[Chem. Pharm. Bull. 17(7)1369—1376(1969)]

UDC 581.19 : 547.918.02 : 547.92.02

Structure of Tokoronin[1]

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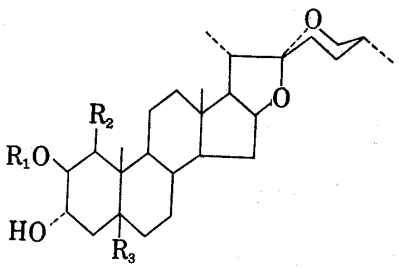
(Received November 18, 1968)

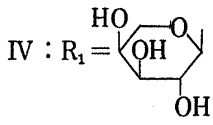
Pure tokoronin, mp 275—277° (decomp.), $[\alpha]_D$ −13.2°, was obtained in an improved method from the ethanol extracts of rhizomes of *Dioscorea tokoro* Makino and the structure was established as tokorogenin (25D, 5β-spirostane-1β, 2β, 3α-triol)-1-O-α-L-arabinopyranoside (XVII).

It represents an additional example of a novel type of spirostanol glycoside in which the sugar moiety is combined with a hydroxyl group other than that at C-3 of the aglycone.

Tokoronin,[3] mp 270—274° (decomp.), $[\alpha]_D$ −10.6°, is a spirostanol glycoside found in the rhizomes[4] of *Dioscorea Tokoro* Makino together with dioscin, gracillin, yononin, and others. It consists[3] of one mole each of L-arabinose and tokorogenin (I) which was first obtained from the same source by Nishikawa, *et al.*[5] and assigned the structure 25D, 5β-spirostane-1β, 2β,3α-triol by Morita.[6] I was isolated[7] by Okanishi, *et al.* and Takeda, *et al.* also from the aerial parts of the same plant along with yonogenin and kogagenin. The structures of the latter two were established by Takeda, *et al.* as 2β,3α-diol (II)[8] and 1β,2β,3α,5β-tetraol (III),[9] respectively, of 25D,5β-spirostane, and the common structural feature of these two and I, polyhydroxy-5β-spirostane holding an unusual 3α-hydroxyl group,[10] has received considerable attention.



I : R_1=H, R_2=OH, R_3=H
II : R_1=R_2=R_3=H
III : R_1=H, R_2=R_3=OH



IV : R_1= , R_2=R_3=H

1) Presented at the 11th Pacific Science Congress, Tokyo, Aug. 1966 and at the 87th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1967.
2) Location: *1276, Katakasu, Fukuoka.*
3) T. Kawasaki and T. Yamauchi, *Yakugaku Zasshi*, **83**, 757 (1963).
4) From the aerial parts of *D. Tokoro* a glycoside, mp 278—280° (decomp.), was isolated by Akahori,[11a] which was hydrolyzed, according to his private communication, to give tokorogenin. The sample kindly supplied by him yielded on acid hydrolysis L-arabinose and tokorogenin, and, on acetylation, the peracetate, mp 232—234°, $[\alpha]_D^{20}$ −14.9° (*c*=0.60, chloroform). It was identified as tokoronin by direct comparisons (mixed melting points, IR spectra, co-chromatography on thin-layer[21]) of the glycoside and its acetate with the authentic samples. The authors thank Dr. Akahori for the information and the sample.
5) M. Nishikawa, K. Morita, H. Hagiwara, and M. Inoue, *Yakugaku Zasshi*, **74**, 1165 (1954).
6) K. Morita, *Pharm. Bull.* (Tokyo), **5**, 494 (1957); *Bull. Chem. Soc. Japan*, **32**, 791, 796 (1959).
7) T. Okanishi and A. Shimaoka, *Ann. Rept. Shionogi Res. Lab.*, **6**, 78 (1956); K. Takeda, T. Okanishi, and A. Shimaoka, *Yakugaku Zasshi*, **77**, 822 (1957).
8) K. Takeda, T. Okanishi, and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **6**, 532 (1958).
9) *a*) K. Takeda, T. Kubota, and A. Shimaoka, *Tetrahedron*, **7**, 62 (1959); *b*) T. Kubota, *Chem. Pharm. Bull.* (Tokyo), **7**, 898 (1959); T. Kubota and K. Takeda, *Tetrahedron*, **10**, 1 (1960).
10) A new compound of this type which was named diotigenin and assigned the structure 25L,5β-spirostane-2β,3α,4β-triol (M. Ogata, F. Yasuda, and K. Takeda, *J. Chem. Soc.* (C), **1967**, 2397; K. Takeda, T. Okanishi, A. Akahori, and F. Yasuda, *Chem. Pharm. Bull.* (Tokyo), **16**, 421 (1968)) was isolated from *D. tenuipes* complex (A. Akahori, *Phytochemistry*, **4**, 97 (1965); A. Akahori, F. Yasuda, and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968)).

According to Akahori,[11] these novel type of spirostanols are mainly contained in the aerial parts of *D. Tokoro* in a free state[4] in contrast to diosgenin which is found in the underground parts of the same plant as its glycosides.

