Studies on the Sapogenins of Dioscorea tokoro Makino. I.[1]* *The Structure of Tokorogenic Acid*

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Steroidal sapogenin from plants belonging to the family *Dioscoreaceae* in Japan was first reported in detail by Tsukamoto et al.[2] Nevertheless, until we reported the isolation of a new sapogenin from *Dioscorea tokoro* Makino, diosgenin had been the sole sapogenin from these plants. In a previous paper[1] we made clear the following points on the new sapogenin: (1) The elementary analyses of the sapogenin and its acetate coincide with those of trihydroxysapogenin and its triacetate. (2) The infrared absorption spectrum (980, 917, 895 and 862 cm^{-1}) shows that the sapogenin has iso-configuration of ring F[3]. (3) Chloroform solution of the sapogenin rapidly consumes bromine and evolves hydrogen bromide fume. (4) 16-Pregnene-x, y, z-triol-20-one triacetate, U.V. $\lambda_{\text{max}}^{\text{EtOH}}$: 240 m$\mu$ (ε: 1120), can be derived from the sapogenin by the isomerization to the pseudosapogenin followed by oxidation, hydrolysis and re-acetylation.

Since no trihydroxysapogenin possessing the same physical constants with this had so far been announced, we named this sapogenin "tokorogenin" and started to investigate its chemical structure.

Tokorogenin undergoes oxidation with chromic anhydride in acetic acid to give a dibasic acid "tokorogenic acid" and a small amount of a lactone-like substance, the structure of which has not yet been elucidated. Tokorogenin also undergoes oxidation with periodic acid in an ethanol solution giving a crystalline substance, $C_{34}H_{60}O_6$, which tends to become tokorogenic acid by treatment with aqueous acetic acid and subsequent oxidation with chromic anhydride in the same solvent. The I.R.-absorption spectrum of the periodic-acid oxidation product, however, showed that the substance possessed neither a hydroxyl group nor aldehyde functions, and therefore an acetal structure must be necessarily assigned to this substance.

Lead tetra-acetate also affects tokorogenin in 80% acetic acid, but no crystalline substance could be obtained. Quantitative estimation, however, showed that the oxidizing agent consumed in the reaction was almost equivalent to the value calculated for a glycerol structure.

In view of these results, it is quite reasonable to presume that tokorogenin possesses a glycerol moiety in ring A of steroid nucleus and that the chromic anhydride oxidation of this triol possibly causes elimination of one of its carbon atoms giving tokorogenic acid. It is, therefore, almost certain that tokorogenin must be defined as either spirostan-1, 2, 3-triol (I) or spirostan-2, 3, 4-triol (I′) and

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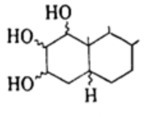
* This is a full paper of the previous communication (K. Morita, *Pharm. Bull.*, **5**, 496 (1957)), and constitutes Part VIII of Nishikawa's paper entitled "Studies in Steroids".

[1] M. Nishikawa, K. Morita, H. Hagiwara and M. Inoue, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **74**, 1165 (1954).

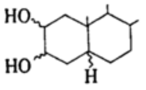
[2] T. Tsukamoto and Y. Ueno, ibid., **56**, 802, 939 (1936).

[3] C. R. Eddy et al., *Anal. Chem.*, **24**, 1337 (1952).

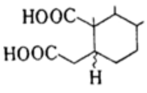
tokorogenic acid as either 1, 3-seco-2-nor-spirostane-1, 3-dioic acid (II) or 2, 4-seco-3-nor-spirostane-2, 4-dioic acid (II′).



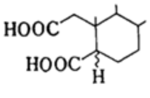
(I)



(I′)



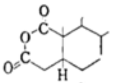
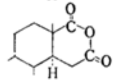
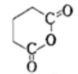
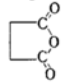
(II)



(II′)

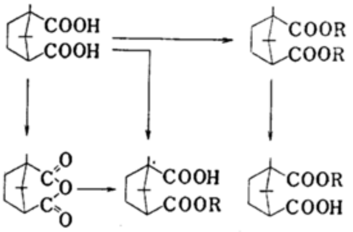
These assumptions are further supported by the fact that tokorogenic acid, on heating with acetic anhydride, forms an anhydride (I.R. λ_{max}^{Nujol}: 5.51, 5.71 μ), for according to the Blanc rule[4] no seven-membered ring anhydride can be formed from the corresponding 1,6-dibasic acids under these conditions. Moreover, the elementary analytical values of tokorogenic acid, its dimethyl ester and the anhydride favor the values calculated for $C_{26}H_{40}O_6$, $C_{28}H_{44}O_6$ and $C_{26}H_{38}O_5$, respectively. Evidence is also given by the fact that the infrared carbonyl bands of tokorogenic anhydride are typical of the 6-membered ring anhydride as shown in Table I.

