parallel relation to the basic nitrogen. This could be explainable by the electrostatic and the steric interferences of an ester group in 1,3-diaxial relation to the lone pair, thus causing some deformation of C-ring from taking the normal chair form.

Generally, interpretation of the absorption in the 2700—2900 cm^{-1} region in term of the Bohlmann band is said to be very careful, otherwise apt to committing misjudging the situation. Thus the application of the Bohlmann band to the ring system other than the quinolizidine system has been known very few, *i.e.*, Bohlmann bands in the oxazine system by Leonard[8] and also in the azabicycloketone system by House[9] were among rare cases reported.

Although the Bohlmann-like absorption on our compounds were obvious, further extensive and quantitative analyses are needed to draw the conclusion over the octahydrobenzo[*f*]-quinoline system.

Experimental

Infrared absorption spectra were measured in chloroform solution of the compounds previously reported[4], using NaCl cell on Hitachi Spectrophotometer EPI-G_2.

8) M.J. Leonard and W.K. Musker, *J. Am. Chem. Soc.*, **82**, 5148 (1960).
9) H.O. House, P.P. Mickham, and H.C. Muller, *J. Am. Chem. Soc.*, **84**, 3139 (1962).

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UDC 581.19 : 547.918.02 : 547.92.02

Isolation and Structure of a New Tokorogenin Glycoside

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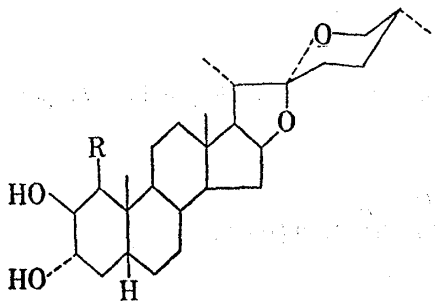
Tokorogenin which was obtained from the underground parts[2] and also from the aerial parts[3a] of *Dioscorea Tokoro* MAKINO and assigned the structure, 25D,5β-spirostane-1β,2β,3α-triol (I),[4] is the first unusual spirostanol having 3α-hydroxyl group so far found in plants. Later the analogous 2β,3α-diol (yonogenin),[3,5] 1β,2β,3α,5β-tetraol (kogagenin)[3a,6] and, quite recently, 25L,5β-spirostane-2β,3α,4β-triol (diotigenin)[3b,7] were isolated, accompanying tokorogenin, from *D. Tokoro*[3a] and *D. tenuipes* complex.[3b]

1) Location: *1276, Katakasu, Fukuoka.*
2) M. Nishikawa, K. Morita, H. Hagiwara, and M. Inoue, *Yakugaku Zasshi*, **74**, 1165 (1954).
3) *a*) 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); A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **10**, 1411 (1960); *ibid.*, **11**, 93 (1961); *ibid.*, **13**, 68 (1963); A. Akahori, I. Okuno, T. Okanishi, and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **16**, 1994 (1968); *b*) A. Akahori, *Phytochemistry*, **4**, 97 (1965); A. Akahori, F. Yasuda, and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968).
4) K. Morita, *Pharm. Bull.* (Tokyo), **5**, 494 (1957); *Bull. Chem. Soc. Japan*, **32**, 791, 796 (1959).
5) K. Takeda, T. Okanishi, and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **6**, 532 (1958).
6) K. Takeda, T. Kubota, and A. Shimaoka, *Tetrahedron*, **7**, 62 (1959); T. Kubota, *Chem. Pharm. Bull.* (Tokyo), **7**, 898 (1959); T. Kubota and K. Takeda, *Tetrahedron*, **10**, 1 (1960).
7) 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).

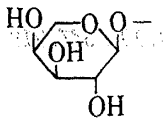
In contrast to an usual (having 3β-hydroxyl group) spirostanol, diosgenin, which is most common in *Dioscorea* plants and contained as its glycosides in the underground parts, this novel type of spirostanols are known to occur mainly in a free state in the epigeous parts.[3)]

As reported earlier, two of them, yonogenin and tokorogenin, were found in the rhizomes of *D. Tokoro* in the form of their glycosides, which were named[8)] yononin and tokoronin and proved to be yonogenin 2-O-α-L-arabinopyranoside[9)] and tokorogenin 1-O-α-L-arabinopyranoside (II),[10)] respectively. They have been noted as a novel type of spirostanol glycosides where the sugar moiety is attached to the hydroxyl group other than that at C-3 of the aglycon.[11)]

