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The Structure of Diosbulbine*

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A novel norditerpene lactone, diosbulbine, has been isolated from the tubers of *Dioscorea bulbifera* Linn. The structure (III) for the diterpene lactone has been assigned on the basis of spectroscopic and degradative evidence.

A REPORT that the boiled tubers of *Dioscorea bulbifera* Linn. are eaten by some of the tribal people in India in times of food scarcity and are believed to have hunger-suppressing property¹ led us to the chemical investigation of this plant. Chakravarti *et al*² have recorded the isolation of diosgenin from the roots.

Habermehl *et al*³ have recently disclosed the isolation of three compounds from *D. bulbifera* and have carried out X-ray structural investigation on one of these and shown it to have the structure (IV). We wish to report in this communication the work on the structure elucidation of a novel diterpene lactone isolated from the tubers of *D. bulbifera*†.

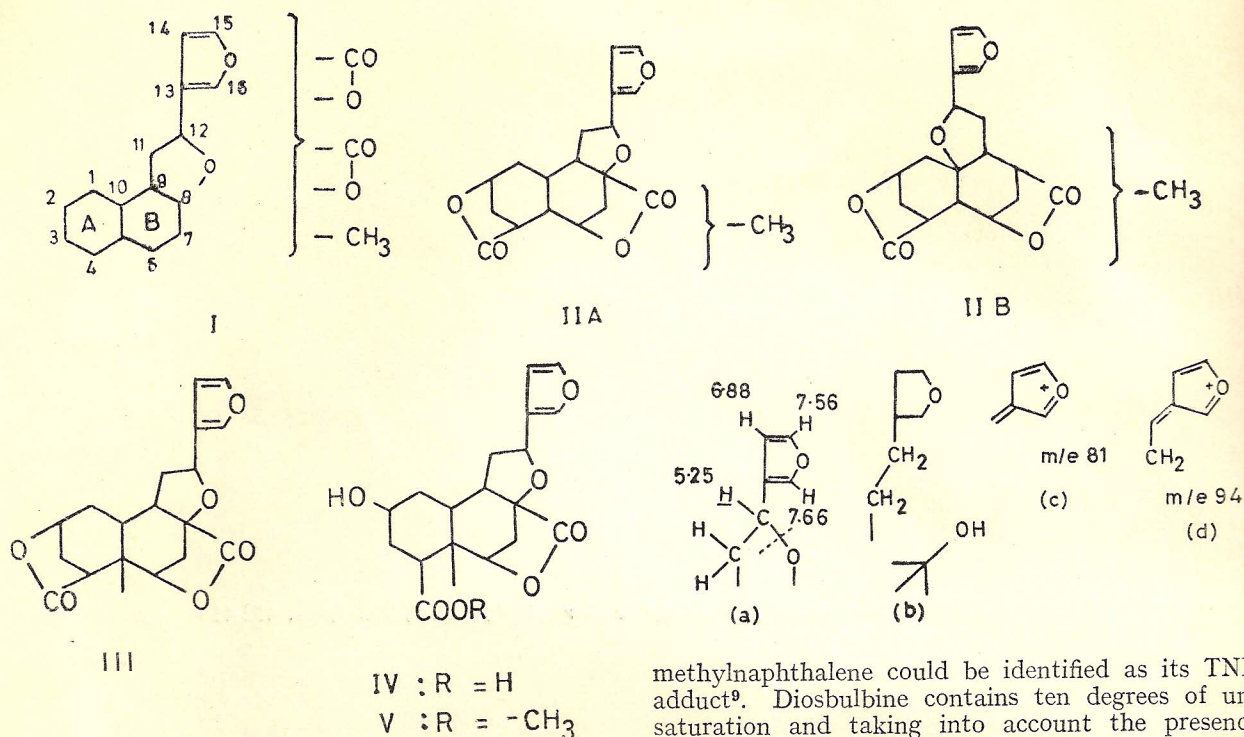
By extracting the boiled tubers (see experimental) with chloroform, a crystalline compound designated diosbulbine has been obtained in 0.16% yield based

