Furanoid Norditerpenes from Dioscorea Plants. VII. 1) Structures of Diosbulbinsides D and F

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Two kinds of furanoid norditerpene glucosides, named diosbulbinsides D (I), mp 169–173° (dec.), [α]D = -31°, and F (II), mp 193–195° (dec.), [α]D = -25°, were isolated from the fresh root tubers of Dioscorea bulbifera L. forma spontanea MAKINO et NEMOTO. On the basis of chemical and spectral evidences I and II were assigned the structures, 6-O-B-D-glucopyranosides of the enol forms, 5-en-6-ols, of coexisting diosbulbins D (IV) and F (V), respectively. They are noted as the non-conjugated enol glucosides.

Keywords—furanoid norditerpene glucoside; non-conjugated enol glucoside; Dioscorea bulbifera L.; bitter principles; snail enzyme; hydrolysis in D2O-CH3OD

In the preceding papers1-3 of this series it was reported that along with two furanoid norditerpenes, diosbulbins A and B, of which structures had previously been established,4) five new analogous compounds, D–H, were isolated and characterized from the root tubers of Dioscorea bulbifera L. forma spontanea MAKINO et NEMOTO (Japanese name, "niga-kashu").

During the course of isolation of these compounds from the methanol extracts of the fresh materials,5) the much more polar fraction ("Fraction B")3) was found on thin-layer chromatogram (TLC) to contain diosbulbin C4) and two new compounds showing similar coloration to those of diosbulbins with anisaldehyde and Ehrlich reagents.

This paper concerns the structure elucidation of the new compounds which were named diosbulbinsides D (I) and F (II).


Both I and II had bitter taste in common with diosbulbins and the former was suggested on infrared (IR) and proton magnetic resonance (PMR) spectra to have hydroxyl, furan, two carbonyl and one tertiary methyl functions, while the latter to have, in addition to the above, one methoxy group. Usually acetylation of I provided the peracetate (III), mp 151–153°, which exhibited on the mass spectrum the characteristic fragment peaks due to the terminal tetra-O-acetyl-hexosyl residue6) and on the PMR spectrum the signals of approximately four acetoxyl groups and a doublet with J = 7 Hz assignable to the anomeric proton of the sugar.

2) Location: 3-1-1 Maedashi, Higashi-ku, Fukuoka, 812, Japan.
5) Dried slices of the root tubers of D. bulbifera L., commercial crude drug available in the Hong-Kong market, are known also to contain diosbulbins,9) but neither diosbulbinside D nor F was detected in their extracts.
unit. Accordingly, I was thought to be a glycoside of furanoid diterpenoid related to diosbulbins and containing one hexose residue. Although an attempted acid hydrolysis of I yielded glucose as detected by paper partition chromatography (PPC) but failed to give a homogeneous aglycone, I was treated with snail enzyme to provide D-glucose and a crystalline substance, mp 229—230°, $[\alpha]_D -49^\circ$, which was identified with diosbulbin D (IV). Taking the behavior on TLC and spectral data into account, II was supposed also to be a glycoside closely related to I, and was hydrolyzed in the same way as for I to give glucose and diosbulbin F (V), mp 211—212°. Since IV is known to be converted into V on alkaline hydrolysis followed by methylation with diazomethane, I was subjected to the same reactions and the sole product was found to be nothing but II. Therefore I and II were thought to have, in situ, the $\delta$-lactone group in their aglycone parts, and, in fact, I was hydrogenated, in the same manner as for IV yielding 8-carboxylic acid (VI) to provide a glycoside (VII), mp 140—141° (dec.), which was composed of glucose and VI.

All the above findings indicate that I and II have one $\beta$-D-glucopyranose unit which is presumed to be combined with 6-hydroxyl derived from the 6-keto group of IV and V, respectively, to form either enol- or hemiacetal glucoside.

The presumption was supported by the IR (Fig. 1) and optical rotatory dispersion (ORD) spectra of I showing neither absorption (1720 cm$^{-1}$) nor Cotton curve (peak at 310 nm) due

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to the ketone carbonyl at C₈ which were observed on those³ of IV. Of the two structures, enol- and hemiacetal glucosides, the former was more likely, because the IR absorptions ascribable to a double bond other than those of furan ring appeared on the spectra of I (1675 cm⁻¹) (Fig. 1), II (1675 cm⁻¹) and VII (1690 cm⁻¹), but not on those⁶ of IV (Fig. 1), V and VI.

Subsequently, in order to confirm the enol structure and to determine either 5-en-6-ol- (I and II) or 6-en-6-ol glucosides (I' and II'), II was hydrolyzed in D₂O-CH₃OD (3:1) with snail enzyme to give a mixture (IX) of V and its monodeutero derivative in the form of colorless needles, mp 224-226°, and the PMR spectra of pure V and IX were compared (Fig. 2). The intensity of the quartet at 2.07 ppm observed in V and assigned⁶ to 5β-H was much decreased in IX and the splitting patterns of signals due to 10α-H and 4β-H both being vicinal to 5β-H in V were distinctly deformed in IX. On the other hand the signals attributable to the protons at C₇ and C₈ of V appeared unchanged in IX. This result indicates that deuterium is introduced at C₅ during the enol-keto transformation accompanying the cleavage of glucosidic linkage, and that the aglycone moiety of II as well as I has the 5-en-6-ol and not 6-en-6-ol system.