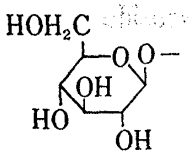
During the course of a further examination of the rhizomes of *D. Tokoro* in an attempt to isolate kogagenin and its glycoside.[3a,8)] a new compound was obtained in a pure state which was hoped to be the expected glycoside on the basis of its behavior on thin-layer chromatograms (TLC). It was found, however, to be a new tokorogenin glycoside and characterized as 1-O-β-D-glucopyranoside (III). This paper is to describe isolation and structure elucidation of the glycoside.



I: R=OH tokorogenin



II: R= tokoronin



III: R= compound x

When the water-insoluble part of ethanol extractives of the rhizomes were treated with dilute methanol and the soluble portion was subjected to repeated chromatographies on silica gel, a fraction was found to contain, though in a very small amount, a new compound (x) which was more polar than tokoronin on TLC[12)] and showed with sulfuric acid a different color from diosgenin glycosides (yellowish green) and tokoronin (dark purplish brown). Subsequently the isoation of the compound in a pure state and in an appreciable amount was investigated and it was achieved successfully by the procedure shown in Chart 1.

Compound x, mp 275—284° (decomp.), $[\alpha]_D^{22}$ −43.0°, was hydrolyzed with acid to give tokorogenin and D-glucose, and its peracetate, mp 181—184°, $[\alpha]_D^{22}$ −27.0°, analyzing for $C_{44}H_{64}O_{16}$, showed in its nuclear magnetic resonance (NMR) spectrum sixacetoxyl signals and one anomeric proton of the sugar moiety at 4.62 ppm as a doublet (J=6 cps). It was negative to the Ehrlich reagent[13)] and the infrared (IR) spectrum had the characteristic absorptions[14)] of the 25D-spiroketal side chain. These data indicate that compound x is a tokorogenin

8) T. Kawasaki and T. Yamauchi, *Yakugaku Zasshi*, **83**, 757 (1963).
9) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).
10) K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1369 (1969).
11) 1,3-Bis-O-glycoside (convallasaponin D), 3,5-bis-O-glycoside (gluco-convallasaponin B), 5-O-glycoside (convallasaponin B) (I. Yoshizawa, M. Tohma, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **15**, 129 (1967); M. Kimura, M. Tohma, I. Yoshizawa, and H. Akiyama, *ibid.*, **16**, 25 (1968); M. Kimura, M. Tohma, and I. Yoshizawa, *ibid.*, **16**, 1228 (1968)) and, quite recently, 6-O-glycosides (paniculonins A and B) (H. Ripperger and K. Schreiber, *Chem. Ber.*, **101**, 2450 (1968)) have been reported.
12) T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), **11**, 1546 (1963).
13) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968).
14) 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).

mono-D-glucoside in which the sugar moiety is β-linked[15] to one of the three hydroxyl groups on A–ring of the aglycon. Permethylation of x by Hakomori method[16] followed by methanolysis provided one kind of methylated sugar and an aglycon. The former was identified as methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside by gas liquid chromatography and the latter was proved to be tokorogenin 2,3-dimethyl ether by direct comparison with the authentic sample[10] derived from tokoronin(II).

Therefore compound x is defined as tokorogenin 1-O-β-D-glucopyranoside (III), another example of natural spirostanol 1-O-glycoside.

Experimental[17]

Isolation of New Compound (x) (Chart 1)——Defatted (with benzene) ethanol extractives[18] (340 g) was treated with hot acetone and the insoluble portion (300 g) was refluxed with ethyl acetate. The insoluble

