Phytochemistry, Vol. 23, No. 3, pp. 623–625, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

DIOSBULBIN D AND 8-EPIDIOSBULBIN E ACETATE, NORCLERODANE DITERPENOIDS FROM *DIOSCOREA BULBIFERA* TUBERS

ROBERT D. H. MURRAY*, ZACARIAS D. JORGE, NURUL H. KHAN*†, MOHAMMAD SHAHJAHAN† and MOHAMMAD QUAISUDDIN†

Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ, U.K.; † Department of Biochemistry, University of Dhaka, Dhaka 2, Bangladesh

(*Revised received* 6 *September* 1983)

Key Word Index—*Dioscorea bulbifera* L. var *sativa*; Dioscoreaceae; diterpenoids; norclerodane derivatives; diosbulbin D; norditerpenoid; 8-epidiosbulbin E acetate.

Abstract—From tubers of *Dioscorea bulbifera* L. var *sativa* a new 18-norclerodane diterpenoid, 8-epidiosbulbin E acetate, has been isolated, besides the previously known norditerpenoid, diosbulbin D, and its structure established by spectroscopic means.

INTRODUCTION

Plants of the Dioscoreaceae are known as a source of diosgenin and related steroid sapogenins, which occur mainly in the underground parts [1–3]. Extensive studies on the root tubers of the Japanese *Dioscorea bulbifera* L. forma *spontanea* have revealed no steroid sapogenins but instead eight furanoid norditerpenoids, the diosbulbins A–H [4–6] and the enol glucosides of two of these, the diosbulbinosides D and F [7].

D. bulbifera L. var *sativa* grows wild in the hilly parts of the northern districts of Bangladesh where the bitter tuber is used by tribal people for treatment of leprosy and tumours. The present study, undertaken to isolate the biologically active constituents of the tuber, has resulted in the isolation of diosbulbin D (1) and a new furanoid norditerpenoid for which the structure 8-epidiosbulbin E acetate (3) is proposed.

RESULTS AND DISCUSSION

Combustion analysis and mass spectrometry indicated the molecular formula $C_{19}H_{20}O_6$ for compound **1**, the base peak at *m/z* 94 suggesting the presence of a 2-(β-furyl) ethylene group [4]. The IR spectrum was consistent with the presence of a furan ring (3150, 1610, 1508, 878 cm^{-1}) and three carbonyl functions, a γ-lactone (1778 cm^{-1}), a δ-lactone (1752 cm^{-1}) and a cyclohexanone (1720 cm^{-1}). The absence of any vinyl proton resonance in the ^1H NMR spectrum, apart from the characteristic signals for a β-substituted furan ring, required the compound which contained one tertiary methyl group to be bicyclic. Analysis of the ^1H NMR spectrum (Table 1) in conjunction with extensive spin decoupling and the optical rotation established conformation **4** for the norditerpenoid (**1**). This norclerodane structure was previously assigned to diosbulbin D [5]. Direct comparison of our compound with an authentic sample revealed their identity.

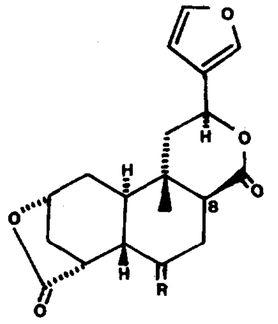
The new compound (**3**) from *D. bulbifera* L. var *sativa* had the molecular formula $C_{21}H_{24}O_7$ and was deduced to be the acetate of a norditerpenoid alcohol from the strong IR absorption at 1740 cm^{-1}, the three-proton singlet at δ2.0 in the ^1H NMR spectrum and the facile loss of ketene from the parent ion in the mass spectrum. The IR and ^1H NMR spectra confirmed the presence of a β-substituted furan ring, a γ-lactone (1778 cm^{-1}), a δ-lactone (1740 cm^{-1}) and one tertiary methyl group.

Signals at δ5.53 (H-12) and 4.88 (H-2) in the ^1H NMR spectrum of **3** revealed that each lactone ring was linked through a secondary hydroxyl group as in diosbulbin D. The δ-lactone in **3** was similarly proximate to the furan ring from the allylic coupling (0.6 Hz) of H-16 with H-12. This, together with a similar isolated ABX system for the C-12 methine and adjacent C-11 methylene, revealed that the six-membered ring lactone was again attached to ring B.

