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HYPOESTENONE: A FUSICOCCANE DITERPENE KETONE FROM *HYPOESTES FORSKALEI*

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Key Word Index—*Hypoestes forskalei*; Acanthaceae; diterpenes; X-ray crystallography; hypoestenone; 8(9)α-epoxyhypoestenone; (+)-sesamin.

Abstract—The aerial parts of *Hypoestes forskalei* yielded a new fusicoccane diterpene ketone, named hypoestenone. Its structural assignment was based on chemical derivatization and ^{1}H and ^{13}C NMR spectroscopic studies, mainly 2D NMR experiments, including long-range COSY and HETCOR correlations. X-Ray crystallographic analysis established the complete structure and relative stereochemistry of the epoxide of hypoestenone.

INTRODUCTION

Hypoestes forskalei Vahl. Roem. & Schult. is a perennial bushy and leafy herb which is widely distributed throughout the southern region of Saudi Arabia [1]. Although plants of the genus *Hypoestes* have found application in East African folk medicine [2] for various chest and other diseases, only two species have received any prior chemical or biological investigations. Thus, a number of cytotoxic phenanthroindolizidine alkaloids have been reported [3] to occur in *H. verticillaris*, while tricyclic diterpene ketones with uncommon fusicoccane (dicyclopenta[*a,d*]cyclooctane) [4–6] and bicyclo[9, 3, 1]pentadecane skeletons were reported [7] to occur in *H. rosea*. Examination of a sample of the aerial part of *H. forskalei* collected in Abha, Saudi Arabia, has now led to the isolation and characterization of a new fusicoccane diterpene derivative, hypoestenone (**1**) as well as the known lignan (+)-sesamin [8–10].

RESULTS AND DISCUSSION

A *n*-hexane extract of *H. forskalei* was partitioned between *n*-hexane and acetonitrile. The latter fraction was flash-chromatographed on silica gel to give diterpene **1** in 0.025% yield. Compound **1** was fluorescent under short-wave UV light and was obtained as crystals that analysed for the molecular formula $C_{20}H_{28}O_2$. Its fusicoccane (dicyclopenta[*a,d*]cyclooctane) carbon skeleton was suggested on the basis of its ^{1}H and ^{13}C NMR spectral data [4, 5] (Tables 1 and 2). Hypoestenone (**1**) demonstrated the presence of two carbonyl groups (ν_{max} 1710 cm^{-1}, δ_C 209.1; ν_{max} 1745 cm^{-1}, δ_C 220.1), a trisubstituted double bond (δ_{C-8} 134.9, δ_{C-9} 128.2) and a tetrasubstituted double bond conjugated with a ketone (δ_{C-3} 172.5, δ_{C-4} 139.0). Upon epoxida-

Table 1. ^{1}H NMR spectral data and coupling constants (in Hz in parentheses) for diterpenes* **1** and **2**

H	**1**	**2**
2	3.28 *d* (15.5)	3.53 *d* (15.5)
	1.83 *d*[a]	1.83 *br d* (15.5)
6	2.50 *br d* (14.1)	2.56 *br d* (15.0)
	2.48 *br d* (14.1)	2.55 *br d* (15.0)
7	3.92 *br s*	2.72 *br s*
9	5.67 *t* (7.7)	2.92 *dd* (5.3, 9.6)
10	2.65 *ddd* (8.5, 11.5, 14.0)	2.60 *ddd* (5.3, 9.3, 14.2)
	2.33 *m*	1.78 *m*
11	2.08 *br dd* (7.3, 11.5)	2.27 *br dd* (7.0, 11.2)
12	1.96 *m*	2.04 *m*
13	2.43 *dd*[b]	2.46 *dd*[c]
	2.38 *dd*[b]	2.49 *dd*[c]
15	0.92 *s*	0.82 *s*
16	1.77 *t* (1.8)	1.80 *t* (1.6)
17	1.49 *s*	1.05 *s*
18	1.83 *m*[a]	1.72 *m*
19	1.06 *d* (6.6)[d]	1.05 *d* (6.7)[d]
20	0.92 *d* (6.5)[d]	0.93 *d* (6.7)[d]

*All spectra recorded at 300 MHz. Assignments were aided by 2D NMR ^{1}H–^{1}H COSY and LR-COSY experiments.

[a,b,c]Signals partially superimposed on each other; *J* unresolved.

[d]Interchangeable signals.

