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Furanoid Norditerpenes from Dioscoreaceae Plants. I. Diosbulbins A, B and C from *Dioscorea bulbifera* L. *forma spontanea* Makino et Nemoto

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Three nonsteroidal compounds, named diosbulbins A, B and C, were isolated from the root tubers of *Dioscorea bulbifera* L. *forma spontanea* Makino et Nemoto (a wild type of *D. bulbifera* L.) in which scarcely steroid sapogenin was found. Diosbulbin A, $C_{20}H_{24}O_7$, mp 265° (decomp.), $[\alpha]_D^{20}$ +26.7°, B, $C_{19}H_{20}O_6$, mp 285° (decomp.), $[\alpha]_D^{20}$ +92.0° and C, $C_{19}H_{22}O_7$, mp 247—250° (decomp.), $[\alpha]_D^{20}$ +64.7°, all having bitter taste, were assigned the partial structural formulae III, I, II, respectively, and regarded as a series of novel type of furanoid norditerpenes. These are first terpenoids reported in Dioscoreaceae and first furanoid diterpenoids in Monocotyledoneae.

The Dioscoreaceae plants are known as the source of diosgenin which is contained mainly in the underground parts in the form of its glycosides (steroid saponins).[2] However, there are some species in japanese Dioscoreaceae plants which have been reported to contain neither diosgenin nor other steroid sapogenins.[2b,3] In the previous study[3] in this laboratory on the root tubers of *Dioscorea bulbifera* L. *forma spontanea* Makino et Nemoto (Japanese name, *"niga-kashu"*)[4] any steroid sapogenin was not found,[5] but, instead, a nonsteroidal, nonglycosidal and nitrogen-free substance was obtained as a crystal separated out from the methanol extract. At that time the substance was thought to be homogeneous, but later examination by means of thin-layer chromatography showed it to consist of three compounds which are positive to both Ehrlich and anisaldehyde reagents.[6] This paper deals with the reinvestigation of the root tubers which has led to the isolation of the three compounds and to the characterization as furanoid norditerpenes closely related to each other.

The sample, mp 275—287° (decomp.), which had been obtained previously from the root tubers collected in October in Oosumi peninsula (Kagoshima prefecture), was shown on thin-layer of silica gel G to be a mixture of three compounds, *Rf* 0.34, 0.25 and 0.02 (solvent, ethyl acetate–cyclohexane 96:4). They were named in order of increasing polarity diosbulbins A, B and C. Extraction was duplicated with the root tubers from the same place in October and the extract was evaporated and treated again with methanol to give a crude crystal. It was recrystallized from methanol to provide colorless plates, mp 275—285° (decomp.) which showed the identical pattern with that of the previous sample on thin-layer chromatography. Separation of the components was then tried and taking advantage

1) Location: *1276, Katakasu, Fukuoka.*
2) *a*) A. Akahori, *Shionogi Kenkyusho Nempo*, **11**, 93 (1961); **10**, 1411 (1960); *b*) *Idem.*, *Phytochemisry*, **4**, 97 (1965).
3) T. Tsukamoto, T. Kawasaki, T. Yamauchi and J. Korenaga, *Kyushu Yakugakukai Kaiho*, **11**, 51 (1955).
4) A wild type of *D. bulbifera* L. of which root tuber tastes bitter. A cultivated one is called *D. bulbifera* L. *forma domestica* Makino et Nemoto (Japanese name, *"kashu-imo"*) and its root tuber does not taste bitter; J. Ohwi, "Flora of Japan," Shibundo, Tokyo, 1965, p. 387; T. Makino, "New Illustrated Flora of Japan," Hokuryukan Co., Ltd., Tokyo, 1966, p. 866.
5) In the present reinvestigation a minute amount of diosgenin was detected in glycoside fraction.
6) E. Stahl, "Dünnschicht-Chromatographie," Springer-Verlag, Berlin, 1962, p. 498, 503.