on dry weight of the tubers. Diosbulbine, mp 295°, $[\alpha]_D +78^\circ$, analyses for $C_{19}H_{20}O_6$ (M^+ at m/e 344) and shows dimorphic crystalline forms depending on the solvent of crystallization. It gives colourless rhombic cubes from acetic acid and needles from methylethyl ketone. The two forms show different IR spectra in nujol or KBr, but their solution spectra in acetonitrile are identical. Diosbulbine gives a pink Ehrlich colour reaction indicating the presence of furan ring⁴. This is also confirmed by its UV spectrum; λ_{max}^{EtOH} 210 m μ ; ϵ 5800; IR bands 3130, 1590, 1510, 880 cm^{-1} and NMR (DMSO- d_6) signals at 7.66, 7.56 (1H each), 6.88 δ (1H). The signals at 7.66 and 7.56 represent the two aromatic α -protons and the peak at 6.88 δ the β -proton of a β -monosubstituted furan⁵.

The IR spectrum (nujol) shows carbonyl peaks at 1798 and 1790 cm^{-1} , indicative of two saturated γ -lactones. The IR spectrum does not show the presence of any other carbonyl or hydroxyl bands and no active hydrogen could be found at rt or at 100°. The sixth oxygen of diosbulbine was, therefore, inferred to be present as an ether oxygen.

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†After submission of the manuscript we have noticed a publication on the constitution and configuration of diosbulbin-A, -B and -C; Kumori, T., Setoguchi, S. & Kawasaki, T., *Chem. Ber.*, **101** (1968), 3096.



The NMR spectrum showed the presence of one tertiary methyl group at 1.2 δ and the X proton of an unsymmetrical ABX quartet centred at 5.25 δ ($J_{AX} + J_{BX} = 15$ cps) which could be ascribed to the proton on the carbon attached to the β -substituted furan ring⁶. This proton should be adjacent to an ether oxygen function and a methylene group as shown in (a). A two-proton complex multiplet appears at 4.8 δ and this could be assigned to hydrogens on carbon carrying ethereal oxygens⁷. A doublet at 2.7 δ (1H) indicates the presence of a proton on carbon adjacent to a carbonyl group⁸.

Hydrogenation of diosbulbine with PtO₂ in ethanol gave a mixture of two compounds which were separated by chromatography on silica gel. The tetrahydro derivative (R_f 0.35; Si gel; Chf—3% MeOH) was formed by reduction of the furan double bonds. This was confirmed by its mass spectrum (M^+ at m/e 348) and the disappearance of the aromatic protons in the NMR spectrum.