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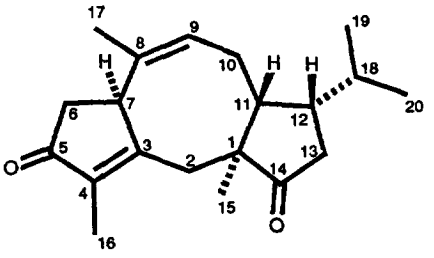
Table 2. ^{13}C NMR spectral data for diterpenes* **1** and **2**

C	1	2
1	49.8 (*s*)†	48.9 (*s*)
2	36.2 (*t*)	37.6 (*t*)
3	172.5 (*s*)	167.7 (*s*)
4	139.0 (*s*)	141.2 (*s*)
5	209.1 (*s*)	207.7 (*s*)
6	37.1 (*t*)	36.0 (*t*)
7	42.7 (*d*)	44.5 (*d*)
8	134.9 (*s*)	59.6 (*s*)
9	128.2 (*d*)	63.9 (*d*)
10	26.1 (*t*)	27.4 (t)
11	55.3 (*d*)	49.8 (*d*)
12	44.4 (*d*)	44.0 (*d*)
13	41.9 (*t*)	42.1 (*t*)
14	220.1 (*s*)	219.6 (*s*)
15	16.6 (*q*)	16.4 (*q*)
16	8.7 (*q*)	8.4 (*q*)
17	18.4 (*q*)	16.5 (*q*)
18	30.4 (*d*)	30.4 (*d*)
19	23.3 (*q*)[a]	23.1 (*q*)[a]
20	24.0 (*q*)[a]	23.7 (*q*)[a]

*All spectra recorded at 75 MHz. Assignments were aided by 2D NMR COSY and HETCOR and long-range HETCOR experiments.

†Letters in parentheses designate multiplicity as established by APT and DEPT experiments.

[a]Interchangeable signals.



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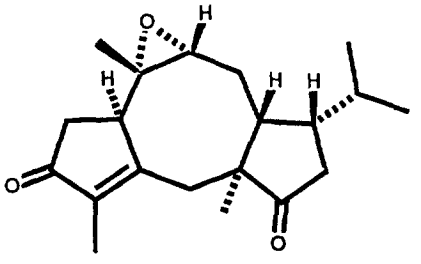

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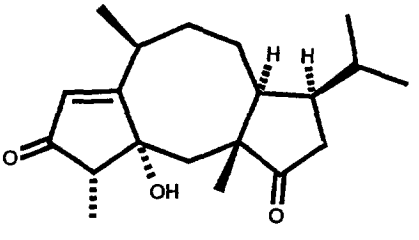

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tion, **1** yielded the corresponding 8(9)α-epoxide **2**, the gross structure of which was substantiated by extensive 2D NMR experiments involving the determination of its COSY, HETCOR, long-range COSY and long-range HETCOR spectra. The latter demonstrated the key three-bond correlations between the signals at δ_H 1.80 (Me-16) and δ_C 207.7 and 167.7 (C-5 and C-3, respectively), δ_H 1.05 (Me-17) and δ_C 63.9 (C-9), and δ_H 0.82 (Me-15) and δ_C 219.6 (C-14). Compound **2** is also formed by the autoxidation of **1** when it is exposed to light at room temperature.

An X-ray crystallographic analysis unambiguously established the structure and relative stereochemistry of **2**. Bond lengths (Fig. 1) are all in accord with expectations [11]. A view of the solid-state conformation is presented in Fig. 2.* The cyclopentenone ring is fairly flat while the cyclopentanone ring approximates to an envelope form with C-12 as the out-of-plane atom. The central cyclooctane ring is best described as being in a distorted boat–chair conformation.

Hypoestenone (**1**) has the same carbon skeleton as the fusicoccins, which are a group of plant growth regulators isolated from the cultures of fungus *Fusicoccum amydali* [12, 13]. The cotylenins are glycosidal leaf growth substances of fungal origin and possess the same carbon skeleton [14]. Anadensin (**3**) is a related metabolite isolated [15] from the liverwort *Anastrepta orcandensis*. It is intriguing to note that hypoestenone (**1**) has a unique stereochemistry at C-1, which carries an α (relative stereochemistry) methyl group, while all other fusicoccanes, including those isolated from higher plants [4–6], are epimeric at this position.

*Endocyclic torsion angles (ω_{ij}, σ 0.2–0.4°) about bonds between atoms *i* and *j* follow: $\omega_{3,4}$ −2.0, $\omega_{4,5}$ -2.4, $\omega_{5,6}$ 5,6, $\omega_{6,7}$ −6.3, $\omega_{7,3}$ 5.4° in the cyclopentenone ring; $\omega_{1,2}$ 89.0, $\omega_{2,3}$ −50.4, $\omega_{3,7}$ −48.6, $\omega_{7,8}$ 90.5, $\omega_{8,9}$ −5.6, $\omega_{9,10}$ −80.7, $\omega_{10,11}$ 89.2, $\omega_{11,1}$ −78.3° in the cyclooctane ring; $\omega_{1,11}$ 29.8, $\omega_{11,12}$ −43.6, $\omega_{12,13}$ 39.5, $\omega_{13,14}$ −23.2, $\omega_{14,1}$ −3,9° in the cyclopentanone ring.