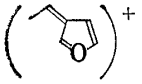
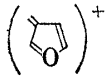
of the different solubility in ethyl acetate the main, diosbulbin B, was obtained from the less soluble fraction in pure form as colorless plates, mp 285° (decomp.), $[\alpha]_D^{20}$ +92.0°, and diosbulbin A from the soluble part in a small amount as colorless needles, mp 265° (decomp.), $[\alpha]_D^{20}$ +26.7°. Diosbulbin C, though detected on chromatogram, could not be isolated. When the extractives of the root tubers collected in the suburbs of Fukuoka city in September were fractionated and examined by thin-layer chromatography, diosbulbins A, B and C were also detected,[7] and the major constituent B was easily obtained in a pure form.

The commercial crude drug, "*oo-yaku-si*," which is regarded[8] as dried slices of the root tubers of *D. bulbifera* L. was subsequently examined in the analogous way as above. The material from Hong-Kong market which was quite similar in appearance and taste[4] to that prepared from the domestic root tubers was found to contain equally diosbulbins A, B and C, and in addition to the considerable amount of B, diosbulbin C was successfully isolated in a homogeneous state as colorless plates, mp 248°(decomp.).

Diosbulbin B, the main constituent in all materials so far examined, analysed for $C_{19}H_{20}O_6$ (mol. wt. 344, Mass). The positive Ehrlich reaction and the spectral data, infrared (IR) absorptions[9] at 3130, 1511, 877, 761 cm^{-1} and ultraviolet (UV) at 210 mμ (ε=6020),[10] suggested a β-substituted furan function in the molecule and the mass spectrum showing two fragment ions, *m/e* 94 and 81, which are characteristic ones common in the spectra of marrubiin and columbin and assigned the formulae $C_6H_6O^+$,[11] and $C_5H_5O^+$,[12] respectively,[13] indicated the presence of 2-(β-furyl)-ethylene grouping. The IR absorption (1782 cm^{-1}, broad) and titration with sodium hydroxide suggested tow γ-lactone rings in the molecule and the remaining one oxygen atom was considered to be that of ether from the IR spectral data (1072, 1063 cm^{-1}). The nuclear magnetic resonance (NMR) spectrum showed the signals of one tertiary methyl group (1.08 ppm, 3H, singlet), two hydrogens (4.69 ppm, 2H) on carbon(s) bearing a lactone ether oxygen, one (5.31 ppm, 1H) on carbon carrying ether oxygen and three (7.02 ppm, 1H; 7.45, 1H; 7.72, 1H) on a β-substituted furan ring. When B was completely reduced by catalytic hydrogenation followed by lithium aluminum hydride reduction and the product was subjected to selenium dehydrogenation, 1,2,5-trimethylnaphthalene was afforded as the major product, while direct dehydrogenation of B with selenium yielded mainly 1-methylnaphthalene.

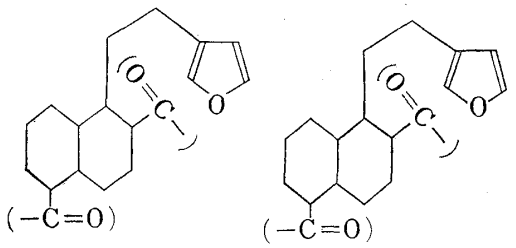
On the basis of the above results diosbulbin B is assigned the partial structure I.

Diosbulbin A, a minor constituent in the root tubers from Oosumi had the molecular formula $C_{20}H_{24}O_7$ (mol.wt. 376, Mass; one methoxyl, Zeisel) and, in common with B, the spectral and chemical data indicated the presence of β-substituted furan, γ-lactone, ether and tertiary methyl groups. However it was different from B in IR (3410, 1793 sharp, 1698 cm^{-1}) and NMR (3.78 ppm, 3H, singlet; 4.03, 1H; 4.70, 1H) spectra which suggested the presence of hydroxyl and methoxycarbonyl groups and one γ-lactone ring. In view of the probable interrelation of A and B, the hydrolytic opening of one of the two γ-lactone rings in B was tried and on treatment with sodium hydroxide in 50% pyridine at room temperature followed