In consequence I and II are formulated respectively as 6-O-β-d-glucopyranosides of 5-en-6-ols tautomeric to IV and V.

A number of glycosides which have the sugar moiety linked to the hydroxyl group of conjugated enol have been documented: for example, indoxyl glucoside, cucurbitacin 2-O-glucosides,⁸ flavonol- and anthocyanidin 3-O-glycosides. However, I and II represent rather novel type of enol glycoside and seem to be noteworthy.

![Chart 1](image)

**Experimental**

Melting points were determined on a YANACO micromelting point apparatus MP-S3 and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter and ORD and CD spectra were

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measured with JASCO ORD/UV-5 recording spectropolarimeter at 23–26°. IR spectra were obtained in KBr disk with a JASCO IR-G spectrometer, and PMR spectra were taken at 100 MHz on a JEOL PS-100 spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 6 kV, ionizing potential of 30 eV and a source temperature of 140°. PPC was conducted on Tōyō Rōshi No. 50 in double ascending method using the upper layer of BuOH–pyridine–water (6:2:3) + pyridine (1) as solvent and aniline hydrogen phthalate for staining. TLC was performed on Kieselgel nach Stahl Type 60 (Merck) using anisaldehyde and Ehrlich reagents as the detector.9 The ratio of solvents in mixture are given in v/v.

Isolation of Diosbulbinosides D (I) and F (II)—Fr. B (12.3 g) in column chromatography of the BuOH soluble part of MeOH extractives of the fresh root tubers (32 kg) described in the preceding paper9 was passed through a silica gel column (250 g) by using CHCl₃–MeOH–water (80:20:2) as eluent. Under monitoring by TLC (solvent, CHCl₃–MeOH–water (70:30:5)), the less polar and more polar (containing diosbulbin C49 and others unidentified) fractions were removed and those containing I and II (RF 0.47 and 0.37, respectively, reddish purple with Ehrlich reagent) were combined. Removal of coloring matters, enrichment and separation of I and II were carried out by successive chromatographies over Sephadex LH-20 (elucent, MeOH) (three times) and silica gel (twenty times quantity of the material) (eluents, CHCl₃–MeOH (85:15) and CHCl₃–MeOH–water (85:15:1) (three times) followed by crystallization from CHCl₃–MeOH. I: yield 970 mg, colorless needles having a bitter taste, mp 169–173° (dec.), [α]D -31° (c=0.8, methanol). ORD ε=1.09, methanol [α] (nm): +1210° (220), 0° (226), -1700° (238) (trough), -1080° (250), -566° (260), -285° (250), -154° (300), -44° (350), -36° (400). IR νmax cm⁻¹: 3500–3400 (OH), 1504, 874 (furan), 1766 (C=O), 1750 (C=O), 1675 (C=C) (Fig. 1). PMR (CD2OD): 0.98 (3H, s, tert-Me), 4.24 (1H, d, J=7 Hz), 4.68 (1H, d, J=7 Hz), 5.05 (1H, m), 5.54 (1H, q, J=4, 12 Hz), 6.57, 7.55, 7.63 (1H each). Anal. Calcd. for C₁₈H₂₄O₇·2H₂O·C; 55.34% C, 56.32% H, 5.99. II: yield 130 mg, colorless needles having a bitter taste, mp 193–198° (dec.), [α]D -25° (c=0.29, methanol). IR νmax cm⁻¹: 3500–3400 (OH), 1506, 874 (furan), 1740 (C=O), 1707 (COOR), 1675 (C=C). PMR (CD2OD): 0.90 (3H, s, tert-Me), 5.74 (OH, s, OMe), 5.50 (1H, q, J=6, 12 Hz), 6.56, 7.53, 7.62 (1H each). Anal. Calcd. for C₁₈H₂₄O₇·2H₂O; 53.43% C, 56.74% H, 6.52. Found: C, 53.43; H, 6.52.

I Peracetate (III)—I (100 mg) was acetylated with acetic anhydride (1 ml) and pyridine (3 ml) at room temperature overnight. The product was purified by passing through a silica gel column (12 g) (eluents, AcOEt–hexane (2:1)) to give III (98 mg) as a white powder (mp 151–153°) (from MeOH). IR νmax cm⁻¹: no OH, 3150, 1507, 876 (furan), 1780 (C=O), 1750–1730 (C=O, AcO), 1685 (C=C). PMR (CDCl₃): 0.95 (3H, s, tert-Me), 2.03 and 2.09 (12–15H, AcOx×4–5), 3.75 (1H, m), 4.01 (1H, d, J=5 Hz), 4.27 (2H, d, J=3 Hz), 4.78 (1H, d, J=7 Hz, anomeric proton of sugar), 4.93 (1H, t, J=6 Hz), 5.41 (1H, q, J=5, 12 Hz), 6.42, 7.41, 7.46 (1H each). MS m/z: 674 (M⁺, C₁₈H₂₄O₇), 344 (M⁺ of IV, C₁₈H₂₂O₈), 331, 229, 169, 127, 109 (fragments due to terminal acetylated hexose unit).9 Anal. Calcd. for C₁₈H₂₄O₇·2H₂O: 58.75% C, 56.79% H. Found: C, 58.95; H, 6.03.