TABLE I

Substance	Structure	I.R. max.	Ref. No.
Tokorogenic anhydride (III)		Nujol 5.55, 5,71	5)
3β-Acetoxy-5-etiobilienic anhydride		Nujol 5.55, 5.70	5)
Glutaric anhydride		$CHCl_3$ 5.55, 5.68	6)
Succinic anhydride		Nujol 5.36, 5.61	7)

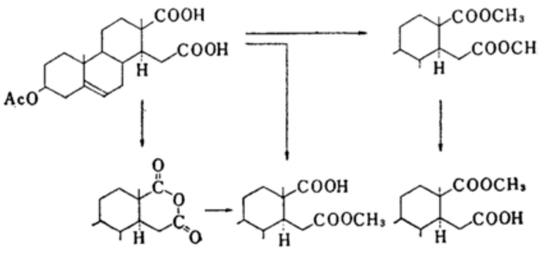
For the determination of the structure of tokorogenic acid, the following facts provide evidence in support of formula II rather than II′: (1) on treatment with methanol and hydrogen chloride tokorogenic acid affords a monomethyl ester (α-monomethyl ester); (2) alkaline hydrolysis of the dimethyl ester gives a different monomethyl ester (β-monomethylester); (3) treatment of the anhydride with one mole of sodium methoxide furnishes the α-monomethyl ester. These results are in complete accord with the earlier observations in the camphoric acid series (Chart 1)[8], where one of the carboxyl groups of the acid is attached to a tertiary carbon atom.

CHART 1



Since the structure II is closely similar to that of etiobilienic acid, which was first prepared from cholesterol by Kuwada[9] in our Laboratories, it can be presumed that etiobilienic acid and its derivatives will undergo reactions similar to those demonstrated in the camphoric acid series (Chart 1). We confirmed that this was true with etiobilienic acid, prepared from 5, 16-pregnadien-3β-ol-20-one acetate according to Adams et al.[10]

CHART 2



On the basis of these findings and the aforementioned discussion, one is led to conclude that the reactions in the tokorogenic acid series can be best represented by Chart 3; that is, tokorogenic acid must have the structure II, and tokorogenic anhydride III, the α-monoester V, and the β-monoester VI, respectively.

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[4] L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene", 3 Ed., Reinhold Publishing Corporation, New York, N. Y. (1949), p. 130.

[5] Presented by the author.

[6] H. H. Wasserman and H. E. Zimmerman, *J. Am. Chem. Soc.*, **72**., 5787 (1950).

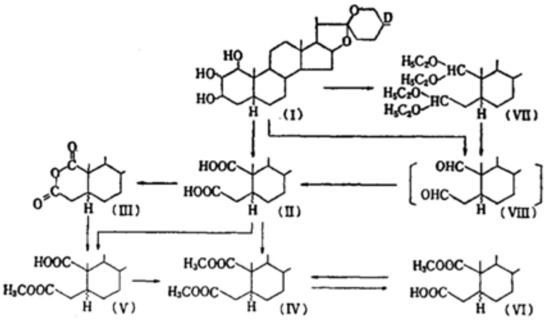
[7] H. M. Randall et al., "Infra-red Determination of Organic Structure" D. Van Norstrand Co., Inc., New York, N. Y. (1949), p. 163.

[8] J. W. Brühl, *Ber.*, **25**, 1796 (1892); W. A. Bone et al., *J. Chem. Soc.*, **85**, 534 (1904).

[9] S. Kuwada, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **56**, 78 (1936).

It is also evident that the periodic acid oxidation product ($C_{34}H_{60}O_6$) must have a dialdehyde diacetal structure VII and that the structure I has to be assigned to tokorogenin.

CHART 3



Experimental

All melting points are corrected.

Tokorogenic Acid (II).—To a solution of 1.0 g. of tokorogenin I in 90 ml. of glacial acetic acid was added with vigorous stirring a solution of 2.0 g. of chromium trioxide in 20 ml. of 80% acetic acid at room temperature over a period of one hour. The solution was then allowed to stand overnight and the excess of the reagent was destroyed with sodium bisulfite. Partial evaporation gave a difficultly soluble crystalline substance of the solution (135 mg., m.p. 241°C (decomp.); I.R.$\lambda_{\max}^{\text{Nujol}}$: 5.60 μ).