In the previous paper[12] the authors reported that yononin (IV), a yonogenin L-arabinoside, is the first spirostanol glycoside in which the sugar moiety is not attached to the C–3 but to C–2 hydroxyl group of the aglycone. Tokoronin (XVII), apparently a coexisting analog of IV, is expected also to have the arabinose residue combined with a hydroxyl group other than that at C–3. This paper is concerned with a study on the structure of XVII, the site of sugar linkage to the aglycone.

Pure XVII, mp 275—277° (decomp.), $[\alpha]_D$ −13.2°, which was obtained in an improved method from the rhizomes of *D. Tokoro* was methylated by the Hakomori method[13] to give the permethylate, which exhibited in the nuclear magnetic resonance (NMR) spectrum five methoxyl signals and an anomeric proton of the sugar moiety at 4.88 ppm as a doublet (J=2 cps).[14] It was subjected to methanolysis to give a methylated I (V), analyzing for $C_{29}H_{48}O_5$ and having two methoxyl signals in the NMR spectrum, and a methylated L-arabinose which was identified with an authentic sample of methyl 2,3,4-tri-O-methyl-L-arabinopyranoside. On heating with acetic anhydride and pyridine XVII gave the peracetate which showed NMR signals of five acetoxyl groups and of an anomeric proton at 4.50 ppm as a doublet (J=6 cps).[14] The infrared (IR) spectra of XVII and its permethylate and peracetate exhibited the characteristic absorptions[15] of the spiroketal side chain. The above data, in association with the previous experimental results,[3] show that XVII has one mole of L-arabinopyranose α-linked[14] with one of the three hydroxyl groups on the A–ring of I.

On acetylation with acetic anhydride and pyridine at room temperature V was recovered unchanged but on boiling with the same reagent it gave a monoacetate (VI), $C_{31}H_{50}O_6$. The NMR spectra (Fig. 1, 2) of V and VI showed a 19-methyl signal at 1.14 and 0.95 ppm,[16] respectively, and a signal of one proton on the carbon atom bearing a hydroxyl group in V and

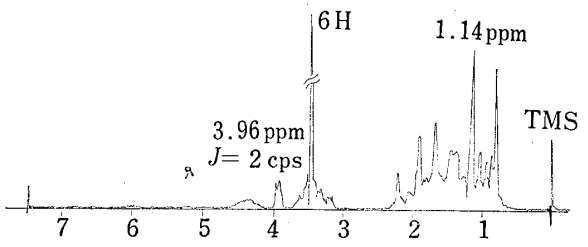


Fig. 1. Nuclear Magnetic Resonance Spectrum of V in $CDCl_3$ (60 Mcps)

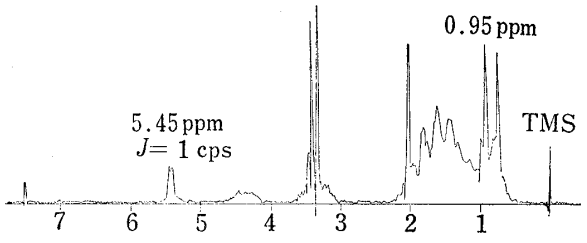


Fig. 2. Nuclear Magnetic Resonance Spectrum of VI in $CDCl_3$ (60 Mcps)

11) *a*) A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **10**, 1411 (1960); *b*) *Idem, ibid.*, **11**, 93 (1961); *ibid.*, **13**, 68 (1963).
12) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).
13) S. Hakomori, *J. Biochem.* (Japan), **55**, 205 (1964).
14) Coupling constant (J) (6 cps) of an anomeric proton signal in the peracetate seems to conflict with that (2 cps) observed in the permethylate. However, the 1,2-*trans*-diaxial conformation indicated by the former J value of C_1–H and C_2–H of the sugar moiety (R.U. Lemieux, R.K. Kullnig, H.J. Bernstein, and W.G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958); N. Mori, S. Omura, O. Yamamoto, T. Suzuki, and Y. Tsuzuki, *Bull. Chem. Soc. Japan*, **36**, 1048 (1963)) is only possible when the L-arabinopyranose residue has C–1 conformation (C_2–H being axial) and is α-linked with the aglycone, and the small J value in permethylate is presumed to be due to 1–C conformation (both C_1–H and C_2–H being equatorial) of the sugar portion. In other L-arabinopyranosides and some L-rhamnopyranosides the same finding was experienced, but in D-glucopyranosides no such difference was observed between acetate and methylate (unpublished result).
15) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); C.R. Eddy, M.E. Wall, and M.K. Scott, *ibid.*, **25**, 266 (1953); E.S. Rothman, M.E. Wall, and C.R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952).

