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**Original**

# Studies on the Constituents of the *Dioscorea tokoro* Rhizome

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**Abstract**: The rhizome of *Dioscorea tokoro* has been used in China to alleviate the pain of rheumatic disorders of the knees and hips, but the active component has not been clarified. In order to determine its pharmacological activity, we isolated and characterized the structure of the constituents of the *Dioscorea tokoro* rhizome by means of column chromatography and spectral analysis. As a result, two new furostanol saponins, protoyononin (D8) and protocompound x (D12), were isolated from the *Dioscorea tokoro* rhizome, along with ten known spirostanol and furostanol derivatives, namely, dioscin, gracillin, yononin, tokorogenin, tokoronin, compound x, protoyonogenin, protodioscin, protogracillin and prototokoronin. The structures of D8 and D12 were determined based on chemical and spectral methods and characterized as 26-*O*-β-D-glucopyranosyl (25*R*)-2β,3α,22ξ,26-tetrahydroxyfurost-2-*O*-α-L-arabinopyranside (protoyononin) and 26-*O*-β-D-glucopyranosyl (25*R*)-1β,2β,3α,22ξ,26-pentahydroxyfurost-1-*O*-β-D-glucopyranside (protocompound x).

**Key words**: *Dioscorea tokoro*, rhizome, spirostanol, furostanol, saponin

## Introduction

*Dioscorea tokoro*, a perennial plant that belongs to the family Dioscoreaceae, is distributed in the sunny mountains and fields of Japan and in the central to southern part of China. This plant belongs to the same species as *Dioscorea villosa* (wild yam)[1] which is used as a raw material for producing steroid hormones, and many steroid glycosides have already been reported as constituents of the rhizome[2-7]. The rhizome of *Dioscorea tokoro* has been used in China to alleviate the pain of rheumatic disorders of the knees and hips[8], but the active component has not yet been clarified. In order to determine the pharmacological activity of its constituents, we carried out the isolation and structural characterization of the constituents of the *Dioscorea tokoro* rhizome.

## Material and methods

The plant (rhizome of *Dioscorea tokoro*) was collected in the city of Fujiyoshida, Yamanashi Prefecture, Japan, in June 2001, and botanically identified by Susumu Isoda (PhD) at Showa University. Silica gel 60 (0.063–0.2 mm) for column chromatography (CC), silica gel plates (Kieselgel 60 F254) and octadecylsilane (ODS) plates (RP-18 WF254S) for thin-layer chromatography

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(TLC) were the products of Merck (Darmstadt, Germany). Chromatorex ODS (DM-1020T) for CC was obtained from Fuji Silysia Co. (Tokyo, Japan). The solvents and reagents used for CC and reactions were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

*Analytical instruments*

The nuclear magnetic resonance (NMR) spectra were recorded in pyridine-$d_5$ on a JNM-LA 500 spectrometer ($^1$H ; 500 MHz, $^{13}$C ; 125 MHz, JEOL Ltd., Tokyo, Japan). Chemical shifts were assigned by means of distortionless enhancement by polarization transfer and two-dimensional NMR methods ($^1$H-$^1$H correlation spectroscopy, $^1$H-$^{13}$C correlation spectroscopy). Mass spectra were recorded by an electrospray ionization-time-of-flight (TOF) mass spectrometer (Micromass LCT (Waters Xevo G2-XS QTof LC/MS)).