Anal. Found: C, 70.06; H, 8.74. Calcd. for $C_{27}H_{40}O_7$: C, 70.40; H, 8.75%.

The mother liquor was diluted with a large volume of water and extracted with ether, and the ethereal solution was repeatedly washed with water. The ethereal solution was then extracted with alkali and the alkaline solution was acidified and re-extracted with ether. Thorough washing of the solution followed by drying and evaporation furnished a solid residue (II, 470 mg.), which was recrystallized from acetic acid containing water yielding 400 mg. of colorless needles; m.p. 255°C (decomp.), $[\alpha]_D^{23}$ $-26.3°$ (in acetone).

Anal. Found: C, 69.65; H, 9.13. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99%.

Tokorogenic Acid Dimethyl Ester (IV).—A solution of tokorogenic acid II in ether was treated with a slight excess of diazomethane in the same solvent and the solution was immediately evaporated to dryness to give a crystalline dimethyl ester IV. Crystallization from methanol raised the melting point to 158~159°C.

Anal. Found: C, 70.75; H, 9.31. Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31%.

Tokorogenic Anhydride (III).—Tokorogenic acid II was heated with acetic anhydride for twenty minutes. After evaporation to a small volume the solution was cooled to give colorless needles of the anhydride III; m.p. 270~273°C, I.R.$\lambda_{\max}^{\text{Nujol}}$: 5.55, 5.71 μ.

Anal. Found: C, 72.47; H, 9.09. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90%.

A small portion of the anhydride III dissolved in methanol containing water and potassium hydroxide was digested for one hour, cooled, and neutralized with hydrochloric acid. Tokorogenic acid (II) was recovered in a good yield.

Tokorogenic Acid α-Monomethyl Ester (V).—a) To a mixture of 80 mg. of tokorogenic acid II and 3 ml. of absolute methanol was added 0.01 ml. of chlorosulfonic acid and the mixture was warmed on a steam bath to ensure complete solution. After being kept at room temperature for two hours the fine needles, which precipitated in the solution, were collected. Recrystallization from methanol afforded the analytical sample, which first melted at 141°C, then soon solidified and finally melted at 184~187°C (sintering 140°C).

Anal. Found: C, 69.88; H, 9.26. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15%.

By the action of diazomethane V gave the dimethyl ester IV.

b) To a solution comprising 5 ml. of methanol and 6 mg. of sodium metal was added 100 mg. of the anhydride III and the mixture warmed on a steam bath for several minutes. The solution was then allowed to stand at room temperature for forty-five minutes. A small quantity of acetic acid was added to this and the solution was evaporated to dryness to give fine needles; m.p. 184~187°C (pre-sintering at 140°C). The sample here obtained did not show depression of melting point on admixture with a specimen prepared according to (a).

Tokorogenic Acid β-Monomethyl Ester (VI).—To a solution of 50 mg. of dimethyl ester IV in 4 ml. of methanol was added 100 mg. of sodium hydroxide dissolved in 1 ml. of water and the solution was refluxed for fifteen minutes on a steam bath and then allowed to stand at room temperature for one hour. The solution was diluted with a large volume of water, extracted with ether and the ethereal solution was discarded. After acidification, the aqueous layer was again extracted with ether and the ethereal solution was washed, dried and evaporated to give a solid substance VI. Recrystallization from methanol afforded colorless thin plates of the β-monomethyl ester VI; m.p. 208~210°C.

Anal. Found: C, 70.18; H, 9.04. Calcd. for $C_{27}H_{42}O_6$: C, 70.10, H, 9.15%.

By the action of diazomethane, the β-monomethyl ester VI gave the dimethyl ester IV melting at 158~159°C, which did not show depression of the melting point on admixture with the sample obtained by the other method.

Periodic Acid Oxidation of Tokorogenin (I).—To a suspension of 500 mg. of tokorogenin I in 10 ml. of absolute ethanol was added 1 g. of periodic acid dissolved in 1 ml. of water. The mixture was stirred vigorously for fifteen minutes and the resulting solution was allowed to stand

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10) W. J. Adams et al., *J. Chem. Soc.*, **1956**, 297.

at room temperature for twenty-four hours to give colorless prisms. Recrytallization from methanol afforded 200 mg. of the diacetal VII; m.p. 150∼151°C.

Anal. Found: C, 72.86; H, 10.55. Calcd. for $C_{34}H_{60}O_6$; C, 72.80; H, 10.71%.