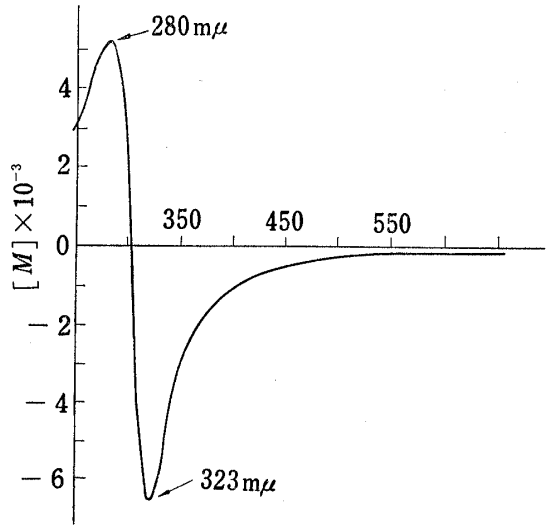


Fig. 3. Optical Rotatory Dispersion Curve of VII (in Dioxane)

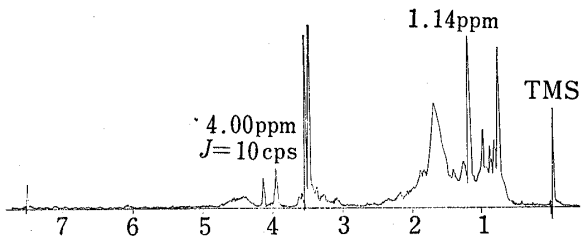


Fig. 4. Nuclear Magnetic Resonance Spectrum of VII in $CDCl_3$ (60 Mcps)

an acetoxyl group in VI appeared at 3.96 and 5.45 ppm, respectively, both as a doublet. These data suggest that V has a free hydroxyl group at C–1 and VI is the corresponding acetate. Chromic acid oxidation of V provided a ketone (VII), $C_{29}H_{46}O_5$, which showed in the optical rotatory dispersion (ORD) spectrum (Fig. 3) a negative Cotton curve with a molecular amplitude of −115.4 and in the NMR spectrum (Fig. 4) a signal of one proton at 4.0 ppm as a doublet (J=10 cps). Comparison of this ORD curve and the amplitude with those reported on 1-one,[17] 2-one[12] and 3-one[12,17] of 5β-steroid suggests VII to be a 1-one and the NMR signal at 4.0 ppm is accounted for by ascribing to that of the proton at C–2 which is next to methoxyl and carbonyl groups.

Therefore V is presumed to be tokorogenin 2,3-dimethyl ether and an unequivocal verification was made by synthesis (Chart 1) and direct comparison of three possible dimethylethers of I, their corresponding acetates and ketones.

Thus, I was methylated with methyl iodide and silver oxide in benzene to give a dimethylether (VIII) which gave, on acetylation, a monoacetate (IX) and, on oxidation, a ketone (X). On the other hand, when I was treated with dihydropyran and p-toluenesulfonic acid it provided a mixture of tetrahydropyranylethers. It was methylated by the Hakomori method,[13] subsequently the tetrahydropyranyl group was removed with acid and the resulting product was chromatographed on silica gel to afford two compounds XI and XII. Acetylation of XI and XII gave their acetates (XIII and XIV). XIII was saponified to give pure XI which was oxidized to afford a ketone (XV), while XIV afforded with alkali pure XII which gave on oxidation another ketone (XVI). Neither XIII nor XIV was identical with IX, and their analytical data and NMR spectra indicated that they were isomeric dimethylether monoacetates. The NMR spectrum (Fig. 5) of XIII showed a signal of X proton as an ABX type quartet (J=9 cps and 3 cps) centering at 4.96 ppm which is attributable to the proton at the carbon holding an acetoxyl group and located between two methine groups. Therefore XIII is regarded as 2-monoacetate. In the spectrum (Fig. 6) of XIV a signal of one proton next to an acetoxyl group was observed at 4.95—5.45 ppm as a broad multiplet suggesting that the acetoxyl group is located at C–3. The ORD spectra (Fig. 7) of the two ketones, XV and XVI, showed similar negative Cotton curves but the amplitude in XV was larger than that

16) K. Tori and K. Aono, *Ann. Rept. Shionogi Res. Lab.*, **14**, 136 (1964).
17) I. Yoshizawa, M. Tohma, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **15**, 129 (1967); M. Kimura, M. Tohma, and I. Yoshizawa, *ibid.*, **16**, 1228 (1968).