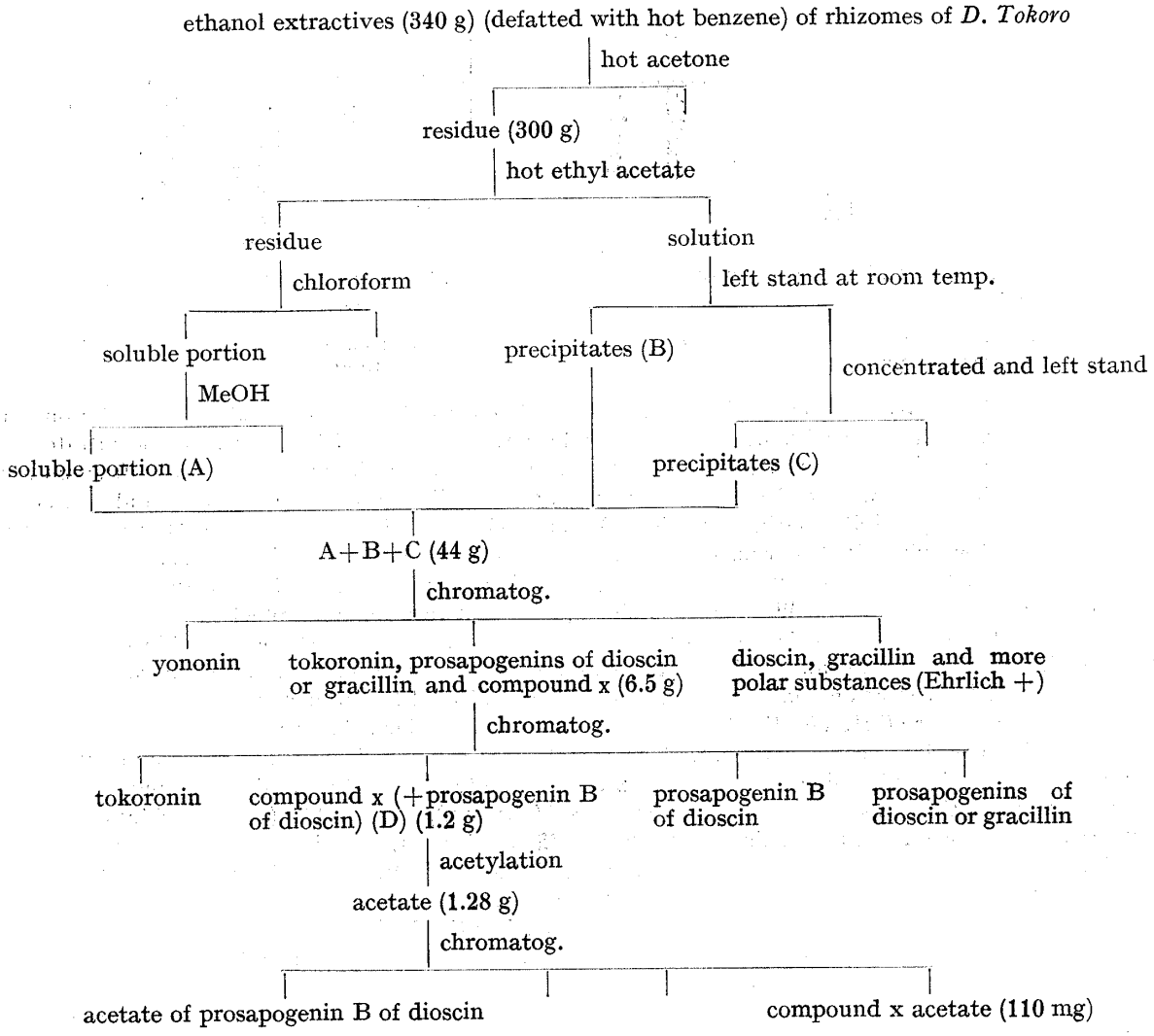


Chart 1. Isolation of Compound x

15) 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).

16) S. Hakomori, *J. Biochem.* (Japan), **55**, 205 (1964).

17) Melting points were taken on a Kofler block and are uncorrected. Optical rotations were measured with a JASCO DI-SL automatic polarimeter. IR spectra were obtained with a JASCO IR-S spectrometer and NMR spectra were recorded at 60 Mcps on a JEOL JNM-C-60H spectrometer with tetramethylsilane as internal standard. In column chromatography "Kanto" silica gel (100—200 mesh) was used.

18) Kindly supplied by Drs. M. Goto and S. Imai of the Research Laboratories of Takeda Chemical Industries, Ltd., to whom the authors are grateful.