*Extraction and isolation of steroidal constituents of the Dioscorea tokoro rhizome*

Fresh rhizomes of *Dioscorea tokoro* (3.32 kg) were extracted with MeOH (2.0 l, 15 days × 3). After removal of the solvent by evaporation, the combined extract (184 g) was dissolved in water and extracted with ether ($Et_2O$). The $Et_2O$ layer was evaporated under reduced pressure, yielding an $Et_2O$-soluble portion weighing 9.52 g. Then, the aqueous layer was chromatographed on ODS ($H_2O$, 30% MeOH, 70% MeOH and MeOH, successively) to give 9 fractions (frs.), I - IX in order of elution. Fr. IV ($H_2O$ eluate ; 3.25 g) was purified by silica gel CC ($CHCl_3$-MeOH-$H_2O$ = 65:35:3.5) to give D11 (1.23 g). Fr. V (30% MeOH eluate ; 26.5 g) was separated and purified by ODS CC (30% acetonitrile) and silica gel CC ($CHCl_3$-MeOH-$H_2O$ = 65:35:3.5) to give D8 (1.84 g), D9 (1.14 g), D11 (1.55 g) and D12 (508 mg). Fr. VI (70% MeOH eluate) was separated and purified by silica gel CC ($CHCl_3$-MeOH-$H_2O$ = 8:2:0.2) to give D7 (3.55 g), D8 (70.0 mg), D9 (1.14 g), D10 (79.0 mg) and D12 (30.0 mg). Fr. VII (70% MeOH eluate) was separated and purified by silica gel CC ($CHCl_3$-MeOH-$H_2O$ = 75:25:1) to give D1 (2.38 g), D2 (37.5 mg), D3 (1.52 g), D4 (115 mg), D5 (2.42 g) and D6 (62.0 mg).

## Results and discussion

The compounds isolated from the rhizome of *Dioscorea tokoro* were identified as dioscin (D1)[2,9], gracillin (D2)[2,10], yononin (D3)[3,11], tokorogenin (D4)[4,12], tokoronin (D5)[5,12], compound x (D6)[6], protoyonogenin (D7)[13], protodioscin (D9)[7,9], protogracillin (D10)[8,13] and prototokoronin (D11)[12] (Fig. 1), based on analysis of the $^{13}$C-NMR spectra (Tables 1–3).

D8 was obtained as an amorphous powder. $^1$H-NMR ($C_5D_5N$, 500 MHz) : $\delta$ 0.83 (3H, s, $C_{18}$-Me), 0.90 (3H, s, $C_{19}$-Me), 0.96 (3H, d, $J$ = 6.9 Hz, $C_{27}$-Me), 1.01 (3H, d, $J$ = 6.3 Hz, $C_{21}$-Me), 4.79 (1H, d, $J$ = 8.0 Hz, Glc H-1), 5.20 (1H, d, $J$ = 6.9 Hz, Ara H-1). $^{13}$C-NMR ($C_5D_5N$, 125 MHz) : Table 2. High resolution TOF mass spectrometry (HR-TOFMS) *m/z* 767.4203 [M+Na]$^+$, calcd. 767.4194.

D8 is positive with Ehrlich reagent[14], suggesting it is a furostane-type glycoside. An aqueous solution of D8 (25 mg) was incubated with almond emulsin (25 mg) at 37°C for 8 hr. The precipitate (D8-EH ; 5 mg) was collected by filtration. D8-EH was identified as yononin (D3) by

