anal. Found: C, 75.49; H, 9.37. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41%.

b) 25D-5β-Spirostan-1β, 2β-diol-3-one acetonide (VI) was subjected to acid isomerization under the conditions described above. The crude product after recrystallization from methanol showed the same melting point as that of the specimen from a.

c) A solution of 70 mg. of 25D-5β-spirost-1-en-2-ol-3-one (VIII) in 5 ml. of methanol containing 0.2 ml. of concentrated hydrochloric acid was boiled under reflux for thirty minutes. Working up of the reaction mixture by the same procedure as described in a and b yielded fine needles melting at 254~255°C.

All the specimens from a, b and c were confirmed to be identical with one another by mixed melting point determination.

25D-5β-Spirostan (XI).—25D-5β-Spirostan-1β, 2β-diol-3-one acetonide (VI) (500 mg.) was dissolved in 20 ml. of diethylene glycol containing 1 ml. of 80% hydrazine hydrate and 2.0 g. of potassium hydroxide. The mixture was heated at 200°C in an oil bath for three hours. After cooling, the reaction mixture was diluted with a large volume of water and extracted with ether. The ethereal solution was washed with water, dried, concentrated, and chromatographed on Florisil. 25D-5β-Spirostan (XI) was eluted from the column by petroleum ether and recrystallization from methanol-petroleum ether gave the analytical sample melting at 136~137°C. Identity of the substance here obtained was confirmed by comparison of the I. R.-spectra and the mixed melting point determination with an authentic specimen. When 25D-5β-spirostan-1β, 2β-diol-3-one (VII) and 25D-5β-spirost-1-en-2-ol-3-one (VIII) were subjected to the same reaction, 25D-5β-spirostan (XI) was obtained.

Samogenic Acid (XIIa).—Thirty per cent hydrogen peroxide (1 ml.) was added to a solution of 300 mg. of 25D-5β-spirost-1-en-2-ol-3-one (diosphenol) (VIII) in 5 ml. of methanol containing 200 mg. of potassium hydroxide and the solution was left standing at room temperature for one hour. A large volume of water was added, the resulting clear solution was extracted once with ether, and the ether solution was discarded. The aqueous solution was acidified and extracted with ether and the ethereal solution was washed with water, dried and evaporated to give a crystalline solid. Recrystallization of the solid from methanol furnished colorless thin plates melting at 271~273°C. Yield, 200 mg.

By the action of diazomethane, the acid (XIIa) afforded the dimethyl ester (XIIb), m. p. 147~148°C, $[\alpha]_D^{31}$: −28° (*c*; 0.5% in $CHCl_3$).

Summary

(1) Reduction of anhydrotokorogenin with lithium aluminum hydride led to a new dihydroxysapogenin, desoxytokorogenin; the structure 25D-5β-spirostan-1β, 3β-diol was assigned on the basis of various reactions and theoretical considerations.

(2) Desoxytokorogenin, 25D-5β-spirostan-1β, 3β-diol, was incidentally found to be identical with isorhodeasapogenin, the structure of which had so far been left unclarified.

(3) Identity of tokorogenic acid with isorhodeasapogenic acid was also confirmed.

(4) Tokorogenin was correlated with samogenic acid and yonogenin via a diosphenol, 25D-5β-spirost-1-en-2-ol-3-one, whose structure was unambiguously established.

The author wishes to express his grateful thanks to Dr. Y. Asahina of the University of Tokyo, Dr. S. Kuwada and Dr. T. Matsukawa of Takeda Research Laboratories for their encouragement throughout the work. Thanks are also due to Dr. K. Takeda of Shionogi Research Laboratories for the gift of a sample of yonogenin, to Mr. H. Kamio for infrared- and ultraviolet-spectral measurements and to Mr. M. Kan for elementary analyses.

Takeda Research Laboratories
Juso, Higashiyodogawa-ku,
Osaka