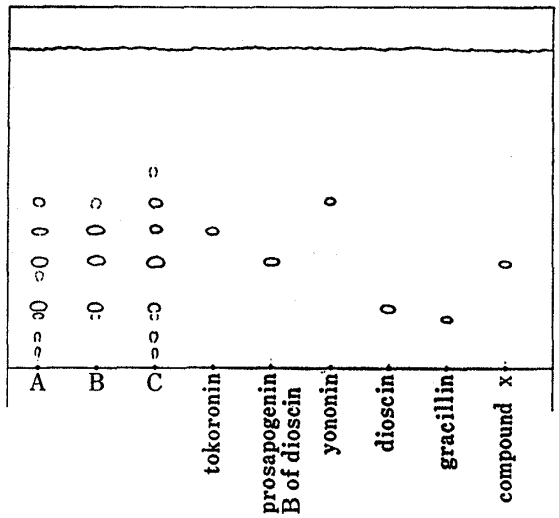


Fig. 1. Thin-Layer Chromatogram of Fractions A, B and C

solvent: chloroform–methanol–water 7:3:1, v/v, silica gel G[18]

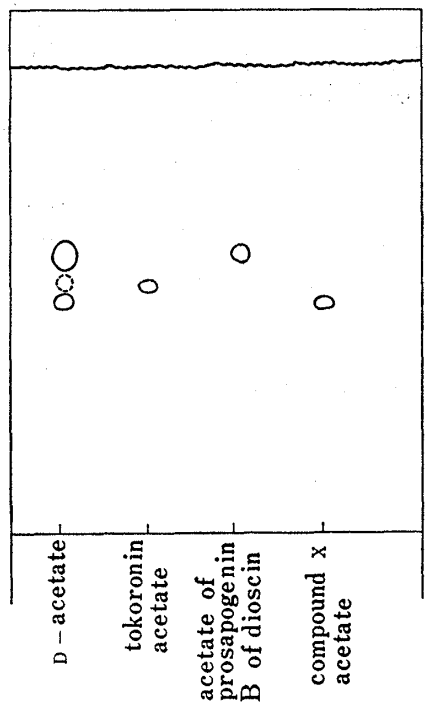


Fig. 2. Thin-Layer Chromatogram of Fraction D Acetate

solvent: benzene–acetone 8:2, v/v, silica gel G[12,19]

substance was collected by filtration while hot and extracted with chloroform. Chloroform solution was evaporated to dryness, treated with methanol, insoluble part was removed by filtration, the filtrate was evaporated to give a resinous mass (A). The hot ethyl acetate solution obtained above was left stand and the precipitates (B) formed were collected by filtration and the filtrate was concentrated and left stand to give another crop of precipitates (C). Fractions A,B, and C (TLC being shown in Fig. 1) were combined (44 g) and chromatographed in two runs (22 g sample on 250 g silicagel in each run) using chloroform–methanol–water (7:2:1, v/v) as a solvent. Fractions containing compound x along with tokoronin and prosapogenins[19] of dioscin or gracillin were combined (6.5 g) and rechromatographed in the same way as above. The fractions which revealed only one spot of compound x on TLC were combined (1.2 g) and acetylated with acetic anhydride and pyridine at room temperature. The acetate (1.28 g) showed two major spots accompanied by a faint one on TLC (Fig. 2) and then subjected to another chromatography on silica gel using benzene–acetone (9:1, v/v).[19] The fraction of the most polar substance was recrystallized from methanol to give colorless needles (110 mg). The acetate was saponified with 2% potassium hydroxide in methanol and the free glycoside was recrystallized from dilute ethanol to afford compound x.

Compound x——Colorless fine needles, mp 275—284° (decomp.), $[\alpha]_D^{22}$ −43.0° (c=0.66, chloroform), IR ν_{max}^{KBr} cm^{-1}: 855, 900>921, 984 (25D-spiroketal), 3250—3450 (hydroxyl), negative to the Ehrlich reagent[13] and dark bluish purple with sulfuric acid on TLC. On refluxing with 2N hydrochloric acid in 50% ethanol and working up as usual it provided an aglycon and a sugar portion. The former was recrystallized from dilute methanol to give colorless prisms, mp 270°, which was identified with the authentic sample (mp 268°) of tokorogenin by direct comparison (mixed melting point, IR spectra and TLC[20]). The sugar portion was examined by paper chromatography using butanol–pyridine–water (20:15:16, v/v) and identified as glucose. Acetylation of x with acetic anhydride and pyridine at room temperature gave acetate, mp 181—184°, $[\alpha]_D^{22}$ −27.0° (c=0.18, chloroform), NMR ppm (in deuterochloroform): 0.79 (3H, 18-CH_3), 1.09 (3H, 19-CH_3), 2.01, 2.03, 2.08, 2.11, 2.13 (18H, $OCOCH_3 \times 6$), 4.62 (1H, doublet, J=6 cps, anomeric proton of glucose). *Anal.* Calcd. for $C_{44}H_{64}O_{16}$ (tokorogenin monoglucoside hexaacetate): C, 62.25; H, 7.60. Found: C, 62.07; H, 7.66.