Hydrolysis of I—a) With Acid: I (30 mg) was boiled with 1 N H₂SO₄ in 50% aqueous MeOH (10 ml) for 30 min. Water being added, the precipitates were collected by filtration and the filtrate was concentrated in vacuo. The former showed several spots on TLC and separation by chromatography over silica gel was in failure. The latter was examined by PPC to show the single spot of glucose.

b) With Snail Enzyme: I (300 mg) was incubated with snail enzymeb (22 mg) in 20% aqueous MeOH (40 ml) at 37° for 20 hr. The precipitates were collected and chromatographed over silica gel (8 g) (eluents, CHCl₃–MeOH (97:3)) to give a homogeneous (TLC) compound (170 mg), which was crystallized from MeOH to provide colorless plates, mp 229–230°, [α]D -49° (c=0.1, methanol). It was identified with diosbulbin D (IV)9 by comparisons of spectral data and by mixed melting point and co-chromatography with an authentic sample. The filtrate of hydrolysate was passed through a Sephadex LH-20 (10 g) column (eluents, MeOH) and evaporated in vacuo to yield a colorless syrup (78 mg), [α]D +50.5° (c=3.3, MeOH), RF (PPC) 0.47, identical to d-glucone.

Hydrolysis of II with Enzyme—II (50 mg) was hydrolyzed and worked up in the same way as above to give glucose (PPC) and a solid substance (23 mg). The latter was crystallized from MeOH to afford colorless needles, mp 211–212°, identical to diosbulbin F (V).9

Conversion of I into II—I (35 mg) was stirred with 5% NaOH in 50% aqueous MeOH (10 ml) at room temperature for 30 min. The reaction mixture was neutralized with 1% H₂SO₄, concentrated in vacuo, and chromatographed over Sephadex LH-20 (10 g) (eluents, MeOH). Colorless syrup (30 mg) thus obtained was treated in MeOH (10 ml) with CH₃CN in ether. Solvent was evaporated and the residue (30 mg) was crystallized from MeOH to give colorless needles, mp 193–195° (dec.), identical to II in every respect (IR and PMR spectra, mixed melting point, co-chromatography on TLC).

Hydrogenation of I to Carboxylic Acid Glucoside (VII) —— I (180 mg) was hydrogenated over 5% Pd-BaSO₄ (90 mg) in dioxane (20 ml). Catalysts were removed by filtration and the filtrate was evaporated in vacuo to dryness. The residue was chromatographed over silica gel (23 g) (eluent, CHCl₃-MeOH—water (85: 15: 0.5)). The fractions showing single spot (Rf 0.35, reddish purple with Ehrlich reagent) on TLC (solvent, CHCl₃-MeOH—water (70: 30: 5)) were combined, evaporated and the residue (65 mg) was crystallized from MeOH to give VII as colorless prisms, mp 140—141° (dec.). IR ν max cm⁻¹: 3500—3300 (OH), 2800—2700, 1720 (COOH), 1768 (C=O), 1690 (C=O), 1504, 875 (furan).

Hydrolysis of VII with Enzyme —— VII (61 mg) was incubated with snail enzyme (14 mg) and worked up in the same way as for I. The filtrate of the hydrolysate showed a single spot of glucose on PFC and the precipitates were chromatographed over silica gel (11 g) (eluent, CHCl₃-MeOH—water (90: 10: 0.5)) to give a homogeneous substance (28 mg). It was crystallized from CHCl₃-MeOH to afford colorless prisms, mp 248—253° (dec.), which were identified with 8-carboxylic acid (VI) corresponding to IV by mixed melting point and comparisons of their spectra.

Hydrolysis of II with Enzyme in D₂O-CH₃DO —— Snail enzyme (16 mg) was kept in D₂O (5 ml) overnight at room temperature and the solution of II (70 mg) in CH₃DO—D₂O (3: 2) (2.5 ml) was added. The mixture was shaken at 37° for 46 hr. The solvent was removed in vacuo, the residue was extracted with CHCl₃, and the soluble part was chromatographed over silica gel (5 g) (eluent, CHCl₃-MeOH (95: 5)) to give a mass (32 mg), which was crystallized from MeOH to provide a mixture (IX) of V and its monodeutero derivative as colorless needles, mp 224—226°. PMR (Fig. 2). MS m/z: 377 (M⁺, C₂₆H₆₅DO₇), 376 (M⁺, C₂₆H₆₄O₇, intensity relative to that of m/z 377 being about 0.7).

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