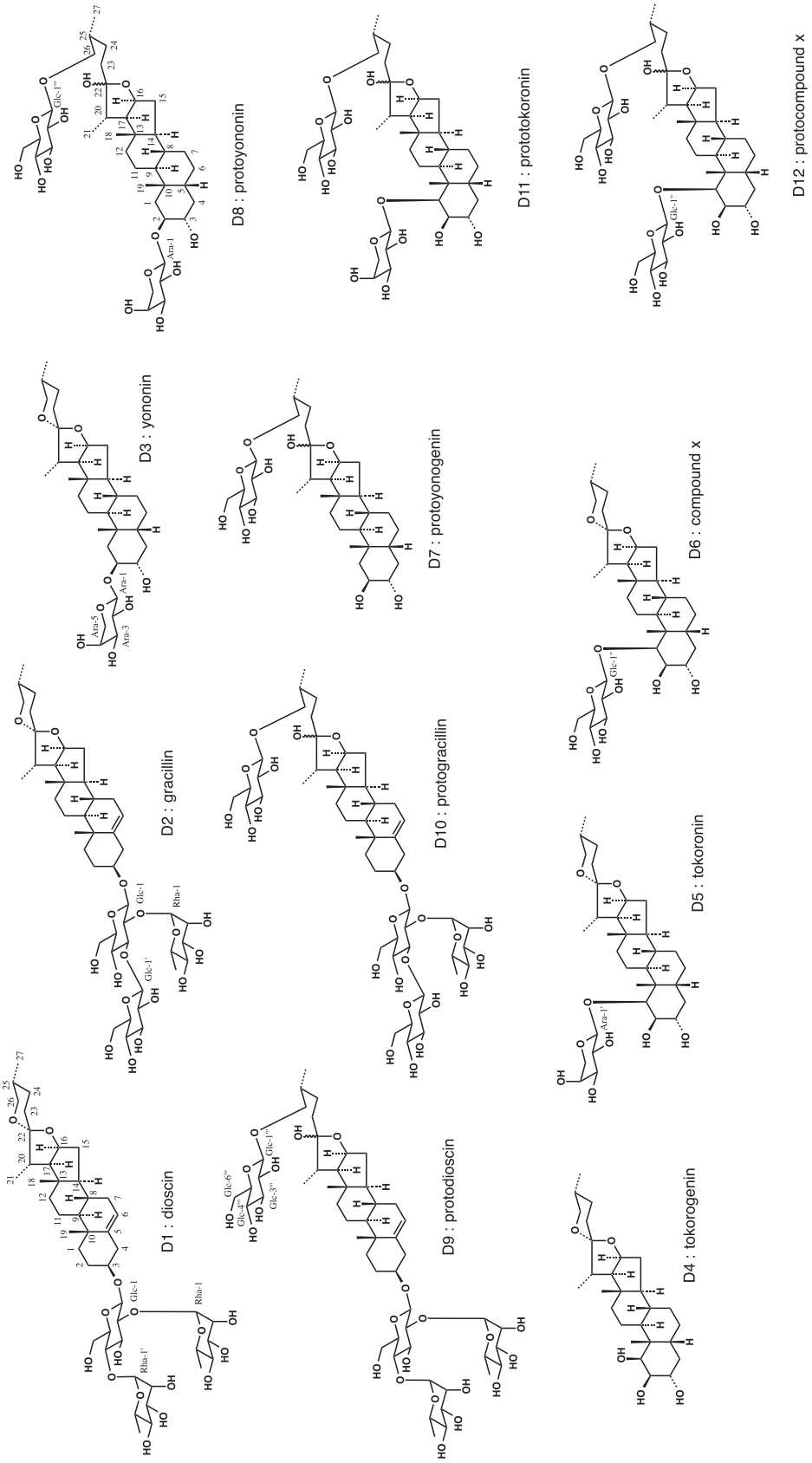


Fig. 1. Steroid compounds isolated from the *Dioscorea tokoro* rhizome

Table 1. $^{13}$C-nuclear magnetic resonance signals of the diosgenin and protodiosgenin glycosides, D1, D2, D9 and D10 in pyridine-$d_5$