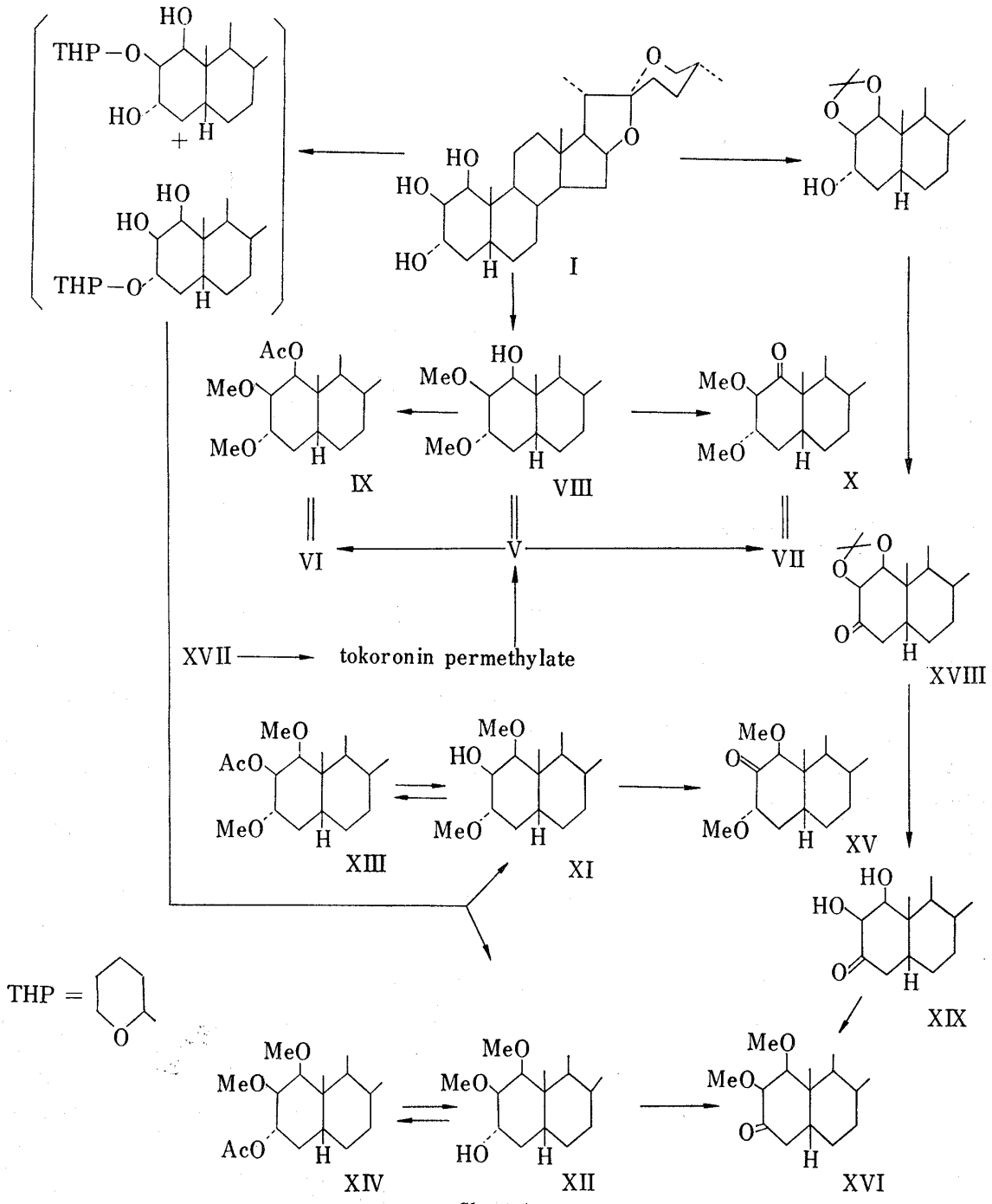


Chart 1

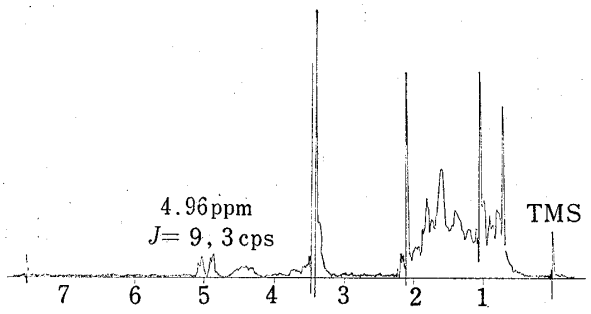


Fig. 5. Nuclear Magnetic Resonance Spectrum of XIII in $CDCl_3$ (60 Mcps)

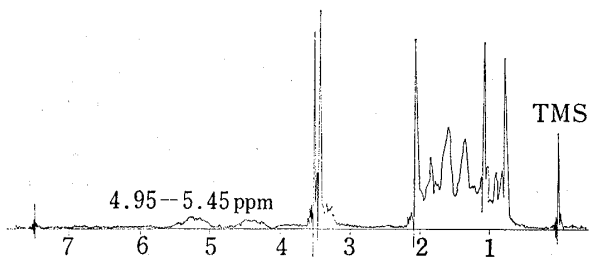


Fig. 6. Nuclear Magnetic Resonance Spectrum of XIV in $CDCl_3$ (60 Mcps)

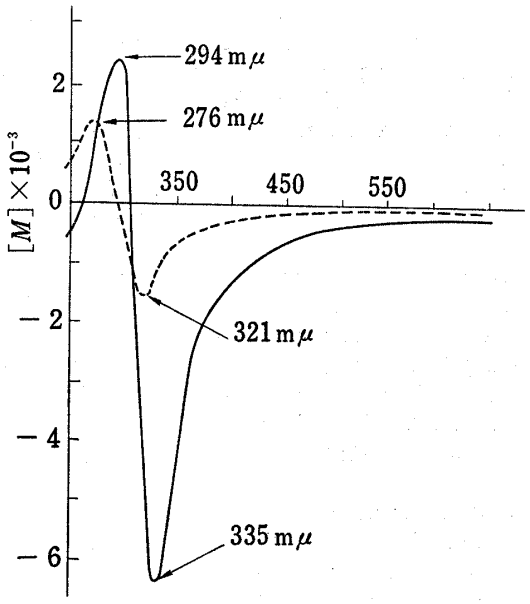
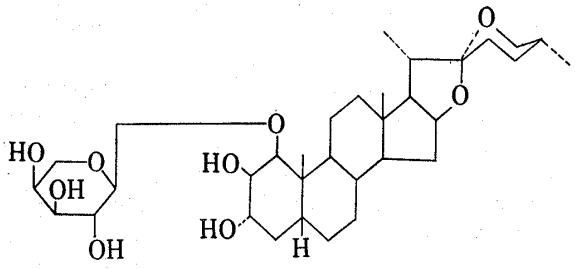