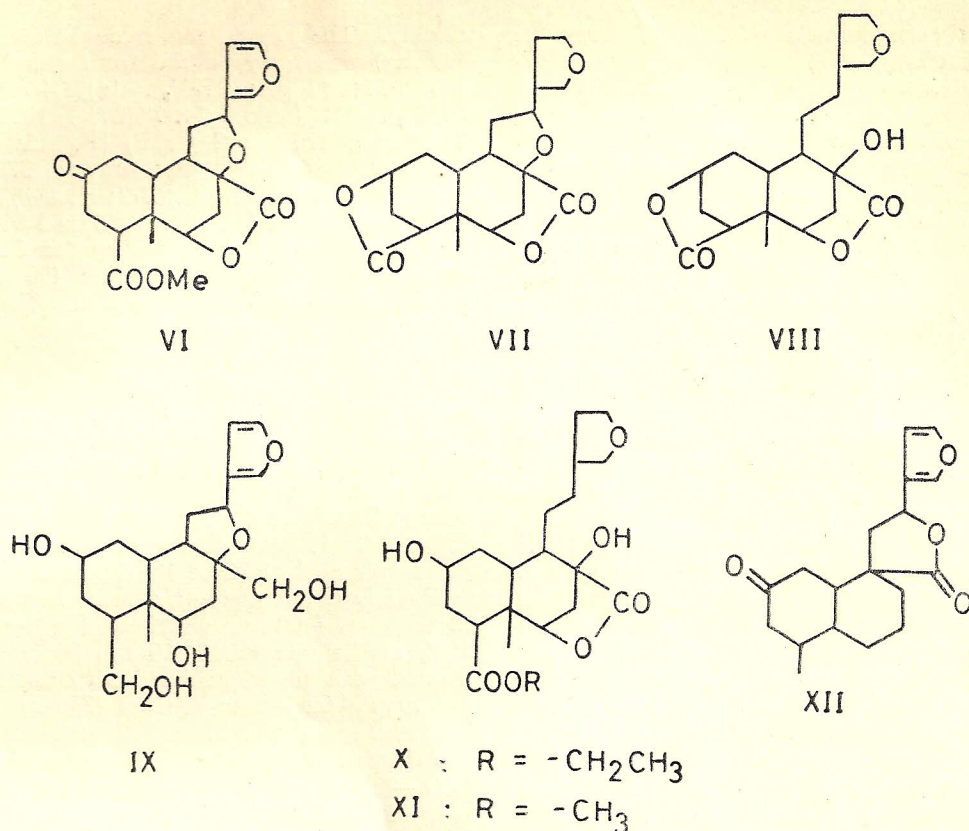
Hexahydrodiosbulbine (R_f 0.25; Si gel; Chf, 3% MeOH) (M^+ at m/e 350) was obtained by reduction of the furan double bonds and hydrogenolysis of the ether oxygen as shown by the dotted lines in (a). The compound showed a hydroxyl band at 3460 cm^{-1} and the γ -lactone bands at 1796 and 1782 cm^{-1} . The hydroxyl group is tertiary as it did not undergo acetylation or oxidation with CrO₃; the group (a) of diosbulbine changes to (b) in the hexahydro derivative. Diosbulbine does not contain any double bonds other than those present in the furan ring. Selenium dehydrogenation* of diosbulbine gave a mixture of methyl-naphthalenes from which a small amount of 1,2,5-tri-

*Examples are known in the literature, where a methyl appears in place of a carboxyl group during selenium dehydrogenations.

methylnaphthalene could be identified as its TNB adduct⁹. Diosbulbine contains ten degrees of unsaturation and taking into account the presence of a furan ring, two γ -lactones and a naphthalene skeleton, it could be deduced that the ether oxygen in (a) forms another ring, which appears to be five membered. An epoxide is ruled out since diosbulbine is unaffected by treatment with BF₃-ether or acids. On biogenetic considerations and the aforesaid evidence, a partial structure (I) could be written for diosbulbine.

Lithium aluminium hydride reduction of diosbulbine gave a tetrol, C₁₉H₂₈O₆, mp 185°, in which the furan and the ring containing the ether oxygen were unaffected. Mass spectrum of diosbulbine exhibits fragments characteristic of the furan ring at m/e 81 and m/e 94 due to the ions (c) and (d)¹⁰⁻¹². The LAH reduction product does not show the molecular ion peak at m/e 352 but shows m/e at 334 due to loss of water molecule. The presence of a β -substituted furan as in (a) in the LAH reduction product can be inferred from the fragments (c) and (d) in the mass spectrum. NMR spectrum shows the furan protons at 7.42 (2H), 6.4 (1H) and a one proton multiplet at 5.2 δ . The tetrol is formed by the reduction of the carbonyls and opening of both the γ -lactone rings. Selenium dehydrogenation of the LAH reduction product gave 1,2,5-trimethylnaphthalene, identified as its TNB adduct. The obtainment of 1,2,5-trimethylnaphthalene by selenium dehydrogenation of the LAH reduction product leads to the placement of the lactone carbonyls at positions 8 and 4 in the formula (I). A partial formula (IIA) or (IIB) could then be written for diosbulbine.