| Carbon No. | D1 | D2 | D9 | D10 | Carbon No. | D1 | D2 | D9 | D10 |
|---|---|---|---|---|---|---|---|---|---|
| aglycone | | | | | sugars | | | | |
| C-1 | 37.6 | 37.6 | 37.5 | 37.5 | Glc-1 | 100.4 | 100.2 | 100.2 | 99.9 |
| C-2 | 30.3 | 30.2 | 30.1 | 30.0 | Glc-2 | 79.5 | 77.2 | 77.9 | 77.0 |
| C-3 | 78.4 | 78.5 | 78.5 | 78.5 | Glc-3 | 77.9 | 89.5 | 78.0 | 89.5 |
| C-4 | 39.1 | 38.9 | 38.9 | 38.7 | Glc-4 | 76.7 | 69.6 | 78.6 | 69.6 |
| C-5 | 141.0 | 141.0 | 140.7 | 140.7 | Glc-5 | 78.1 | 78.0 | 76.8 | 77.8 |
| | | | | | Glc-6 | 61.6 | 62.5 | 61.2 | 62.4 |
| C-6 | 121.8 | 121.8 | 121.8 | 121.9 | | | | | |
| C-7 | 32.4 | 32.4 | 32.3 | 32.4 | Rha-1 | 101.9 | 102.2 | 102.0 | 102.2 |
| C-8 | 31.9 | 31.8 | 31.6 | 31.7 | Rha-2 | 72.4 | 72.5 | 72.5 | 72.4 |
| C-9 | 50.6 | 50.5 | 50.3 | 50.3 | Rha-3 | 72.8 | 72.8 | 82.8 | 72.7 |
| C-10 | 37.3 | 37.3 | 37.1 | 37.2 | Rha-4 | 74.2 | 74.2 | 74.1 | 74.1 |
| | | | | | Rha-5 | 69.4 | 69.8 | 69.5 | 69.5 |
| C-11 | 21.2 | 21.2 | 21.1 | 21.1 | Rha-6 | 18.8 | 18.7 | 18.5 | 18.7 |
| C-12 | 40.1 | 40.0 | 39.9 | 39.9 | | | | | |
| C-13 | 40.6 | 40.6 | 40.7 | 40.6 | Rha-1' | 103.0 | | 102.8 | |
| C-14 | 56.9 | 56.8 | 56.5 | 56.6 | Rha-2' | 72.4 | | 72.5 | |
| C-15 | 32.3 | 32.3 | 32.4 | 32.3 | Rha-3' | 72.7 | | 72.7 | |
| | | | | | Rha-4' | 73.8 | | 73.9 | |
| C-16 | 81.2 | 81.2 | 81.1 | 81.1 | Rha-5' | 70.5 | | 70.4 | |
| C-17 | 63.1 | 63.1 | 63.8 | 63.8 | Rha-6' | 18.4 | | 18.6 | |
| C-18 | 16.4 | 16.4 | 16.4 | 16.4 | | | | | |
| C-19 | 19.5 | 19.4 | 19.4 | 19.4 | Glc-1' | | 104.5 | | 104.5 |
| C-20 | 42.1 | 42.1 | 40.6 | 40.8 | Glc-2' | | 75.1 | | 74.9 |
| | | | | | Glc-3' | | 77.8 | | 77.6 |
| C-21 | 15.0 | 15.0 | 16.3 | 16.4 | Glc-4' | | 71.6 | | 71.6 |
| C-22 | 109.3 | 109.3 | 110.6 | 110.6 | Glc-5' | | 78.7 | | 78.7 |
| C-23 | 32.0 | 32.0 | 37.2 | 37.1 | Glc-6' | | 62.6 | | 62.6 |
| C-24 | 29.4 | 29.4 | 28.3 | 28.3 | | | | | |
| C-25 | 30.7 | 30.7 | 34.2 | 34.2 | Glc-1''' | | | 104.9 | 104.9 |
| | | | | | Glc-2''' | | | 75.2 | 75.2 |
| C-26 | 67.0 | 67.0 | 75.2 | 75.2 | Glc-3''' | | | 78.5 | 78.4 |
| C-27 | 17.3 | 17.4 | 17.4 | 17.4 | Glc-4''' | | | 71.6 | 71.4 |
| | | | | | Glc-5''' | | | 78.6 | 78.6 |
| | | | | | Glc-6''' | | | 62.8 | 62.8 |

comparison with the $^{13}$C-NMR spectra for D3 (Table 2). The aqueous filtrate was evaporated to dryness in vacuo and the residue was examined by silica gel TLC with $CHCl_3$-MeOH-$H_2O$ (6:4:1) and compared with an authentic sample, and glucose ($R_f$ 0.19) was detected.