7) Additional three compounds were detected. They were positive to anisaldehyde reagent but negative in Ehrlich reaction, located on thin-layer between B and C, and tentatively named diosbulbins B_1, B_2 and B_3 according to their increasing polarity. *cf.* experimental part.
8) A. Akahori, *Shionogi Kenkyusho Nempo*, **13**, 71 (1963).
9) T. Kubota, *Tetrahedron*, **4**, 68 (1958).
10) T. Tokoroyama, *Nippon Kagaku Zasshi*, **79**, 316 (1958).
11) 
12) 
13) R.I. Reed and W.K. Reid, *J. Chem. Soc.*, **1963**, 5933.

by acidification, B was converted to a monohydroxy monocarboxylic acid, named diosbulbinic acid, mp 247—250° (decomp.), $[\alpha]_D^{20}$ +64.7°, $C_{19}H_{22}O_7$ (mol.wt. 362, Mass). It had, besides a hydroxyl and a carboxyl functions, a β-substituted furan ring, an ether and a tertiary methyl group and a γ-lactone ring as evidenced by spectral data and can be formulated as II. The carboxylic acid (II) was methylated with diazomethane to give the corresponding methylester (III), mp 265° (decomp.), $[\alpha]_D^{20}$ +26.4°, which was identical in all respects with A. Therefore diosbulbin A is represented by the partial formula III.

Diosbulbin C which was isolated in a minute amount from *"oo-yaku-si"* showed the same melting point and *Rf* values as those of diosbulbinic acid (II) and the identity was proved by mixed melting point determination and co-chromatography on thin-layer.



1 *tert*-Me, 2 γ-lactone, 1 ether

1 *tert*-Me, 1 γ-lactone, 1 OH, 1 COOR, 1 ether

diosbulbin B (I) diosbulbin C (II): R=H (diosbulbinic acid)
diosbulbin A (III): R=Me

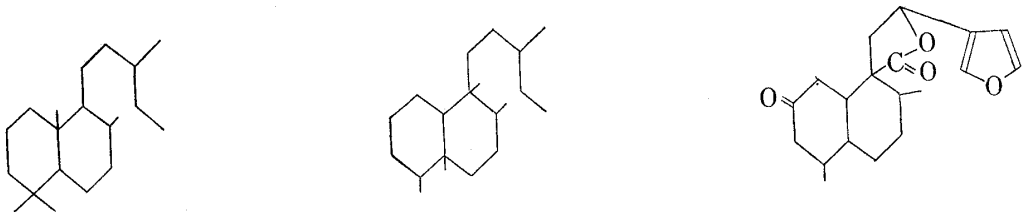
Chart 1

Consequently, diosbulbins A, B and C are regarded as a series of novel type of furanoid norditerpenes[14] possibly related to the known furanoid diterpenes[13,15-25] which possess the secopimarane (labdane) skeleton (IV) or its modification (V).

Since these compounds were also found in acetone extract of the fresh tubers and their inter-conversion on treatment with methanol could not be effected, none of them is thought to be an artefact formed during the procedures of extraction and purification. Diosbulbins B, A and, in particular, C taste bitter and are likely to be responsible for the bitterness[4] of the root tubers.

As the constituents of Dioscoreaceae plants, spirostanols, sterols,[2,3] alkaloids, polyphenols (tannins, anthocyanins[26]), carbohydrates and others have been recorded,[27] but diosbulbins A, B and C are first terpenoids reported in this family. To our knowledge about twenty furanoid diterpenes have appeared in the literatures, however, except a few in Pinaceae[15]

