[Chem. Pharm. Bull. 26(2) 435—439 (1978)]

UDC 547.918.02 : 581.192

Furanoid Norditerpenes from Dioscorea Plants. VII.[1] Structures of Diosbulbinosides D and F

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(Received June 20, 1977)

Two kinds of furanoid norditerpene glucosides, named diosbulbinosides D (I), mp 169—173° (dec.), $[\alpha]_D$ −31°, and F (II), mp 193—195° (dec.), $[\alpha]_{350}$ −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-β-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 D_2O–CH_3OD

In the preceding papers[1,3] 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 C[4b] 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 diosbulbinosides D (I) and F (II).

Repeated chromatographic separation of the constituents of "Fraction B" over silica gel and Sephadex LH-20 followed by crystallization of the resulting homogeneous fractions from a chloroform–methanol mixture afforded I as colorless needles, mp 169—173° (dec.), $[\alpha]_D$ −31°, and II, colorless needles, mp 193—195° (dec.), $[\alpha]_{350}$ −25°.

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 methoxyl group. Usual 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 residue[6] 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

1) Part VI: Y. Ida, T. Komori, and T. Kawasaki, *Ann. Chem.*, in press.
2) Location: *3-1-1 Maedashi, Higashi-ku, Fukuoka, 812, Japan.*
3) Y. Ida, S. Kubo, M. Fujita, T. Komori, and T. Kawasaki, *Ann. Chem.*, in, press.
4) *a*) K. Kamiya, Y. Wada, T. Komori, M. Arita, and T. Kawasaki, *Tetrahedron Lett.*, **1972**, 1869; T. Komori, T. Kawasaki, K. Kamiya, and Y. Wada, *Chem. Pharm. Bull.* (Tokyo), **25**, 1701 (1977); *b*) T. Komori, M. Arita, Y. Ida, T. Fujikura, T. Kawasaki, and K. Sekine, *Ann. Chem.*, **1973**, 978.
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,[3] but neither diosbulbinoside D nor F was detected in their extracts.
6) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 204; Y. Ida, T. Komori, T. Kawasaki, K. Yoshida, and K. Kato, *Yakugaku Zasshi*, **91**, 119 (1971).

unit.[7] 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°, which was identified with diosbulbin D (IV).[3] 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),[3] mp 211—212°. Since IV is known to be converted into V on alkaline hydrolysis followed by methylation with diazomethane,[3] 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 δ-lactone group in their aglycone parts, and, in fact, I was hydrogenated, in the same manner as for IV yielding 8-carboxylic acid (VI),[3] 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 β-D-glucopyranose[7] 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

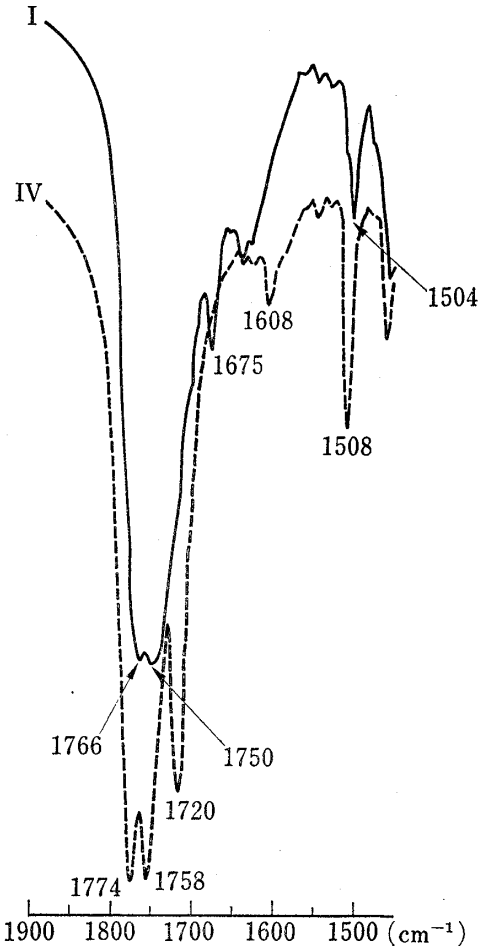

Fig. 1. IR Spectra of I and IV (KBr Disk)