The γ-lactone ring was fused to ring A, the low-field methine proton being equatorial and showing four couplings, two of 5.5 Hz with the equatorial protons at C-1 and C-3, one of 0.8 Hz with the axial proton at C-1 and a long-range coupling of 1.2 Hz with H-4, the single proton adjacent to the γ-lactone carbonyl group. Examination of a model of the conformer **5** revealed two dihedral angles of 90° between the axial proton at C-3 and H-2 and H-4, which accounts for the absence of coupling between these protons. Comparison with the spectrum of diosbulbin D revealed that the seven protons attached to C-1, C-2, C-3, C-4 and C-10 had similar chemical shifts and coupling patterns (Table 1) and thus the γ-lactone function was fused to ring A in the same manner in both compounds. However, the upfield shift of H-4 by 0.81 ppm and downfield shift of H-10 by 0.48 ppm must arise from the different functionality in ring B. That the ketone group at C-6 in diosbulbin D was replaced by an acetoxyl group attached axially on the α-face followed from the following observations. The axial C-5 proton was upfield by 0.52 ppm from its position in the spectrum of diosbulbin D and showed one additional coupling (2.7 Hz) with the

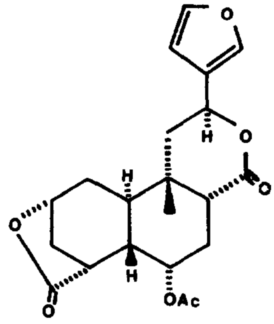
---

*To whom correspondence should be addressed.

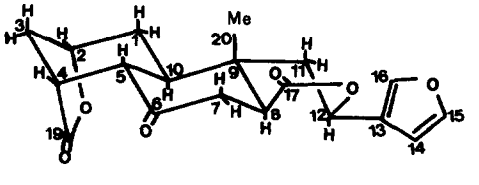


1 R = O

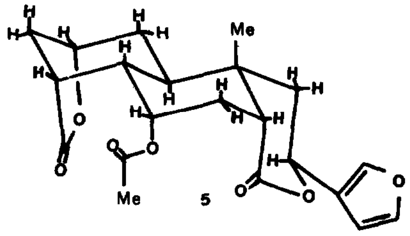
2 R = α-OH,β-H



3



4



5

acetoxyl methine proton at δ5.17, which was thus equatorial. This methine proton showed two further equatorial–axial and equatorial–equatorial couplings of 2.6 and 2.8 Hz respectively with the β(δ1.9) and α(δ2.87) C-7 protons, the geminal coupling constant (15.1 Hz) of these being consistent with a methylene group in a chair cyclohexane. The remaining proton, at C-8, upfield by 0.64 ppm from its position in the spectrum of diosbulbin D, was coupled only to the protons at C-7 and was equatorial from its coupling constants of 6.1 Hz (eq–ax) to H-7β and 2.0 Hz (eq–eq) to H-7α. The configuration at C-8 in the acetate was thus opposite to that of the co-occurring ketone, diosbulbin D (**1**) and the corresponding alcohol, diosbulbin E (**2**). The δ-lactone is therefore *cis*-fused to ring B and probably adopts a chair-like conformation with the furyl residue at C-12 equatorial. The axial nature of H-12 was revealed by its couplings of 12.6 and 3.5 Hz to the axial and equatorial protons at C-11, respectively. These spectroscopic findings and the ^{13}C NMR spectrum (Table 2) taken with the co-occurrence of diosbulbin D of known absolute configuration [6] were consistent with structure **3** and conformation **5** for the new compound, 8-epidiosbulbin E acetate. This norclerodane diterpenoid is unique in having the δ-lactone ring *cis*-fused to ring B since in the diosbulbins D–H and the two diosbulbinosides this ring is *trans*-fused.

EXPERIMENTAL

Mps were determined on a Kofler hot-stage apparatus. Microanalyses were obtained by Mrs. W. Harkness and her staff. Mass spectra were recorded by Mr. A. Ritchie. IR spectra of KBr discs were recorded by Mrs. F. Lawrie and her staff. UV spectra were for solns in MeOH. ^{1}H NMR spectra were recorded by Dr. D. S. Rycroft on a Bruker WP 200SY spectrometer and the ^{13}C NMR spectrum by Mr. J. Gall on a Varian XL 100 spectrometer for solns in CDCl$_3$. Signals are reported in δ-values (ppm) from TMS. TLC was performed on silica gel 60G (Merck). The following systems were used to develop the chromatographs: A, EtOAc; B, EtOAc–Me$_2$CO (8:1); C, EtOAc–C$_6$H$_6$ (3:1); D, C$_6$H$_6$–EtOAc–HOAc (4:2:1).