14) Only one furanoid norditerpene (crotonin (VI)) has been reported (ref. 20*b*). It is structurally related to the cascarillins (ref. 20*a*) of modified secopimarane series.
15) W.G. Dauben and V.F. German, *Tetrahedron*, **22**, 679 (1966).
16) M. Sumimoto, *Tetrahedron*, **19**, 643 (1963).
17) M.P. Cava, W.R. Chan, L.J. Haynes, L.F. Johnson and B. Weinstein, *Tetrahedron*, **18**, 397 (1962).
18) K.W. Gopinath, T.R. Govindachari, P.C. Parthasarathy and N. Viswanathan, *Helv. Chim. Acta*, **44**, 1040 (1961).
19) J.T. Pinhey and R.F. Simpson, *Chem. Commun.*, **1967**, 9; L. Canonica, B. Rindone, C. Scolastico, G. Ferrari and C. Casagrande, *Tetrahedron Letters*, **1967**, 2639.
20) *a*) J.M. Robertson, *Proc. Chem. Soc.*, **1963**, 235; T.G. Halsall, A.W. Oxford and W. Rigby, *Chem. Commun.*, **1965**, 218; *b*) W.R. Chan, D.R. Taylor and C.R. Willis, *ibid.*, **1967**, 191.
21) R.A. Appleton, J.W.B. Fulke, M.S. Henderson and R. McCrindle, *J. Chem. Soc.* (C), **1967**, 1943; C.H. Brieskorn and T. Pfeuffer, *Chem. Ber.*, **100**, 1998 (1967).
22) R. Misra, R.C. Pandey and Sukh Dev, *Tetrahedron Letters*, **1964**, 3751; W. Cocker, A.L. Moore and A.C. Pratt, *ibid.*, **1965**, 1983; J. Haeuser and R. Lombard, *Tetrahedron*, **12**, 205 (1961); F.E. King, D.H. Godson and T.J. King, *J. Chem. Soc.*, **1955**, 1117; L. Canonica, G. Jommi, P. Manitto and F. Pelizzoni, *Tetrahedron Letters*, **1963**, 2079.
23) S.K. Balasubramanian, D.H.R. Barton and L.M. Jackman, *J. Chem. Soc.*, **1962**, 4816; T. Hori, A.K. Kiang, K. Nakanishi, S. Sasaki and M.C. Woods, *Tetrahedron*, **23**, 2649 (1967); K.H. Overton, N.G. Weir and A. Wylie, *J. Chem. Soc.*, **1966**, 1482; D.H.R. Barton and D. Elad, *ibid.*, **1956**, 2085, 2090; K.K. Cheung, D. Melville, K.H. Overton, J.M. Robertson and G.A. Sim, *J. Chem. Soc.* (B), **1966**, 853; G.A. Santos, *Chem. Ind.* (London), **1965**, 1074.
24) P.R. Jefferies and T.G. Payne, *Tetrahedron Letters*, **1967**, 4777.
25) D.H.R. Barton, H.T. Cheung, A.D. Cross, L.M. Jackman and M. Martin Smith, *J. Chem. Soc.*, **1961**, 5061.



secopimarane (Labdane) skelton (IV) modified secopimarane skeleton (V) crotonin (VI)

Chart 2

and Taxodiaceae[16] of Gymnospermae, all occur in Dicotyledoneae (Acanthaceae,[17] Anonaceae,[18] Compositae,[19] Euphorbiaceae,[20] Labiatae,[21] Leguminosae,[22] Menispermaceae,[23] Sapindaceae,[24] Verbenaceae[25]) and it is noteworthy that a novel type of furanoid diterpenoids are found in Dioscoreaceae, Monocotyledoneae.

Experimental

Melting points were taken on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-SL automatic polarimeter. UV spectra were recorded on a Shimadzu RSF-2A and IR spectra on a KOKEN BS-301 spectrometers. NMR spectra were determined on a JEOL JNM-C-60H (60 Mc) and a JEOL JNM-4H-100 (100 Mc) spectrometers with tetramethylsilane as internal standard. Mass spectra were determined on a Atlas CH-4 and a Hitachi RMU-6D spectrometers. *Rf* values refer to thin-layer chromatography (TLC) on silica gel G (Merck) using (a) the bottom layer of chloroform–methanol–water (7:3:1), (b) ethylacetate–ethanol–cyclohexane (20:2:1) and (c) ethylacetate–cyclohexane (96:4) as developing solvents and (1) Ehrlich reagent[6] and (2) anisaldehyde reagent[6] for staining. Gas liquid chromatography (GLC) was performed with Shimadzu gas chromatograph GC-1C equipped with hydrogen flame ionization detector.