Fig. 7. Optical Rotatory Dispersion Curves of XV and XVI (in Dioxane)
——: XV -----: XVI



XVII

in XVI indicating[12] that the former is 2-one and the latter 3-one. The structure of XVI was further confirmed by identification with a synthetic sample from I *via* its 1,2-acetonide.[6] Accordingly compounds XI and XII are tokorogenin 1,3- and 1,2-dimethylethers, respectively, and another isomer VIII is 2,3-dimethylether. The direct comparison of melting points (and mixed melting points), optical rotations and IR, NMR and ORD spectra of VIII, IX and X respectively with those of V, VI and VII derived from XVII showed their complete identity.

Consequently the site of sugar linkage in XVII must be the hydroxyl group at C–1 of the aglycone and XVII is defined as tokorogenin 1-O-α-L-arabinopyranoside.

As a novel type of spirostanol glycoside in which the sugar moiety is attached to the hydroxyl group other than that at C–3 of the aglycone, yononin[12] has been followed by convallasaponin-B, 25L,5β-spirostane-1β,3β,4β,5β-tetraol-5-O-α-L-arabinopyranoside,[17,18] and tokoronin here reported represents an additional example.[19]

Experimental[20]

Isolation of Tokoronin (XVII)——Defatted (with benzene) ethanol extractives (10 g)[21] of the rhizomes of *D. Tokoro* was dissolved in butanol (200 ml) saturated with water and heated on a water bath with aqueous 10% KOH solution (50 ml) for 2 hr. Butanol layer was washed three times with water (30 ml) and evaporated *in vacuo* to give pale yellow powder (1.96 g). The powder was treated several times with refluxing hexane (100 ml) and insoluble portion (pale yellow powder, 1.09 g) was collected by filtration. The residue which

18) M. Kimura, M. Tohma, I. Yoshizawa, and H. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **16**, 25 (1968).
19) 1,3-Bis-O-glycoside (convallasaponin-D) and 3,5-bis-O-glycoside (gluco-convallasaponin-B),[17,18] and, quite recently, 6-O-glycosides (paniculonin-A and -B) (H. Ripperger and K. Schreiber, *Chem. Ber.*, **101**, 2450 (1968)) have been reported.
20) All melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform unless otherwise stated. IR spectra were obtained with a JASCO IR-S spectrophotometer and ORD curves were taken using a JASCO ORD/UV-5 recording spectropolarimeter. NMR spectra were recorded at 60 Mcps in deuterochloroform on a JEOL JNM-C-60H and 3H-60 spectrometers with tetramethylsilane as internal standard. In column chromatography "Kanto" silica gel (100—200 mesh) was employed.
21) Kindly supplied by Drs. M. Goto and S. Imai of the Research Laboratories of Takeda Chemical Industries, Ltd., to whom the authors' thanks are due.

revealed on thin-layer chromatography (TLC)[22] the spots corresponding to yononin (IV), XVII, prosapogenin-B of dioscin, dioscin and gracillin was chromatographed on silica gel (160 g) using chloroform–methanol–water (7:3:1, v/v) as a solvent to give six fractions: Fr. 1, oil (30 mg); Fr. 2, IV (168 mg); Fr. 3, IV and XVII (78 mg); Fr. 4, XVII (223 mg); Fr. 5, prosapogenin-B of dioscin (185 mg); Fr. 6, dioscin and gracillin (380 mg). Fr. 4 was recrystallized from methanol to give pure XVII as colorless needles (205 mg), mp 275—277° (decomp.), $[\alpha]_D^{20}$ −13.2° (c=0.81, pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3400—3200 (OH), 983, 915<898, 872 (25D-spiroketal). It (10.5 mg) was hydrolyzed on refluxing with 1N HCl in 50% ethanol to give I (8.25 mg) and L-arabinose, and was identified with the authentic sample[3] by mixed melting point, co-chromatography[22] and comparison of IR spectra.

Tokoronin Permethylate——XVII (250 mg) was methylated twice in dimethyl sulfoxide–dioxane (1:1, v/v) (5 ml) according to Hakomori.[13] The product was passed through an alumina column (Brockmann, 20 g) and chloroform eluate was evaporated to give a homogeneous compound (202 mg) which was recrystallized from hexane to provide the permethylate as colorless prisms, mp 101—103°, $[\alpha]_D^{20}$ −54.4° (c=0.95). *Anal.* Calcd. for $C_{37}H_{62}O_9$ (XVII pentamethyl ether): C, 68.27: H, 9.60. Found: C, 68.11; H, 9.70. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH absorptions, 981, 926, 920<900, 867 (25D-spiroketal). NMR ppm: 0.76 (3H, 18-CH_3), 0.96 (3H, doublet, J=6 cps, 21-CH_3), 1.13 (3H, 19-CH_3), 3.40—3.48 (15H, $OCH_3\times5$), 4.88 (1H, doublet, J=2 cps, anomeric H).