Furthermore, when the $^{13}$C-NMR spectra of D8 was compared with yononin obtained by the enzymatic hydrolysis of D8 and protoyonogenin (D7), the signals due to arabinose attached to

Table 2. $^{13}$C-nuclear magnetic resonance signals of the yonogenin and protoyonogenin glycosides, D3, D7 and D8 in pyridine-$d_5$

| Carbon No. | yonogenin [11] | D3 | D8-EH | D7 | D8 | Carbon No. | D3 | D8-EH | D7 | D8 |
|---|---|---|---|---|---|---|---|---|---|---|
| aglycone | | | | | | sugars | | | | |
| C-1 | 44.7 | 43.2 | 43.1 | 44.8 | 43.1 | Ara-1 | 106.8 | 106.8 | | 106.7 |
| C-2 | 71.3 | 82.0 | 81.9 | 71.3 | 81.8 | Ara-2 | 73.4 | 73.3 | | 73.2 |
| C-3 | 77.0 | 76.1 | 76.1 | 77.0 | 76.0 | Ara-3 | 74.8 | 74.7 | | 74.7 |
| C-4 | 35.5 | 34.9 | 34.8 | 35.5 | 34.8 | Ara-4 | 69.6 | 69.6 | | 69.5 |
| C-5 | 42.3 | 42.0 | 41.9 | 42.3 | 41.9 | Ara-5 | 67.2 | 67.1 | | 67.1 |
| C-6 | 26.9 | 26.7 | 26.6 | 26.8 | 26.6 | Glc-1''' | | | 104.4 | 104.9 |
| C-7 | 26.9 | 27.0 | 26.9 | 26.9 | 26.9 | Glc-2''' | | | 74.6 | 75.3 |
| C-8 | 35.7 | 35.7 | 35.6 | 35.6 | 35.6 | Glc-3''' | | | 78.0 | 78.5 |
| C-9 | 42.3 | 42.1 | 42.0 | 42.4 | 42.0 | Glc-4''' | | | 71.1 | 71.6 |
| C-10 | 37.2 | 37.5 | 37.4 | 37.2 | 37.4 | Glc-5''' | | | 77.9 | 78.4 |
| | | | | | | Glc-6''' | | | 62.3 | 62.7 |
| C-11 | 21.1 | 21.2 | 21.1 | 21.1 | 21.1 | | | | | |
| C-12 | 40.2 | 40.1 | 40.0 | 40.2 | 40.1 | | | | | |
| C-13 | 40.8 | 40.9 | 40.8 | 41.1 | 41.1 | | | | | |
| C-14 | 56.3 | 56.3 | 56.2 | 56.1 | 56.1 | | | | | |
| C-15 | 32.1 | 32.2 | 32.1 | 32.3 | 32.3 | | | | | |
| C-16 | 81.2 | 81.3 | 81.3 | 81.1 | 81.1 | | | | | |
| C-17 | 63.1 | 63.2 | 63.1 | 64.0 | 64.0 | | | | | |
| C-18 | 16.6 | 16.6 | 16.5 | 16.7 | 16.6 | | | | | |
| C-19 | 23.6 | 23.5 | 23.5 | 23.6 | 23.4 | | | | | |
| C-20 | 42.0 | 42.0 | 41.9 | 40.6 | 40.5 | | | | | |
| C-21 | 15.0 | 15.2 | 15.1 | 16.5 | 16.5 | | | | | |
| C-22 | 109.2 | 109.3 | 109.2 | 110.6 | 110.6 | | | | | |
| C-23 | 31.9 | 31.9 | 31.8 | 37.2 | 37.2 | | | | | |
| C-24 | 29.3 | 29.3 | 29.3 | 28.4 | 28.4 | | | | | |
| C-25 | 30.6 | 30.7 | 30.6 | 34.2 | 34.2 | | | | | |
| C-26 | 66.9 | 67.0 | 66.9 | 75.3 | 75.3 | | | | | |
| C-27 | 17.3 | 17.3 | 17.3 | 17.4 | 17.4 | | | | | |

the C2 hydroxyl group of aglycone and C1 to C16, C18 and C19 of aglycone were in good agreement with yononin. Additionally, the signals due to glucose attached to the C26 hydroxyl group of aglycone and C17 and C20 to C27 of aglycone were in good agreement with protoyonogenin (Table 2). Based on the above results, D8 was characterized as 26-*O*-$\beta$-D-glucopyranosyl (25*R*)-2$\beta$,3$\alpha$,22$\xi$,26-tetrahydroxyfurost-2-*O*-$\alpha$-L-arabinopyranside (protoyononin) (Fig. 1).