Extraction and Separation of Diosbulbins——a) The root tubers of *D. bulbifera* L. *forma spontanea* Makino et Nemoto collected in Oosumi peninsula, Kagoshima prefecture, in October were sliced, air-dried, powdered and the material (1.86 kg) was extracted with boiling methanol (5 liters) for 30 hr. The extract was filtered while hot and the filtrate was evaporated to give a resinous mass, which was dissolved in boiling methanol (450 ml), treated with active charcoal and filtered. The filtrate was left stand in a refrigerator overnight, the precipitates were collected and recrystallized twice from methanol to give colorless plates (2 g), mp 275—285° (decomp.), *Rf* values: 0.34 (diosbulbin A), 0.25 (B), 0.02 (C) (solv. c); 0.39 (A), 0.30 (B), 0.02 (C) (solv. b); 0.83 (A), 0.76 (B), 0.15 (C) (solv. a) (all showed reddish purple with reagent 1 and purple with 2). The chromatogram was identical with that of the previous sample,[3] mp 275—287° (decomp.), run in parallel. The crystals were treated with hot acetone (2 liters), insoluble substance was removed by filtration and the filtrate was concentrated to 1/3 volume and left stand overnight. The precipitates were collected and fractionated wih hot ethyl acetate. The less soluble fraction was recrystallized repeatedly from acetone to give diosbulbin B as colorless plates (1.043 g), while the soluble part was recrystallized from ethyl acetate to provide A as colorless needles (0.132 g).

b-1) The material (2.1 kg) prepared as in a) from the tubers collected in the suburbs of Fukuoka city in September was extracted with boiling methanol (4 liters) for 34 hr and the extract was left stand in a refrigerator. The precipitates (9 g) formed were collected by filtration and the filtrate was evaporated to a resinous mass. The residue was examined by TLC. *Rf* values: 0.83 (diosbulbin A), 0.76 (B), 0.53 (B_1), 0.46 (B_2), 0.39 (B_3), 0.15 (C) (solv. a). The precipitates were taken up in hot acetone, the solution was treated with charcoal and filtered. The acetone solution gave, on cooling, colorless crystals which were filtered off and recrystallized from acetone to yield B (1.339 g).

b-2) The fresh root tubers (1 kg) collected in October in the same place as above was extracted with acetone (2 liters) at room temperature for 10 days. Acetone solution was concentrated, left stand and the precipitates were recrystallized from acetone to give pure B (1.6 g). The mother liquor was shown on thin-layer chromatgram to contain diosbulbins A, B and C accompanied by B_1, B_2 and B_3.

26) V. Rasper and D.G. Coursey, *Experientia*, 23, 611 (1967).
27) R. Hegnauer, "Chemotaxonomie der Pflanzen," Bd. II, Birkhäuser Verlag, Basel and Stuttgart, 1963, p. 133.

c) Powdered *"oo-yaku-si"* (3 kg) was extracted with boiling methanol (5 liters) for 33 hr and the extract was left stand overnight at room temperature. The crystals separated out were collected by filtration, the filtrate was concentrated to 1/5 volume and allowed to stand in a refrigerator to yield another crop of crystals. The mother liquor was evaporated to give a resinous mass (A, B, C, B_1, B_2 and B_3 were detected on thin-layer), while the crystals were combined and recrystallized from acetone to afford pure B (3.6 g). The residue was treated with acetone–ethyl acetate (2:1) mixture, the soluble part (about 40 g) was placed on a silica gel (Kanto, 750 g) column and eluted successively with hexane (1 liter), ethyl acetate (2 liters) and the bottom layer (2 liters) of chloroform–methanol–water (7:3:1). One of the fractions eluted with the last solvent afforded diosbulbin C as colorless plates (from acetone), mp 248° (decomp.) in a very small amount.