Air dried, powdered tubers (3.5 kg) of *Dioscorea bulbifera* var *sativa*, collected from the hilly parts of Rajshashi district, with the help of local people, in November 1981, were exhaustively extracted with C$_6$H$_6$ (8 l.). The solvent was removed under red. pres., the residue (7.5 g) dissolved in Me$_2$CO (15 ml) and Et$_2$O added dropwise until the soln became slightly turbid. Cooling in a refrigerator gave yellow crystals which were recrystallized from Me$_2$CO–Et$_2$O and then MeOH to give needles (0.95 g) mp 210–212°, TLC of which in solvent A showed two spots (R_f 0.63 and 0.53). The two compounds were separated by prep. TLC to give diosbulbin D (**1**), prisms (100 mg) from EtOH (R_f 0.63, 0.72, 0.38 and 0.70 in solvent systems A–D respectively) and 8-epidiosbulbin E acetate (**3**), needles (350 mg) from EtOH (R_f 0.53, 0.63, 0.27 and 0.18 in solvent systems A–D respectively).

Diosbulbin D. Mp 235–237° (lit. [5] 229–230°); $[\alpha]_D^{20}$ $-21°$ (*c* 0.110, MeOH) (lit. [5] $-49°$; found for authentic sample, $-18°$ *c* 0.107, MeOH); (Found: C, 66.09; H, 5.97. C$_{19}$H$_{20}$O$_6$ requires: C, 66.27; H, 5.85%); MS *m/z*: 344.1267 [M]$^+$ (17%; C$_{19}$H$_{20}$O$_6$ requires 344.1260) and 94.0418 (100%; C$_6$H$_6$O requires 94.0418); IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1} 3150, 1610, 1508, 878 (furan), 1778 (γ-lactone), 1752 (δ-lactone), 1720 (ketone); UV $\lambda_{\max}^{\mathrm{MeOH}}$: 207 nm (log ε3.84); identical (mmp, IR, UV and TLC) with an authentic sample (mp 234–236°).

8-Epidiosbulbin E acetate. Mp 223–225°; $[\alpha]_D^{20}$ $+46°$ (*c*, 0.145, MeOH); (Found: C, 64.82; H, 6.25. C$_{21}$H$_{24}$O$_7$ requires: C, 64.93; H, 6.23%); MS *m/z*: 388.1531 [M]$^+$ (8%; C$_{21}$H$_{24}$O$_7$ requires 388.1522), 346.1430 [M $-$ C$_2$H$_2$O]$^+$ (17%; C$_{19}$H$_{22}$O$_6$ requires 346.1416) and 94.0415 (100%); IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3140, 1605, 1508, 878 (furan), 1778 (γ-lactone), 1740 (δ-lactone, acetate); UV $\lambda_{\max}^{\mathrm{MeOH}}$: 208 nm (log ε3.76).

Acknowledgements—We thank Mr. Abdul Hassan, Department of Botany, University of Dhaka and Dr. M. Hag, National Herbarium of Bangladesh, for botanical classification of the plant

Table 1. ^{1}H NMR (200 MHz) chemical shifts (δ) for diosbulbin D (**1**) and 8-epidiosbulbin E acetate (**3**)

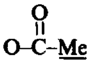
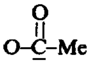
Proton	Compound 1	Compound 3
1β	1.55, *ddd*	1.49, *ddd*
1α	2.19, *dddd*	2.14, *dddd*
2β	4.88, *dddd*	4.88, *dddd*
3β	1.73, *d*	1.80, *d*
3α	2.54, *dddd*	2.53, *dddd*
4β	3.39, *ddd*	2.58, *ddd*
5β	2.48, *dd*	1.96, *ddd*
6β	—	5.17, *ddd*
7β	2.68, *dd*	1.90, *ddd*
7α	2.78, *dd*	2.87, *ddd*
8β	—	2.35, *dd*
8α	2.99, *dd*	—
10α	2.13, *ddd*	2.61, *ddd*
11β	1.90, *dd*	1.81, *dd*
11α	2.17, *dd*	2.14, *dd*
12α	5.35, *ddd*	5.53, *ddd*
14	6.42, *dd*	6.44, *dd*
15	7.44, *dd*	7.42, *dd*
16	7.47, *ddd*	7.51, *ddd*
20	1.23, *s*	1.61, *s*
OAc	—	2.00, *s*

All the assignments were confirmed by double resonance experiments.