D12 was obtained as an amorphous powder. $^1$H-NMR ($C_5D_5N$, 500 MHz): $\delta$ 0.84 (3H, s,

Table 3. $^{13}$C-nuclear magnetic resonance signals of the tokorogenin and prototokorogenin glycosides, D4, D5, D6, D11 and D12 in pyridine-$d_5$

| Carbon No. | D4 | D5 | D6 | D12-EH | D11 | D12 | Carbon No. | D5 | D6 | D12-EH | D11 | D12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aglycone | | | | | | | sugars | | | | | |
| C-1 | 76.6 | 89.7 | 90.3 | 90.4 | 89.7 | 90.3 | Ara-1' | 108.1 | | | 108.1 | |
| C-2 | 74.2 | 75.0 | 74.7 | 74.8 | 75.0 | 74.7 | Ara-2' | 74.0 | | | 74.0 | |
| C-3 | 71.3 | 71.7 | 71.7 | 71.7 | 71.7 | 71.5 | Ara-3' | 75.2 | | | 75.2 | |
| C-4 | 35.4 | 35.1 | 35.0 | 35.1 | 35.1 | 35.0 | Ara-4' | 69.9 | | | 69.9 | |
| C-5 | 35.9 | 36.3 | 36.2 | 36.3 | 36.3 | 36.2 | Ara-5' | 67.7 | | | 67.7 | |
| C-6 | 26.5 | 26.1 | 26.0 | 26.0 | 26.0 | 26.0 | Glc-1'' | | 107.6 | 107.7 | | 107.6 |
| C-7 | 26.5 | 26.3 | 26.3 | 26.3 | 26.3 | 26.3 | Glc-2'' | | 76.6 | 76.7 | | 76.6 |
| C-8 | 35.6 | 35.5 | 35.5 | 35.5 | 35.5 | 35.4 | Glc-3'' | | 78.8 | 78.8 | | 78.8 |
| C-9 | 42.1 | 42.0 | 42.0 | 42.0 | 42.0 | 42.1 | Glc-4'' | | 71.5 | 71.6 | | 71.6 |
| C-10 | 41.3 | 40.6 | 41.5 | 41.5 | 40.9 | 41.5 | Glc-5'' | | 78.5 | 78.6 | | 78.5 |
| | | | | | | | Glc-6'' | | 62.8 | 62.9 | | 62.7 |
| C-11 | 21.2 | 21.1 | 21.0 | 21.0 | 21.1 | 21.0 | | | | | | |
| C-12 | 40.1 | 40.0 | 39.9 | 40.0 | 40.0 | 40.0 | Glc-1''' | | | | 104.9 | 104.9 |
| C-13 | 40.6 | 41.5 | 40.5 | 40.6 | 41.5 | 40.9 | Glc-2''' | | | | 75.2 | 75.2 |
| C-14 | 56.2 | 56.1 | 56.1 | 56.1 | 56.0 | 56.0 | Glc-3''' | | | | 78.6 | 78.5 |
| C-15 | 32.2 | 32.1 | 32.0 | 32.1 | 32.3 | 32.3 | Glc-4''' | | | | 71.6 | 71.7 |
| | | | | | | | Glc-5''' | | | | 78.5 | 78.4 |
| C-16 | 81.1 | 81.1 | 81.0 | 81.0 | 81.0 | 81.0 | Glc-6''' | | | | 62.8 | 62.8 |
| C-17 | 63.1 | 63.0 | 63.0 | 63.0 | 64.0 | 63.9 | | | | | | |
| C-18 | 16.6 | 16.6 | 16.5 | 16.6 | 16.7 | 16.7 | | | | | | |
| C-19 | 19.1 | 19.2 | 19.1 | 19.1 | 19.2 | 19.1 | | | | | | |
| C-20 | 42.0 | 42.0 | 41.9 | 41.9 | 40.6 | 40.6 | | | | | | |
| C-21 | 15.1 | 15.1 | 15.0 | 15.1 | 16.5 | 16.5 | | | | | | |
| C-22 | 109.2 | 109.2 | 109.2 | 109.2 | 110.6 | 110.6 | | | | | | |
| C-23 | 31.9 | 31.9 | 31.8 | 31.8 | 37.2 | 37.2 | | | | | | |
| C-24 | 29.3 | 29.2 | 29.2 | 29.2 | 28.4 | 28.3 | | | | | | |
| C-25 | 30.6 | 30.6 | 30.5 | 30.6 | 34.2 | 34.2 | | | | | | |
| C-26 | 66.9 | 66.9 | 66.8 | 66.9 | 75.2 | 75.3 | | | | | | |
| C-27 | 17.3 | 17.3 | 17.4 | 17.3 | 17.4 | 17.4 | | | | | | |