Diosbulbine on treatment with ethanolic sodium hydroxide gave a hydroxycarboxylic acid, diosbulbic acid, C₁₉H₂₂O₇. The IR spectrum showed the presence of a saturated γ -lactone (1790 cm^{-1}), hydroxyl (3400 cm^{-1}) and carboxylic acid (1690 cm^{-1}) bands. On treatment with acetic anhydride and pyridine



or heating at about 270° under high vacuum, diosbulbic acid cyclized to give diosbulbine. Methylation of the acid with diazomethane provided the corresponding methylester, C₂₀H₂₄O₇. The methyl ester could be obtained in almost quantitative yield by refluxing diosbulbine with sodium borohydride in methanol or by passing dry HCl in a solution of methanol. NMR spectrum of diosbulbic acid showed aromatic protons at 7.65, 7.55 (furan α -protons), and 6.9 δ (furan β -proton). A quartet at 5.25 δ (1H) in diosbulbic acid showed that there was no change in the surroundings of the proton on C-12; the complex two-proton multiplet at 4.8 observed in diosbulbine had disappeared in diosbulbic acid and instead, a one-proton doublet (very slightly split) was clearly seen at 4.77 δ ($J = 5$ cps). The proton on the carbon bearing the hydroxyl group of diosbulbic acid appeared at 4.0 δ . The broad peak at 5.9 δ (2H) which disappeared on deuteration indicated the hydroxyl and carboxylic acid protons. The one-proton doublet observed at 2.7 δ in diosbulbine now appeared downfield at 3.0 δ as a broad peak. These facts could only be accommodated by excluding structure (IIB) and placing the methyl group at C-5 in the partial formula (IIA). The disappearance of the complex multiplet at 4.8 δ and the appearance of an upfield proton at 4.0 δ shows that the γ -lactone on ring A has opened giving diosbulbic acid. Diosbulbine and diosbulbic acid could thus be represented as (III) and (IV) respectively.

The methylester (V) on oxidation with chromic acid in pyridine gave the ketone (VI). It formed a 2,4-dinitrophenylhydrazone; mp 299°. The UV

spectrum of the ketone indicates that the carbonyl group is not conjugated with the furan double bonds. Tetrahydro- and hexahydrodiosbulbines should, therefore, be formulated as (VII) and (VIII) respectively. Lithium aluminium hydride reduction of diosbulbine gives the tetrol (IX). Hydrogenation of the methylester (V) using PtO₂ in ethanol gave (X) by exchange of the ester methyl with an ethyl group, reduction of the furan double bonds and hydrogenolysis. This was based on its analysis, NMR and mass spectral data. NMR signals at 4.6 (1H, C-6), 4.1 (2H; t , $J = 7$ cps; COO-CH₂-CH₃), 1.25 (3H, t , $J = 7$ cps; COO-CH₂-CH₃) and 0.92 δ (3H, s , C-5-CH₃). However, reduction of (V) using Pd/C in ethanol gave the product (XI), C₂₀H₃₀O₇; mp 220°. The compound (XI) was also obtained by the treatment of (VIII) with a trace of methanolic KOH and diazomethane.

Diosbulbine is biogenetically interesting, as it is the first example of a diterpene isolated from a Dioscoreaceae. Moreover, the only other example of a norditerpene is that of crotonin (XII) isolated from the leaves of *Croton lucidus* L. (Euphorbiaceae)⁶.

Experimental Procedure

All melting points are uncorrected. UV spectra were measured with a Beckman DK-2A spectrophotometer and IR spectra were taken using a Perkin-Elmer infracord spectrophotometer. NMR spectra were taken on Varian A-60 instrument using TMS as an internal reference.