Diosbulbin B (I)——Analytical sample (recrystallized from acetone) showed mp 285° (decomp.), $[\alpha]_D^{20}$ +92.0° (c=0.75, pyridine). *Anal.* Calcd. for $C_{19}H_{20}O_6$: C, 66.3; H, 5.9; mol. wt., 344. Found: C, 66.4; H, 6.0; mol. wt. (Mass), 344. Slightly soluble in pyridine, methanol and acetone. Tastes bitter. UV λ_{max}^{EtOH} mμ (ε): 210 (6020). IR ν_{max}^{KBr} cm^{-1}: 3130, 1511, 877, 761 (β-substituted furan), 1782 (broad, γ-lactone), 1072, 1063 (ether). NMR (60 Mc, in C_5D_5N at 60°, ppm): 1.08 (3H, singlet, ≡C–C<u>H</u>$_3$), 4.69 (2H, –COO–C<u>H</u>=), 5.31 (1H R–O–C<u>H</u>=), 7.02 (1H), 7.45 (1H), 7.72 (1H) (β, α and α'-hydrogens of furan). Mass Spectrum m/e: 344 (M^+), 94, 81. B (4.88 mg) was refluxed with s.c./100 potassium hydroxide solution (25.00 ml) in ethanol for 4 hr and excess alkali was back titrated (methyl red) with 1/100 N hydrochloric acid; 1/100 N potassium hydroxide consumed, 2.02 ml, lactone 1.4.

Diosbulbin A (III)——Analytical sample recrystallized from ethyl acetate showed mp 265° (decomp.), $[\alpha]_D^{20}$ +26.7° (c=0.98, pyridine). *Anal.* Calcd. for $C_{20}H_{24}O_7$: C, 63.8; H, 6.4; mol. wt., 376. Found: C, 63.7; H, 6.4; mol. wt. (Mass), 376. Fairly soluble in pyridine, methanol, acetone and ethyl acetate. Tastes bitter. UV λ_{max}^{EtOH} mμ (ε): 209 (6000). IR ν_{max}^{KBr} cm^{-1}: 3410 (hydroxyl), 3140, 1508, 874, 765 (β-substituted furan), 1793 (sharp, γ-lactone), 1698 (ester), 1088, 1072 (ether). NMR (100 Mc, in $CDCl_3$, ppm): 1.17 (3H, singlet, ≡C–C<u>H</u>$_3$), 3.78 (3H, singlet, –COOC<u>H</u>$_3$), 4.03 (1H, HO–C<u>H</u>=), 4.70 (1H, –COO–C<u>H</u>=), 5.28 (1H, R–O–C<u>H</u>=), 6.93 (1H), 7.40 (1H), 7.55 (1H) (β, α and α'-hydrogens of furan). Mass Spectrum m/e: 376 (M^+), 94, 81.

Complete Reduction of B (I) and Selenium Dehydrogenation of the Product——B (860 mg) was shaken in a hydrogen atmosphere with platinum oxide (200 mg) in a mixture of ethanol (300 ml) and acetic acid (300 ml) until the hydrogen absorption stopped. Catalysts were filtered off and the solvent was evaporated to give a residue, which was dried *in vacuo* and dissolved in anhydrous tetrahydrofuran (300 ml). To the solution lithium aluminum hydride (1.2 g) was added and the mixture was refluxed for 30 hr and then stirred at room