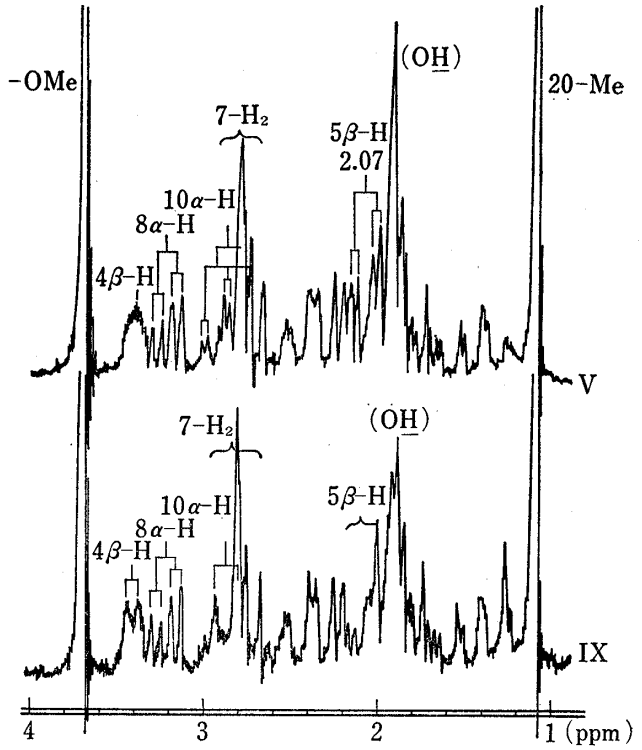

Fig. 2. PMR Spectra of IX and V ($CDCl_3$)

7) 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. Tsuzuki, and Y. Tsuzuki, *Bull. Chem. Soc. Jpn.*, **36**, 1048 (1963); K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1735 (1969).

to the ketone carbonyl at C_6 which were observed on those[3] 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 those[3] 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 D_2O–CH_3OD (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 assigned[3] 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 C_7 and C_8 of V appeared unchanged in IX. This result indicates that deuterium is introduced at C_5 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.

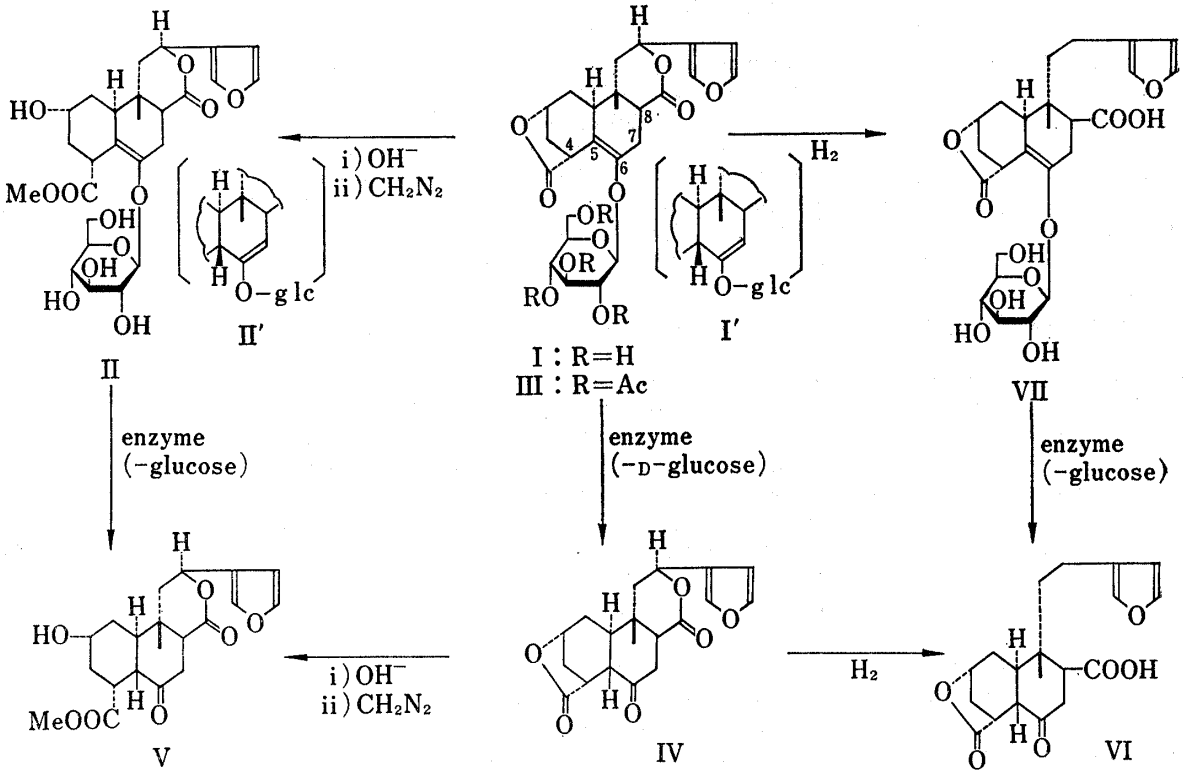

Chart 1

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

8) H. Ripperger, *Tetrahedron*, **32**, 1567 (1976).