$C_{18}$-Me), 1.38 (3H, s, $C_{19}$-Me), 0.96 (3H, d, $J$ = 6.3 Hz, $C_{27}$-Me), 1.00 (3H, d, $J$ = 6.3 Hz, $C_{21}$-Me), 4.80 (1H, d, $J$ = 8.0 Hz, Glc H-1'), 5.24 (1H, d, $J$ = 8.0 Hz, Glc H-1). $^{13}$C-NMR ($C_5D_5N$, 125 MHz) : Table 3. HR-TOFMS (negative) *m/z* 813.4255 [M+Na], calcd. 813.4249. An aqueous solution of D12 (25 mg) was incubated with almond emulsin (25 mg) at 37°C for 8 hr. The precipitate (D12-EH ; 6 mg) was collected by filtration. D12-EH was identified as compound x (D6) by comparison with the $^{13}$C-NMR spectra for D6 (Table 3).

The aqueous filtrate was evaporated to dryness in vacuo and the residue was examined by silica gel TLC with $CHCl_3$-MeOH-$H_2O$ (6:4:1) and compared with an authentic sample, and glucose ($R_f$ 0.19) was detected.

Furthermore, when the $^{13}$C-NMR spectra of D12 was compared with compound x obtained by the enzymatic hydrolysis of D12 and protocompound x, the signals due to glucose attached to the C1 hydroxyl group of aglycone and C1 to C16, C18 and C19 of aglycone were in good agreement with compound x. In addition, the signals due to glucose attached to the C26 hydroxyl group of aglycone and C17 and C20 to C27 of aglycone were in good agreement with protocompound x, a furostanol-type bisglycoside of tokorogenin (Table 3). Based on the above results, D12 was characterized as 26-*O*-$\beta$-D-glucopyranosyl (25*R*)-1$\beta$,2$\beta$,3$\alpha$,22$\xi$,26-pentahydroxyfurost-1-*O*-$\beta$-D-glucopyranside (protocompound x) (Fig. 1).

Protoyononin and protocompound x were new compounds isolated from natural sources.

Among the compounds isolated from *Dioscorea tokoro*, dioscin, protodioscin, gracillin and protogracillin are distributed in many plants and many aspects of their biological activity have already been reported[15-18]. However, there are no reports on the bioactivities of yononin, protoyononin, protoyonogenin, tokoronin, prototokoronin, tokorogenin, compound x and protocompound x, whose distributions are limited. In the future, we are planning to start bioactivity studies of mainly these compounds.

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**Conflict of interest disclosure**

The author has no conflict of interest.

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