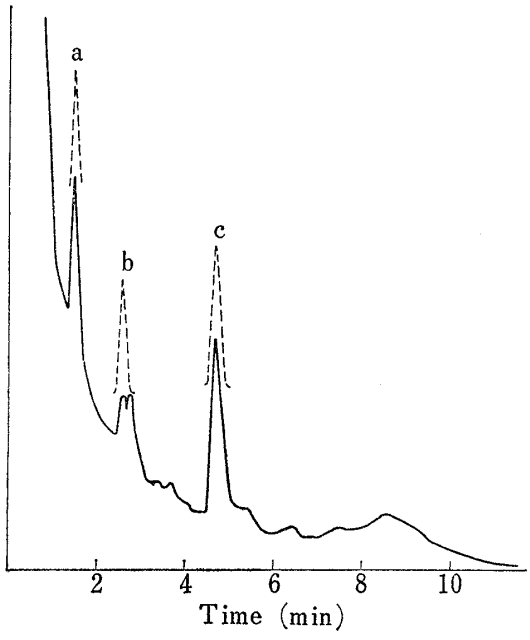


Fig. 1. Gas Chromatogram of the Product obtained by the Selenium Dehydrogenation of completely reduced Diosbulbin B

a: 1-methylnaphthalene b: 1,5-dimethylnaphthalene
c: 1,2,5-trimethylnaphthalene (4.70 min)
column: 4 mm×2.25 m; 1.5% SE-30-Chromosolb W (60—80 mesh)
flash heater temperature: 250°
detector temperature: 210°
column temperature: 150° N_2 flow rate: 120 ml/min

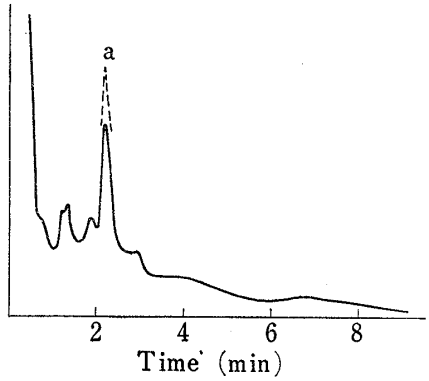


Fig. 2. Gas Chromatogram of the Product obtained by the Selenium Dehydrogenation of completely reduced Diosbulbin B

a: 1,2,5-trimethylnaphthalene (2.18 min)
column: 4 mm×2.25 m, 0.7% QF-1-Chromosolb W (60—80 mesh)
flash heater temperature: 250°
detector temperature: 170°
column temperature: 130° N_2 flow rate: 120 ml/min

temperature for additional 20 hr. Aqueous (50%) ethyl acetate (600 ml) was added to the reaction mixture and the product was extracted with chloroform–acetone (7:1) mixture (640 ml). The bottom layer was washed with water, dried over sodium sulfate and evaporated to give a solid (470 mg). The reduction product was heated with selenium (1 g) in a sealed tube (filled with nitrogen gas) at 310—325° for 15 hr. The resulting violet–black mass was extracted (Soxhlet) with petroleum ether (bp 40—60°)–ether (1:3) mixture (100 ml). The extract was evaporated and the residue (200 mg) was chromatographed on alumina (Brockmann, activity 2—3). Petroleum ether eluate was distilled to give an yellow oil (45 mg), bp 110—175° (bath temp.) (1 mmHg). It was again placed on alumina (Woelm, grade 1) column and eluted with petroleum ether. The eluate, pale yellow oil, UV λ_{max}^{Hexane} mμ: 231, 278, 326 (authentic sample of 1,2,5-trimethylnaphthalene, UV λ_{max}^{Hexane} mμ: 231, 278.5, 325), was examined by GLC (Fig. 1, 2).

Selenium Dehydrogenation of B——B (1 g) and selenium (1 g) were heated in a sealed tube (nitrogen atmosphere) at 320—340° for 32 hr. The product was extracted with petroleum ether–ether (1:3), placed on alumina (Brockmann) column and eluted with petroleum ether. The eluate was distilled to give an yellow oil, bp 72° (4.5 mm Hg), which was examined by GLC (Fig. 3).