Tokoronin Peracetate——XVII (80 mg) was heated with pyridine (3 ml) and acetic anhydride (3 ml) on a water bath for 3 hr. The mixture was evaporated *in vacuo* to dryness and the residue was recrystallized from methanol to give the peracetate as colorless needles, mp 234—235°, $[\alpha]_D^{20}$ −16.4° (c=0.64). *Anal.* Calcd. for $C_{42}H_{62}O_{14}$ (XVII pentaacetate): C, 63.78; H, 7.90. Found: C, 63.33; H, 7.85. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH absorptions, 1761, 1247, 1229 ($OCOCH_3$), 979, 925<900, 865 (25D-spiroketal). NMR ppm: 0.75 (3H, 18-CH_3), 1.12 (3H, 19-CH_3), 2.01, 2.04, 2.07, 2.13 (15H, $OCOCH_3\times5$), 4.50 (1H, doublet, J=6 cps, anomeric H).

Methanolysis of Tokoronin Permethylate——Tokoronin permethylate (171 mg) was refluxed with 2N HCl in methanol (8 ml) for 4.5 hr. A precipitate formed on cooling was collected by filtration and the filtrate was evaporated *in vacuo*. Water was added to the residue, the mixture was shaken with chloroform and the organic layer was evaporated to dryness. The residue was treated with cold methanol and an insoluble portion was combined with the above precipitate and recrystallized from methanol to give colorless needles (V) (67 mg). A methanol soluble portion was chromatographed on a silica gel column using chloroform–methanol (9:1, v/v) to yield a syrup (33 mg).

Tokorogenin Dimethyl Ether (V)——mp 235—239° (turned from needles to prisms at 217—218°), $[\alpha]_D^{30}$ −20.7° (c=1.74). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3570 (OH).[23] NMR ppm (Fig. 1): 0.78 (3H, 18-CH_3), 0.97 (3H, doublet, J=6 cps, 21-CH_3), 1.14 (3H, 19-CH_3), 3.22 (1H, quartet, J=2.5 and 9 cps, C_2–H), 3.44 (6H, $OCH_3\times2$), 3.96 (1H, doublet, J=2 cps, C_1–H). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 73.11; H, 10.12.

Methyl 2,3,4-Tri-O-methyl-L-arabinopyranoside——The syrup obtained above was examined by gas liquid chromatography: Yanagimoto GCG 550F gas chromatograph equipped with a hydrogen flame ionization detector, U-shaped column (2.5 mm×1.2 m) packed with 5% 1,4-butanediol succinate on Chromosorb W (60—80 mesh), column temperature 131°, N_2 flow rate 21 ml/min. Retention time 3.80 min (methyl 2,3,4-tri-O-methyl-L-arabinopyranoside, 3.80 min; methyl tetra-O-methyl-D-glucopyranoside, 5.45 min; methyl tri-O-methyl-L-rhamnopyranoside, 1.63 min).

Tokorogenin Dimethyl Ether Monoacetate (VI)——V was recovered unchanged on leaving stand overnight at room temperature with acetic anhydride–pyridine (1:1, v/v). V (80 mg) was heated with the same reagent (5 ml) on a water bath for 3 hr, the mixture was evaporated *in vacuo* and the residue was recrystallized from methanol to provide VI as colorless needles, mp 178—179°, $[\alpha]_D^{30}$ −23.3° (c=1.50). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1742, 1239 ($OCOCH_3$). NMR ppm (Fig. 2): 0.76 (3H, 18-CH_3), 0.95 (3H, 19-CH_3), 2.08 (3H, $OCOCH_3$), 3.36, 3.44 (6H, $OCH_3\times2$), 4.15—4.65 (1H, broad, C_{16}–H), 5.45 (1H, doublet, J= 1 cps, C_1–H). *Anal.* Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.78; H, 9.76.

Dehydrotokorogenin Dimethyl Ether (VII)——To a solution of V (50 mg) in 90% acetic acid (20 ml) CrO_3 (30 mg) was added. The mixture was stirred at room temperature for 4.5 hr, then diluted with water and excess CrO_3 was decomposed with aqueous $NaHSO_3$ solution. A precipitate was collected by filtration and recrystallized from methanol to afford VII as colorless needles (35 mg), mp 223—225°. ORD (c=0.33, dioxane) $[M]^{32}$ (mμ): +5127 (280) (peak), −6408 (323) (trough) (Fig. 3). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1715 (C=O). NMR ppm (Fig. 4): 0.75 (3H, 18-CH_3), 0.94 (3H, doublet, J=6 cps, 21-CH_3), 1.14 (3H, 19-CH_3), 3.43, 3.47 (6H, $OCH_3\times2$), 4.00 (1H, doublet, J=10 cps, C_2–H). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.22; H, 9.85.