EXPERIMENTAL

Mp: uncorr.; IR: KBr; ^1H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$, TMS as int. standard; standard Varian software was used for APT, DEPTGL, 2D NMR COSY, HETCOR, long-range COSY and long-range HETCOR, which aided structural assignments; CI-MS: recorded on a Finnigan 3300 MS, using CH_4 as ionizing gas; $[\alpha]_D$: at ambient temp. in $CHCl_3$,

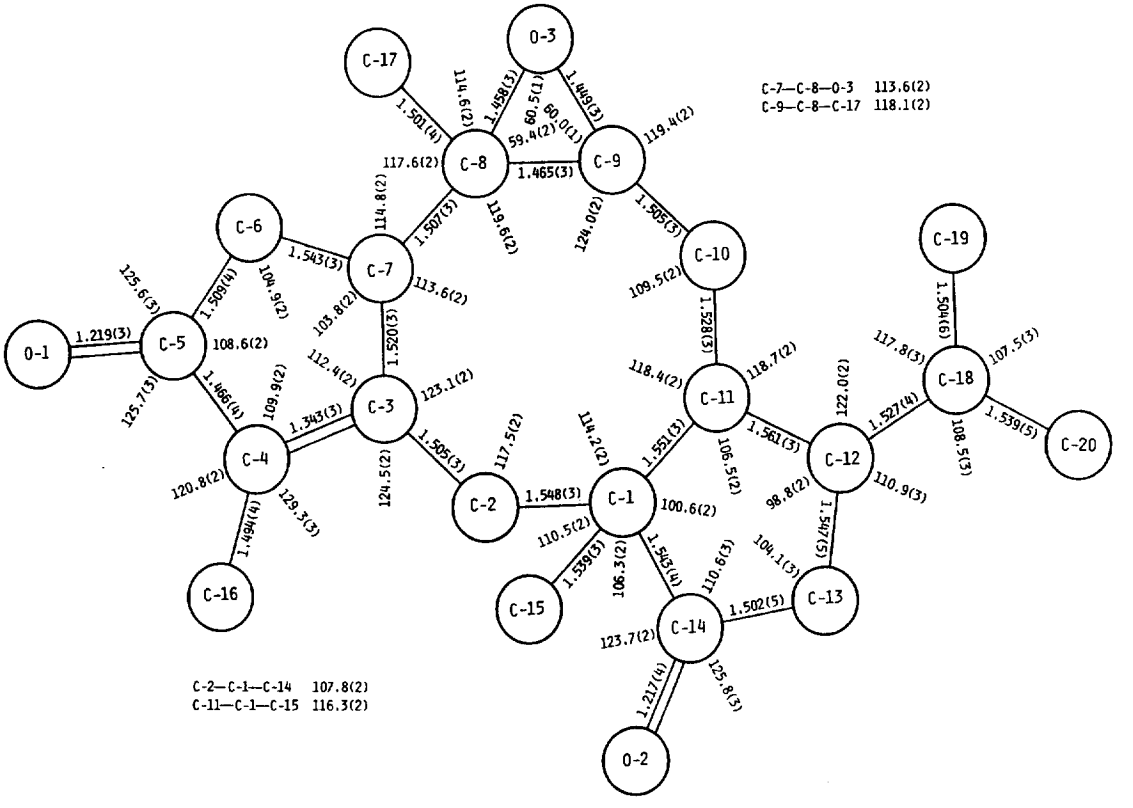

Fig. 1. Bond lengths (Å) and bond angles (°) for compound **2**; estimated standard deviations are in parentheses.