Isolation of diosbulbine (III)—The roots of *D. bulbifera* were boiled in water, sliced and dried in the shade. The powdered material (10 g) was

extracted with petroleum ether (45 litres) to remove the fatty material (18 g). This was further extracted with chloroform (45 litres). The solvent was removed under vacuum and the resulting mass was diluted with a small amount of chloroform and left in the refrigerator overnight. The crystalline material which had separated was filtered and washed with ether (16.0 g). Quick recrystallization of the compound (2.0 g) from glacial acetic acid gave cubes; mp 295° (decomp). Recrystallization of the crude compound from methyl-ethyl ketone gave needles; mp 285° (decomp). There was no depression in the mmp of the two forms and their TLC R_f 0.45 (Si gel; CHCl_3 - 2% MeOH) remained the same.

The compound gave a purple colour with Ehrlich reagent [Found: C, 66.1; H, 5.9; mol wt by mass spectrometry 344. $\text{C}_{19}\text{H}_{20}\text{O}_6$ (mol wt 344) requires C, 66.3; H, 5.8%].

Tetrahydro- (VIII) and *hexahydrodiosbulbine* (IX) — Diosbulbine (5 g) in ethanol (1.5 litres) was hydrogenated over PtO_2 (1 g) at 60°, till about 2 moles of hydrogen was absorbed. Removal of the solvent gave a mixture (3.5 g) of two products (R_f 0.35 and 0.25; Si gel; CHCl_3 - 3% MeOH), which were separated by chromatography on silica gel. Elution of the column with CHCl_3 + 1% MeOH gave tetrahydrodiosbulbine (800 mg). Recrystallization from ethanol gave needles; mp 334°; $[\alpha]_D^{25} +70^\circ$ (C, 1%; CHCl_3) [Found: C, 65.9; H, 7.0; mol wt by mass spectrometry 348. $\text{C}_{19}\text{H}_{24}\text{O}_6$ (mol wt 348) requires C, 65.5; H, 6.9%].

Elution of the column with CHCl_3 + 2% MeOH gave hexahydrodiosbulbine (2.5 g). It crystallized from ethanol in colourless needles; mp 295°; $[\alpha]_D^{25} +59^\circ$ (C, 1%, pyridine). IR (nujol) 3460 (OH), 1796, 1780 cm^{-1} (saturated γ -lactones) [Found: C, 65.1; H, 7.7; mol wt by mass spectrometry 350. $\text{C}_{19}\text{H}_{26}\text{O}_6$ (mol wt 350) requires C, 65.1; H, 7.5%].

Diosbulbic acid (IV) — Diosbulbine (1 g) was heated with 50% ethanolic NaOH (100 ml) for 1 hr. The precipitate obtained by acidification with dil HCl was crystallized from ethanol (900 mg); mp 272°; $[\alpha]_D^{25} +74^\circ$ (C, 1%; pyridine) (R_f 0.3; Si gel; CHCl_3 - 15% MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ 210 m μ ; ϵ 5290; IR (nujol) 3440 (OH), 1790 (saturated γ -lactone) 1680 cm^{-1} (-COOH) [Found: C, 62.6; H, 6.3; mol wt by mass spectrometry, 362. $\text{C}_{19}\text{H}_{22}\text{O}_7$ (mol wt, 362) requires C, 62.9; H, 6.1%].

Conversion of diosbulbic acid to diosbulbine — (a) Diosbulbic acid (100 mg) dissolved in pyridine (1 ml) and acetic anhydride (10 ml) was left overnight. The solution was poured into water and the residue was crystallized from methylethyl ketone to give needles; mp 292°. This was identical with diosbulbine in its mmp, TLC and IR spectrum.

(b) Diosbulbic acid (100 mg) was heated in a sublimation block at 265° at 5×10^{-4} mm. The residue insoluble in NaHCO_3 , crystallized from methylethyl ketone, mp 292°, and was found to be identical with diosbulbine.