Proton coupling constants in Hz: Compound 1 1β,1α 14.2; 1β,10α 12.8; 1β,2β 1.5; 1α,10α 5.0; 1α,2β 5.5; 1α,3α 1.5; 2β,3α 5.5; 2β,3β 0; 2β,4β 1.2; 3β,3α 12.0; 3β,4β 0; 3α,4β 5.5; 4β,5β 1.5; 5β,10α 12.8; 7β,7α 14.6; 7β,8α 12.0; 7α,8α 5.6; 11β,11α 14.2; 11β,12α 11.2; 11α,12α 6.0; 12α,16 0.6; 14,15 1.9; 14,16 0.8; 15,16 1.6. Compound 3: 1β,1α 14.2; 1β,10α 12.1; 1β,2β 0.8; 1α,10α 5.8; 1α,2β 5.5; 1α,3α 1.6; 2β,3α 5.5; 2β,3β 0; 2β,4β 1.2; 3β,3α 11.0; 3β,4β 0; 3α,4β 5.5; 4β,5β 1.7; 5β,10α 12.1; 5β,6β 2.7; 6β,7β 2.6; 6β,7α 2.8; 7β,7α 15.1; 7β,8β 6.2; 7α,8β 2.0; 11β,11α 14.7; 11β,12α 12.6; 11α,12α 3.5; 12α,16 0.6; 14,15 1.9; 14,16 0.8; 15,16 1.6.

material. We are grateful to Professor Tetsuya Komori, Kyushu University for kind gifts of authentic diosbulbins D and G. The work was supported in part by grants from the Centre for Advanced Studies and Research in Biological Sciences, University of Dhaka and the Spanish Ministry of Education and Science.

Table 2. ^{13}C NMR (100 MHz) spectral data of 8-epidiosbulbin E acetate (**3**)

Carbon	δ
1	28.4, *t*[*]
2	69.8, *d*[†]
3	34.5, *t*[‡]
4	42.1, *d*[§]
5	41.2, *d*[§]
6	69.3, *d*[†]
7	26.8, *t*[*]
8	45.9, *d*
9	39.8, *s*
10	38.7, *d*[§]
11	31.6, *t*[‡]
12	76.6, *d*
13	124.9, *s*
14	108.4, *d*
15	143.8, *d*
16	139.9, *d*
17	170.9, *s*
19	176.0, *s*
20	21.8, *q*[‖]
 $\mathrm{O{-}\overset{\overset{O}{\parallel}}{C}{-}\underline{Me}}$	20.9, *q*[‖]
 $\mathrm{O{-}\overset{\overset{O}{\parallel}}{\underline{C}}{-}Me}$	170.9, *s*

*, †, ‡, §, ‖: These assignments may be reversed but those given are considered to be the most likely.

REFERENCES

1. Hegnauer, R. (1963) *Chemotaxonomie der Pflanzen* Vol. 2, p. 133. Birkhauser, Basel.
2. Barua, A. K., Chakravarti, D. and Chakravarti, R. N. (1956) *J. Indian Chem. Soc.* **33**, 799 (and references cited therein).
3. Akahori, A. (1965) *Phytochemistry* **4**, 97.
4. Komori, T., Arita, M., Ida, Y., Fujikura, R. and Kawasaki, T. (1973) *Annalen* 978 (and references cited therein).
5. Ida, Y., Kubo, S., Fujita, M., Komori, T. and Kawasaki, T. (1978) *Annalen* 818.
6. Ida, Y., Komori, T. and Kawasaki, T. (1978) *Annalen* 834.
7. Ida, Y., Noda, N., Kubo, S., Komori, T. and Kawasaki, T. (1978) *Chem. Pharm. Bull.* **28**, 435.