Three hundred mg. of the diacetal dissolved in 80% acetic acid (40 ml.) was gently warmed on a steam bath for thirty minutes and cooled. To this was added a solution of 0.5 g. of chromic anhydride in 5 ml. of 80% acetic acid in small portions. Working up of the reaction mixture as in the case of tokorogenic acid II led to needles melting at 255°C; yield 190 mg., mixture m.p. with tokorogenic acid II, 255°C.

Lead Tetra-acetate Oxidation of Tokorogenin (I).—To a solution of lead tetra-acetate in 90% acetic acid (40 ml., 0.71 M) was added 444 mg. (1 mmol.) of tokorogenin I and the mixture was stirred vigorously to ensure complete solution. The solution was then allowed to stand at 20°C for two hours and a half. During this time, one ml. of the reaction mixture was taken out at regular intervals, a solution of potassium iodide was added to each of the solutions, and the solution was titrated with 0.1 N sodium thiosulfate with results shown in Table II, which show that tokorogenin consumed about two moles of the reagent.

TABLE II

Time	0.1N $Na_2S_2O_3$ ml.	HIO_4 Consumed m mol.
0 min.	0	0
15 min.	0.47	0.94
30 min.	0.67	1.34
45 min.	0.78	1.56
60 min.	0.81	1.62
90 min.	0.87	1.74
120 min.	0.91	1.82
16 hr.	1.21	2.42

3β-Hydroxy-Δ^5-etiobilienic Acid α-Monomethyl Ester.—a) A solution of 500 mg. of 3β-acetoxy-Δ^5-etiobilienic acid9) in 30 ml. of methanol containing 0.5 ml. of chlorosulfonic acid was allowed to stand overnight at room temperature. After partial evaporation of the solution in vacuo water was added in small portions to give needles of the α-monomethyl ester. Recrystallization from acetone and water afforded the analytical sample melting at 175∼178°C.

b) A solution of 350 mg. of 3β-acetoxy-Δ^5-etiobilienic anhydride9) in 20 ml. of methanol containing 300 mg. of potassium hydroxide was refluxed on a steam bath for fifteen minutes. After standing at room temperature for thirty minutes a small quantity of acetic acid was added and the solution was evaporated in vacuo to a small volume. Water was added to this and the mixture was extracted with ether and the ethereal solution was extracted with alkali.

After acidification of the alkaline solution, it was extracted with ether, and then ethereal solution washed with water, dried and evaporated to give a syrup. Recrystallization from acetone and water afforded colorless needles melting at 175∼178°C; mixed m. p. with the sample prepared according to (a), 175∼178°C.

3β-Hydroxy-Δ^5-etiobilienic Acid β-Monomethyl Ester.—A solution of 500 mg. of 3β-acetoxy-Δ^5-etiobilienic acid dimethyl ester10) in 30 ml. of methanol containing 300 mg. of patassium hydroxide was refluxed on a steam bath for four hours. After evaporation in vacuo a large volume of water was added to this and the mixture was extracted with ether. From the ether solution, 200 mg. of the material melting at 110∼112°C was recovered. The aqueous solution, after acidification with diluted hydrochloric acid, was extracted with ether, and the ethereal solution was washed with water, dried and evaporated to give fine needles melting at 214∼215°C*.

The β-monomethyl ester, on treatment with diazomethane, gave the dimethyl ester.

Summary

(1) Oxidation of tokorogenin by means of chromic anhydride in acetic acid, periodic acid in ethanol and lead tetraacetate in aqueous acetic acid led to the conclusion that tokorogenin must have a glycerol moiety in ring A of the steroid nucleus.

(2) Tokorogenic acid, an oxidation product from tokorogenin, forms an anhydride by heating with acetic anhydride and gives rise to a monomethyl ester (α-monoester) by treatment with methanol and hydrogen chloride, while alkaline hydrolysis of the dimethyl ester of the acid furnishes a different monomethyl ester (β-monoester). The same α-monoester also results from tokorogenic anhydride by the action of alkali in a methanol solution.

(3) On the basis of the theoretical considerations and the afore-mentioned observations in the tokorogenic acid series compared with those in the camphoric acid and etiobilienic acid series, it is concluded that tokorogenic acid has to be defined as 1,3-seco-2-nor-spirostan-1,3-dioic acid (II) and hence tokorogenin as spirostan-1, 2, 3-triol (I).

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[*] Kuwada9) assigned the m. p. 176∼177°C (sinter. at 169°C) to the same monomethyl ester. Discrepancy between the melting points may would be attributed to the solvents ethanol being used by Kuwada.

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