Methylester of diosbulbic acid (V) — (a) Diosbulbic acid (100 mg) was dissolved in methanol (20 ml), ether (50 ml) and was treated with excess of ethereal diazomethane. Colourless needles separated imme-

diately. The solvent was removed and the residue (90 mg) was recrystallized from ethanol; mp 295° (R_f 0.33; Si gel; CHCl_3). $[\alpha]_D^{25} +75^\circ$ (C, 1.1%; CHCl_3). IR (nujol) 3400 (OH), 1800 (saturated γ -lactone), 1700 cm^{-1} (ester) [Found: C, 63.8; H, 6.6; mol wt by mass spectrometry, 376. $\text{C}_{20}\text{H}_{24}\text{O}_7$ (mol wt, 376) requires C, 63.8; H, 6.4%].

(b) Diosbulbine (10 g) was refluxed with methanol (2.5 litres) and NaBH_4 (10 g) for 30 min. The solution was diluted with water (50 ml) and the methanol removed under vacuum. The precipitate was collected, washed with water and recrystallized from methanol (10.0 g); mp 295°. The compound was identical with the methyl ester obtained by the procedure (a).

(c) Diosbulbine (50 mg) was dissolved in methanol (150 ml) and dry HCl gas was passed for 30 min. The solution was poured into water, the solvent removed and the residue crystallized from ethanol; mp 295°. It was identical with methylester of diosbulbic acid obtained by the earlier methods.

Hydrolysis of diosbulbic acid methylester — The methylester (400 mg) was heated under reflux with 10% methanolic KOH (10 ml) for 1 hr. It was diluted with water, acidified with dil HCl and the ppt crystallized from ethanol (250 mg); mp 272°. This was found to be identical with diosbulbic acid.

Oxidation of diosbulbic acid methylester with CrO_3 (VI) — To a solution of the methylester (200 mg) in pyridine (10 ml), CrO_3 -pyridine complex (0.5 g in 10 ml)¹³, was added the solution kept overnight. It was diluted with water, extracted with chloroform and washed with 2N HCl, aq NaHCO_3 and water. The chloroform layer was dried (Na_2SO_4) and the solvent removed. The residue was crystallized from ethanol to give colourless needles (100 mg); mp 272° (R_f 0.35; Si gel; CHf - 1% MeOH). $\lambda_{\text{max}}^{\text{EtOH}}$ 210 m μ ; ϵ 5834; IR (KBr) 1782 (saturated γ -lactone), 1725 (ester), 1695 cm^{-1} (six-membered ketone) [Found: C, 64.0; H, 6.2; mol wt by mass spectrometry, 374. $\text{C}_{20}\text{H}_{22}\text{O}_7$ (mol wt, 374) requires C, 64.1; H, 5.9%]. It formed a crystalline 2,4-dinitrophenylhydrazone; mp 299-300° (decomp) (Found: C, 56.2; H, 4.9; N, 10.0. $\text{C}_{26}\text{H}_{26}\text{N}_4\text{O}_{10}$ requires C, 56.3; H, 4.7; N, 10.1%).

Hydrogenation of diosbulbic acid methylester —

(a) *With PtO_2 in ethanol* (X) — The methylester (400 mg) in ethanol (40 ml) was hydrogenated over PtO_2 (200 mg) at 60°. Removal of the solvent gave a residue which was chromatographed over Si gel in benzene. Elution with chloroform - 2% MeOH gave a homogenous product (R_f 0.4; Si gel; CHCl_3 - 2% MeOH). It crystallized from CH_2Cl_2 - hexane in colourless needles; mp 193° (X). IR (nujol) 3420 (OH), 1778 (γ -lactone), 1702 (ester) [Found: C, 63.5; H, 8.4; mol wt by mass spectrometry, 396. $\text{C}_{21}\text{H}_{32}\text{O}_7$ (mol wt, 396) requires C, 63.6; H, 8.1%].