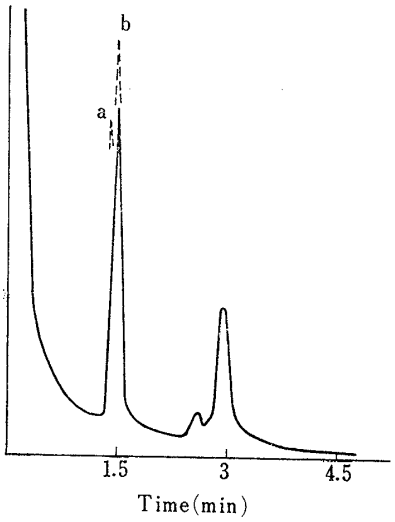


Fig. 3. Gss Chromatogram of the Product obtained by the Selenium Dehydrogenation of Diosbulbin B

a: 2-methylnaphthalene (1.38 min)
b: 1-methylnaphthalene (1.50 min)
column: 4 mm×2.25 m, 1.5% SE-30-Chromosolb W (60—80 mesh)
flash heater temperature: 250°
detector temperature: 210°
column temperature: 150°
N_2 flow rate: 120 ml/min

Conversion of B (I) to Diosbulbinic Acid (=C) (II) and A (III)——B (68.8 mg, 0.2 mM) was dissolved in pyridine (14 ml), water (14 ml) and sodium hydroxide (20 mg, 0.5 mM) were added and the mixture was stirred at room temperature for 3.5 hr. The reaction mixture was evaporated *in vacuo* to give a white solid, which was suspended in chloroform–acetone (5:1) mixture (20 ml) and acidified (to pH 1) with dilute hydrochloric acid. Water layer was extracted twice with chloroform–acetone (5:1), an interfacial solid was collected by filtration and dissolved in acetone. Both, extract and acetone solution, were combined with the filtrate (chloroform–acetone solution), washed three times with water, dried over sodium sulfate and the solvent was removed to give a white solid (72 mg). Recrystallization from acetone provided diosbulbinic acid (II) as a colorless plate, mp 247—250° (decomp.), *Rf* values: 0.02 (solv. c), 0.02 (solv. b), 0.15 (solv. a) (reddish purple with reagent 1 and purple with 2). Mixed melting point and co-chromatography with C showed no depression and single spot, respectively. $[\alpha]_D^{20}$ +64.7° (c=0.35, pyridine). *Anal.* Calcd. for $C_{19}H_{22}O_7$: C, 63.0; H, 6.1; mol. wt., 362. Found: C, 62.9; H, 6.3; mol. wt. (Mass), 362. Slightly soluble in pyridine, acetone and methanol. Tastes bitter. IR ν_{max}^{KBr} cm^{-1}: 3400—3300 (hydroxyl), 3140, 1508, 878, 763 (β-substituted furan), 2540, 1780 (carboxyl), 1790 (γ-lactone), 1090, 1070 (ether). NMR (100 Mc, in C_5D_5N, ppm): 1.07 (3H, singlet, ≡C–C$\underline{H}_3$), 4.10 (1H, HO–C$\underline{H}$=), 4.85 (1H, –COO–C$\underline{H}$=), 5.30 (1H, R–O–C$\underline{H}$=), 7.12 (1H), 7.50 (1H), 7.76 (1H) (β, α and α′-hydrogens of furan). Mass Spectrum *m/e*: 362 (M^+), 94, 81. Diosbulbinic acid (=C) in acetone was methylated with diazomethane in ether to yield a white solid, which was recrystallized from ethyl acetate to give the methyl ester as colorless needles, mp 264° (decomp.), *Rf* values: 0.34 (solv. c), 0.39 (solv. b) (reddish purple with reagent 1, purple with 2). Mixed melting point determination and co-chromatography with A and comparison of their IR spectra indicated the identity.

Treatment of A, B and Diosbulbinic Acid (=C) with Methanol——A, B and C were respectively boiled in methanol and the product was examined by thin-layer chromatography. In all cases only the starting material was detected.

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