Tokorogenin 2,3-Dimethyl Ether (VIII)——I (500 mg) in absolute benzene (30 ml) was stirred with methyl iodide (0.5 g) and Ag_2O (1 g) at room temperature in the dark for 2 days. A precipitate was removed

22) T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), **11**, 1546 (1963).
23) The characteristic absorptions of 25D-spiroketal side chain were observed in all IR spectra of tokorogenin derivatives described herein.

by filtration and the filtrate was passed through an alumina column. The eluate was evaporated and the residue was recrystallized from methanol to give VIII as colorless needles, mp 237—239°, (turned to prisms at 217—218°), $[\alpha]_D^{30}$ −21.3° (c=1.78). Mixed melting point with V showed no depression and IR and NMR spectra were identical with those of V.

Tokorogenin 2,3-Dimethyl Ether 1-Acetate (IX)——VIII was acetylated in the same way as V to give IX as colorless needles (from methanol), mp 178—179°, $[\alpha]_D^{30}$ −24.7° (c=1.78). Mixed melting point and the comparison of IR and NMR spectra showed the identity with VI.

1-Dehydrotokorogenin 2,3-Dimethyl Ether (X)——VIII (70 mg) was oxidized with CrO_3 in the same way as V to give X as colorless needles (48 mg) (from methanol), mp 226—228°. Mixed melting point with VII showed no depression and the IR and NMR spectra were identical with those of VII.

Tokorogenin 1,3-Dimethyl Ether 2-Acetate (XIII)——To a solution of I (1.55 g, 3.5×10^{-3} mole) in tetrahydrofuran (40 ml) dihydropyran (0.33 g, 3.5×10^{-3} mole) and *p*-toluenesulfonic acid (10 mg) were added and the mixture was left stand at room temperature for 4 hr. The reaction mixture was neutralized with aqueous $NaHCO_3$ solution, evaporated *in vacuo* and extracted with chloroform. The solution was washed with water, dried, concentrated and passed through a silica gel column. A fraction (0.53 g) which consisted of mainly three substances less polar than I on TLC[24] was dried, dissolved in dimethylsulfoxide–tetrahydrofuran (1:1, v/v) (10 ml) and methylated by Hakomori method.[13] The product was extracted with chloroform, the organic layer was washed with water, dried, and evaporated *in vacuo* to dryness. The residue was methylated again in the same way. The product was then heated with 1N HCl in methanol (20 ml) on a water bath for 1 hr, the mixture was neutralized with aqueous $NaHCO_3$ solution and evaporated under a reduced pressure. The residue was dissolved in chloroform, washed with water, dried and the solvent was removed to give a solid (0.42 g) (showing five spots on TLC[24]). The mixture was chromatographed on silica gel (30 g) using hexane–ethyl acetate (2:1, v/v) as a solvent to give six fractions. Fr. 3 (78 mg) (XI) was acetylated with acetic anhydride and pyridine at room temperature and the product was passed through a silica gel column (solvent, hexane–ethyl acetate (4:1, v/v)) and recrystallized from methanol to give XIII as colorless plates (62 mg), mp 194—196°, $[\alpha]_D^{30}$ −19.5° (c=2.05). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1742, 1245 ($OCOCH_3$). NMR ppm (Fig. 5): 0.74 (3H, 18-CH_3), 0.96 (3H, doublet, J=6 cps, 21-CH_3), 1.04 (3H, 19-CH_3), 2.10 (3H, $OCOCH_3$), 3.40, 3.44 (6H, $OCH_3\times2$), 4.40 (1H, broad, C_{16}–H), 4.96 (1H, quartet, J=9 and 3 cps, C_2–H). *Anal.* Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.86; H, 9.68.

Tokorogenin 1,2-Dimethyl Ether 3-Acetate (XIV)——Fr. 4 (120 mg) (XII) of the above chromatography was acetylated to give XIV as colorless plates (from methanol) (95 mg), mp 210—212°, $[\alpha]_D^{30}$ −10.8° (c=1.60). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1747, 1238 ($OCOCH_3$). NMR ppm (Fig. 6): 0.79 (3H, 18-CH_3), 0.97 (3H, doublet, J=6 cps, 21-CH_3), 1.07 (3H, 19-CH_3), 2.07 (3H, $OCOCH_3$), 3.44, 3.48 (6H, $OCH_3\times2$), 4.20—4.60 (1H, broad, C_{16}–H), 4.95—5.45 (1H, broad, C_3–H). *Anal.* Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.36; H, 9.91.

Tokorogenin 1,3-Dimethyl Ether (XI)——XIII (55 mg) was refluxed with 2% KOH in methanol (5 ml) for 1 hr and then diluted with water. A precipitate was collected and recrystallized form methanol to give XI as colorless needles (40 mg), mp 199—201°, $[\alpha]_D^{28}$ −34.5° (c=1.16). NMR ppm: 0.77 (3H, 18-CH_3), 0.95 (3H, doublet, J=6 cps, 21-CH_3), 1.07 (3H, 19-CH_3), 3.42, 4.03 (6H, $OCH_3\times2$), 4.40 (1H, broad, C_{16}–H). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 72.89; H, 10.08.