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 Tôyô Roshi 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.[3] Column chromatography was carried out with Kieselgel (0.05—0.2 mm) (Merck). The ratios 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 paper[3] was passed through a silica gel column (250 g) by using $CHCl_3$–MeOH–water (80: 20: 2) as eluent. Under monitoring by TLC (solvent, $CHCl_3$–MeOH–water (70: 30: 5)), the less polar and more polar (containing diosbulbin C[4b] 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 final separation of I and II were carried out by successive chromatographies over Sephadex LH-20 (eluent, MeOH) (three times) and silica gel (twenty times quantity of the material) (eluents, $CHCl_3$–MeOH (85: 15) and $CHCl_3$–MeOH–water (85: 15: 1) (three times) followed by crystallization from $CHCl_3$–MeOH. I: yield 970 mg, colorless needles having a bitter taste, mp 169—173° (dec.), $[\alpha]_D$ −31° (c=0.8, methanol). ORD (c=0.09, methanol [α] (nm): +1210° (220), 0° (226), −1700° (238) (trough), −1080° (250), −566° (260), −285° (280), −154° (300), −44° (350), −36° (400). IR ν_{max} cm^{-1}: 3500—3400 (OH), 1504, 874 (furan), 1766 (C=O), 1750 (C=O), 1675 (C=C) (Fig. 1). PMR (CD_3OD): 0.98 (3H, s, *tert*-Me), 4.24 (1H, d, J=4.5 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 $C_{25}H_{30}O_{11}\cdot 2H_2O$: C, 55.34; H, 6.32. Found: C, 55.55; H, 5.99. II: yield 130 mg, colorless needles having a bitter taste, mp 193—195° (dec.), $[\alpha]_{350}$ −25° (c=0.29, methanol). IR ν_{max} cm^{-1}: 3500—3400 (OH), 1505, 874 (furan), 1740 (C=O), 1707 (COOR), 1675 (C=C). PMR (CD_3OD): 0.90 (3H, s, *tert*-Me), 3.74 (3H, s, OMe), 5.53 (1H, q, J=6, 12 Hz), 6.56, 7.53, 7.62 (1H each). *Anal.* Calcd. for $C_{26}H_{34}O_{12}\cdot 2.5H_2O$: C, 53.51; H, 6.74. 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) (eluent, AcOEt–hexane (2: 1)) to give III (98 mg) as a white powder (mp 151—153°) (from MeOH). IR ν_{max} cm^{-1}: no OH, 3150, 1507, 876 (furan), 1780 (C=O), 1750—1730 (C=O, AcO), 1685 (C=C). PMR ($CDCl_3$): 0.95 (3H, s, *tert*-Me), 2.03 and 2.09 (12—15H, AcO×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),[7] 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/e*: 674 (M^+, $C_{33}H_{38}O_{15}$), 344 (M^+ of IV, $C_{19}H_{20}O_6$), 331, 229, 169, 127, 109 (fragments due to terminal acetylated hexose unit).[8] *Anal.* Calcd. for $C_{33}H_{38}O_{15}$: C, 58.75; H, 5.69. Found: C, 58.95; H, 6.03.

Hydrolysis of I——a) With Acid: I (30 mg) was boiled with 1 N H_2SO_4 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 enzyme[9] (22 mg) in 20% aqueous MeOH (40 ml) at 37° for 20 hr. The precipitates were collected and chromatographed over silica gel (8 g) (eluent, $CHCl_3$–MeOH (97: 3)) to give a homogeneous (TLC) compound (170 mg), which was crystallized from MeOH to provide colorless plates, mp 229—230°, $[\alpha]_D$ −49° (c=0.1, methanol). It was identified with diosbulbin D (IV)[3] 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 (eluent, MeOH) and evaporated *in vacuo* to yield a colorless syrup (78 mg), $[\alpha]_D$ +50.3° (c=3.3, MeOH), *Rf* (PPC) 0.47, identical to D-glucose.

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).[3]

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% H_2SO_4, concentrated *in vacuo*, and chromatographed over Sephadex LH-20 (10 g) (eluent, MeOH). Colorless syrup (30 mg) thus obtained was treated in MeOH (10 ml) with CH_2N_2 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).

9) Prepared according to the reported procedure (O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **34**, 68 (1951); A. Okano, K. Hoji, T. Miki, and K. Miyatake, *Pharm. Bull.* (Tokyo), **5**, 167 (1957).

Hydrogenation of I to Carboxylic Acid Glucoside (VII)——I (180 mg) was hydrogenated over 5% Pd–$BaSO_4$ (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, $CHCl_3$–MeOH–water (85: 15: 0.5)). The fractions showing single spot (*Rf* 0.35, reddish purple with Ehrlich reagent) on TLC (solvent, $CHCl_3$–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=C), 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 PPC and the precipitates were chromatographed over silica gel (11 g) (eluent, $CHCl_3$–MeOH–water (90: 10: 0.5)) to give a homogeneous substance (28 mg). It was crystallized from $CHCl_3$–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 D_2O-CH_3DO——Snail enzyme (16 mg) was kept in D_2O (5 ml) overnight at room temperature and the solution of II (70 mg) in CH_3DO—D_2O (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 $CHCl_3$, and the soluble part was chromatographed over silica gel (5 g) (eluent, $CHCl_3$–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/e*: 377 (M^+, $C_{20}H_{23}DO_7$), 376 (M^+, $C_{20}H_{24}O_7$, intensity relative to that of *m/e* 377 being about 0.7).

Acknowledgement The authors thank Dr. T. Nohara for PMR measurements, Miss M. Kawamura for mass spectra, and members of the Central Analysis Room of this University for microanalysis.