(b) *Pd/C in ethanol* (XI) — The methyl ester (500 mg) in ethanol (40 ml) was hydrogenated over 10% Pd/C (500 mg) at 60°. Evaporation of the solvent gave a residue which after chromatographic separation on Si gel provided the crystalline compound (XI) (120 mg) (CH_2Cl_2 - hexane); mp 220°. [Found: C, 63.1; H, 8.1; mol wt by mass spectro-

metry, 382. $C_{20}H_{30}O_7$ (mol wt, 382) requires C, 62.8; H, 7.9%].

Hexahydrodiosbulbic acid methylester (XI) from hexahydrodiosbulbine — Hexahydrodiosbulbine (200 mg) was dissolved in methanol (25 ml), 10% KOH (0.5 ml) warmed for 5 min and to the cooled solution, an excess of ethereal diazomethane was added. The solution was kept overnight and the solvent removed. The residue was crystallized from CH_2Cl_2 — hexane to afford colourless needles (80 mg); mp 220° (TLC, Si gel; R_f , 0.3; $CHCl_3$ — 3% MeOH). It was found to be identical with hexahydrodiosbulbic acid methylester (XI) in its mmp, TLC and IR spectra.

Lithium aluminium hydride reduction of diosbulbine (IX) — Diosbulbine (5 g) was heated under reflux with dry tetrahydrofuran (200 ml) in a three-neck flask and a suspension of LAH (5 g) in tetrahydrofuran (50 ml) was added dropwise, under vigorous stirring during 30 min. The solution was further refluxed for 2 hr. It was decomposed by pouring over crushed ice and acidified with 2N H_2SO_4 . It was extracted with tetrahydrofuran, washed with water and the extract dried over Na_2SO_4 . Removal of the solvent under vacuum gave a crude product which crystallized from ethanol-hexane giving a colourless needles (2.6 g); mp 185° (R_f 0.33; Si gel; $CHCl_3$ — 5% MeOH). UV λ_{max}^{EtOH} 210 $m\mu$, ϵ 4832; IR (nujol) 3320 cm^{-1} (OH) (Found: C, 64.5; H, 8.1. $C_{19}H_{28}O_6$ requires C, 64.7; H, 8.0%).

Selenium dehydrogenation of diosbulbine — A mixture of diosbulbine (2 g) and selenium (10 g) was heated in a sealed tube at $290\text{--}320^\circ$ for 36 hr. Five such batches were combined and the residue extracted with hexane. Removal of the solvent gave an oil (1.2 g) which was chromatographed over alumina. Elution with hexane gave a yellowish oil (690 mg) which was distilled at $130^\circ/0.2\text{ mm}$ to provide an oil (55 mg). UV λ_{max}^{EtOH} 225, 255, 277, 282, 293 (inflec) and 322 $m\mu$ ($\log \epsilon$ 4.71, 3.62, 3.73, 3.74, 3.55 and 2.64). It gave a crystalline TNB adduct which after three crystallizations gave mp $143\text{--}5^\circ$ (Found: C, 59.2; H, 4.4. $C_{19}H_{17}N_3O_6$ requires C, 59.5; H, 4.5%). The UV spectrum indicated peaks closely resembling the TNB adduct of 1,2,5-trimethyl naphthalene.

Selenium dehydrogenation of the tetrol (IX) — An intimate mixture of (IX) (2 g) and selenium (4 g) was heated in a sealed tube at $290\text{--}320^\circ$ for 36 hr. The residue obtained from four such batches was combined and extracted with hexane. The hexane was washed with 5% $NaHCO_3$, 5% NaOH and the neutral hexane extract was dried and evaporated. This gave an oil (1.2 g) which was passed through a short column of alumina. The eluate was distilled at $140^\circ/0.2\text{ mm}$. The pale yellow oil gave a crystalline TNB adduct; mp $153\text{--}5^\circ$ (Found: C, 60.0; H, 4.4. $C_{19}H_{17}N_3O_6$ requires C, 59.5; H, 4.5%). It was found to be identical in its mmp, UV and IR spectra with the TNB adduct of an authentic sample of 1,2,5-trimethyl naphthalene, kindly provided by Prof B. R. Pai.

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