Tokorogenin 1,2-Dimethyl Ether (XII)——In the same manner as above XIV (80 mg) gave XII as colorless prisms (60 mg) (from methanol), mp 196—198°, $[\alpha]_D^{28}$ −14.1° (c=2.06). NMR ppm: 0.76 (3H, 18-CH_3), 0.95 (3H, doublet, J =6 cps, 21-CH_3), 1.08 (3H, 19-CH_3), 3.45, 3.48 (6H, $OCH_3\times2$). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 73.29; H, 10.28.

2-Dehydrotokorogenin 1,3-Dimethyl Ether (XV)——XI (30 mg) in 90% acetic acid (30 ml) was oxidized under stirring with CrO_3 (20 mg) in 90% acetic acid (2 ml) at room temperature for 3 hr. Excess CrO_3 was decomposed, the mixture was extracted with chloroform and the extract was washed with water, dried, concentrated, placed on a silica gel (20 g) column and eluted with hexane–ethyl acetate (4:1, v/v). The eluate was evaporated and the residue was recrystallized from methanol to give XV as colorless plates (20 mg), mp 191—194°. ORD (c=0.41, dioxane) $[M]^{32}$ (mμ): +2413 (294) (peak), −6551 (335) (trough) (Fig.7). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1742 (C=O). NMR ppm: 0.74 (3H, 18-CH_3), 0.94 (3H, doublet, J=6 cps, 21-CH_3), 1.11 (3H, 19-CH_3), 3.27, 3.43 (6H, $OCH_3\times2$). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 72.92; H, 9.85.

3-Dehydrotokorogenin 1,2-Dimethyl Ether (XVI)——In the same way as above XII gave XVI as colorless needles (from methanol), mp 169—171°. ORD (c=0.31, dioxane) $[M]^{32}$ (mμ): +1491 (276) (peak), −1668 (321) (trough) (Fig. 7). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1727 (C=O). NMR ppm: 0.80 (3H, 18-CH_3), 0.97 (3H, doublet, J=6 cps, 21-CH_3), 1.14 (3H, 19-CH_3), 3.48 (6H, $OCH_3\times2$), 3.61 (1H, doublet, J=2 cps, C_1 or C_2–H), 4.00 (1H, doublet, J=2 cps, C_1 or C_2–H), 4.15—4.65 (1H, broad, C_{16}–H). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.41; H, 9.54.

3-Dehydrotokorogenin 1,2-Acetonide (XVIII)——Tokorogenin 1,2-acetonide (530 mg) preparared from I according to Morita[6] was dissolved in pyridine (50 ml) and oxidized by adding a solution of CrO_3 (1 g) in

24) Solvent, hexane–ethyl acetate (4:1, v/v).

pyridine (20 ml). The reaction mixture was left stand at 65° for 30 hr and excess CrO_3 was decomposed with aqueous $NaHSO_3$ solution. The product was extracted with chloroform and the solutioh was washed with water, dried and evaporated to dryness. The residue was recrystallized from ethanol to yield XVIII as colorless plates (178 mg), mp 231—233° (lit.[6] mp 229—231°). ORD ($c=0.32$, dioxane) $[M]^{32}$ (mμ): +1693 (276) (peak), −2103 (321) (trough).[25] IR ν_{max}^{Nujol} cm^{-1}: 1735 (C=O). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.04; H, 9.53. Found: C, 73.91; H, 9.80.

3-Dehydrotokorogenin (XIX)——A solution of XVIII (100 mg) in 80% acetic acid (30 ml) was refluxed for 20 min, then ice-cooled and a precipitate was collected. Recrystallization from hexane gave XIX as colorless plates (78 mg), mp 223—224° (lit.[6] mp 224—227°). IR ν_{max}^{Nujol} cm^{-1}: 3580 (OH), 1729 (C=O). *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.55; H, 9.61.

3-Dehydrotokorogenin 1,2-Dimethyl Ether (XVI) from XIX——XIX (71 mg) was stirred with methyl iodide (4 ml) and Ag_2O (0.7 g) at room temperature overnight. The mixture was then refluxed for 30 min and after cooling a precipitate was removed by filtration. The filtrate was evaporated, the residue was placed on a silicic acid (Mallinkcrodt, 100 mesh) (30 g) column and eluted with hexane–ethyl acetate(1:1, v/v): Fr. 1, less polar substance (trace); Fr. 2, expected compound (25.7 mg); Fr. 3, XIX (35.5 mg). Fr. 2 was recrystallized from methanol to give XVI as colorless needles (23 mg), mp 170—172°. Comparison (mixed melting point, IR and co-chromatography on thin-layer[24]) with XVI derived from XII showed their complete identity.

Acknowledgement The authors are grateful to the members of the Central Analysis Room of this University for microanalysis. The work was supported in part by a Grant-in-Aid of Scientific Research from the Ministry of Education of Japan, to which the authors' thanks are due.

25) *cf.* Fig. 1 in ref. 9a.