Methanolysis of Compound x Permethylate and Examination of the Products

Compound x was methylated by Hakomori method[16] in the same manner as reported[10] and the product was passed through a silica gel column using benzene–acetone (9:1, v/v) as a solvent to give the homogeneous

19) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1070 (1968).
20) K. Takeda, S. Hara, A. Wada, and N. Matsumoto, *J. Chromatog.*, **11**, 562 (1963); N. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **11**, 1189 (1963).

permethylate. It was refluxed with 2N hydrochloric acid in methanol for 4 hr and the reaction mixture was concentrated *in vacuo*, diluted with water and extracted with chloroform. The organic layer was washed with water, dried and evaporated. The residue was placed on a silica gel column and eluted with benzene–acetone (9:1, v/v). The first fraction was recrystallized from methanol to give colorless needles, mp 237°. *Anal.* Cacld. for $C_{29}H_{48}O_5$ (tokorogenin dimethylether): C, 73.07; H, 10.01. Found: C, 73.23; H, 10.08. It was identified with tokorogenin 2,3-dimethyl ether (mp 239°)[10] by mixed melting point determination and cochromatography on thin-layer of silica gel: *Rf* 0.21 (1,2-dimethyl ether[10] 0.17, 1,3-dimethyl ether[10] 0.29; solvent, hexane–ethyl acetate (4:1, v/v)). The second fraction was examined by gas liquid chromatography: t_R 6.0 min (methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside 6.0 min, methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside 7.2 min; Yanagimoto Gas Chromatograph GCG-550F equipped with a hydrogen flame ionization detector, glass column, 1.2 m long, 2 mmϕ packed with 5% 1,4-butanediol succinate[21] on Chromosorb W (60—80 mesh), flash heater temp. 240°, column temp. 145°, detector temp. 220°, nitrogen gas flow rate 15 ml/min).

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21) G.O. Aspinall, *J. Chem. Soc.*, **1963**, 1676.

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In Vivo and *in Vitro* Antitumor Activity of Sugars containing Sulfur

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Recently Akagi, *et al.*[2] have reported that β-D-xylopyranosyl ethylxanthate and β-D-mannopyranosyl ethylxanthate showed a marked antitumor effect on ascites type of Ehrlich carcinoma cells. This paper deals with the antitumor activity on ascitic form of Ehrlich carcinoma cells *in vivo* and Yoshida sarcoma cells *in vitro* of some new compounds.

As shown in Table I, forty compounds were screened for antitumor activity as a reference to β-D-xylopyranosyl ethylxanthate (I). The compounds I, II, X, XII, XIII, XV, XVII, XXIV, XXVI, XXVII, XXVIII and XXXVII were slightly effective on Ehrlich ascites carcinoma cells with or without toxicity to the mice. The compounds XI, XII, XIV, XVIII, XX, XXII, XXIV, XXV, XXVI, XXX, XXXI and XXXII were weakly active on Yoshida sarcoma cells *in virto*. On the contrary, the compounds I, V, IX, X, XVI and XXIII had no activity to the Yoshida sarcoma. It is interesting that the compounds I and X have been effective *in vivo* but not *in vitro*.

It is difficult to judge a parallelism between *in vivo* and *in virto*, moreover, relationship between chemical constitution and antitumor activity of thes ecompounds. However, it

1) Locat on: *a*) *1-3, Ukima, Kita-ku, Tokyo*; *b*) *Kita-15-jo, Nishi-7-chome, Sapporo.*

2) M. Akagi, S. Tejima, M. Haga, Y. Hirokawa, M. Yamada, M. Ishiguro and D. Mizuno, *Yakugaku Zasshi*, **87**, 287 (1967).