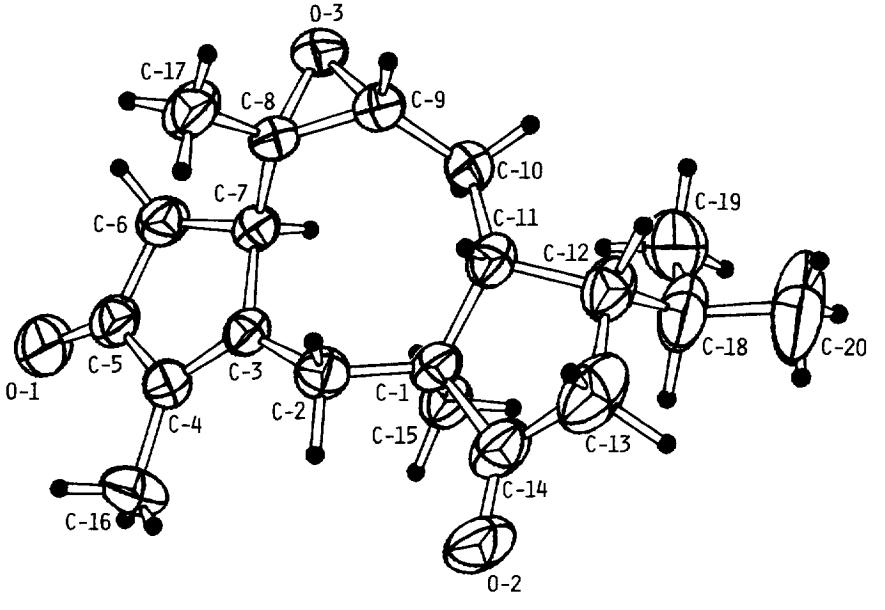

Fig. 2. ORTEP diagram (50% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of compound **2**; small filled circles represent hydrogen atoms.

using a Perkin-Elmer 241 MC polarimeter. TLC: silica gel, *n*-hexane–EtOAc (15:5) as solvent, with visualization by short-wave (λ_{max} 254 nm) UV light and 1% vanillin–H_2SO_4 spray reagent; Centrifugal preparative TLC (CPTLC, using Chromatotron®, Harrison Research Inc. model 7924): 1 mm silica gel P_{254} disk, using a flow rate of 2 ml min^{-1}. The aerial parts of *H. forskalei* were collected in Abha, Saudi Arabia, in June 1993. A voucher specimen has been deposited at the herbarium of MAPPRC, College of Pharmacy, KSU, Riyadh, Saudi Arabia.

Isolation of hypoestenone (**1**). Ground aerial parts of *H. forskalei* (500 g) were continuously extracted with *n*-hexane in a Soxhlet for 48 hr. The gummy residue (15 g), obtained after evapn *in vacuo*, was partitioned between *n*-hexane (300 ml) and MeCN (4 × 75 ml) presatd with each other. The MeCN partition (6.5 g) was subjected to flash CC over silica gel using *n*-hexane–EtOAc (19 : 1) as a solvent to afford a pale yellow fr. (480 mg) that was subjected to CC over silica gel using 3% Me_2CO in petrol (bp 60–80°) as solvent to yield hypoestenone (**1**) as needles (125 mg, R_f 0.35), followed by (+)-sesamin as plates [105 mg, R_f 0.31, mp 126–127°, $[\alpha]_D$ +75° (*c* 0.04, $CHCl_3$); Lit. mp 123–124°, $[\alpha]_D$ +78.4° [10]. The identity of (+)-sesamin was confirmed by comparison of its physical (mp and OR) and spectroscopic (^1H and ^{13}C NMR, and MS) data with lit. values [8, 9].

Hypoestenone (**1**). Plates from *n*-hexane–EtOAc and sharp needles from petrol (40–60°)-Me_2CO; mp 98–99°; $[\alpha]_D$ +194° (*c* 0.64; $CHCl_3$); UV λ_{max}^{MeOH} nm: 207 (log ε 4.15) and 237 (log ε 2.93); IR ν_{max}^{KBr} cm^{-1}: 1745 (CO), 1710 ($\alpha\beta$-unsaturated CO), 1635, 1460, 1395, 1070 and 860; ^1H- and ^{13}C-NMR: Tables 1 and 2; CI-MS *m/z* (rel. int.): 301 $[MH]^+$ (100), 203 (10) and 110 (25).

Preparation of 8(9)α*-epoxyhypoestenone* (**2**). Compound **1** (75 mg) in CH_2Cl_2 (10 ml) was treated by dropwise addition of *m*-chloroperbenzoic acid (100 mg) in CH_2Cl_2 (10 ml) at room temp. and stirred for 1 hr. The reaction mixt. was then stirred with a 5% aq. sodium metabisulphite soln for 25 min, followed by 10% $NaHCO_3$, and the crude epoxide was obtained from the organic phase as a gum (70 mg). The major product **2** was purified from minor side-products by chromatography (CPTLC, 1 mm silica gel P_{254} disc; solvent: 6% EtOAc–*n*-hexane) to provide sharp needles (*n*-hexane–Me_2CO; 50 mg); mp 178–179°; $[\alpha]_D$ +77° (*c* 0.06, $CHCl_3$); UV λ_{max}^{MeOH} nm: 207 (log ε 4.25) and 236 (log ε 2.98): IR ν_{max}^{KBr} cm^{-1}: 1735 (CO), 1700 (α,β-unsaturated CO), 1640, 1460, 1385, 1070, 1060 and 885; ^1H- and ^{13}C-NMR: Tables 1 and 2; CI-MS *m/z* (rel. int.): 317 $[MH]^+$ ($[C_{20}H_{28}O_3 + H]^+$, 100).

X-ray crystallographic analysis of 8(9)α*-epoxyhypoestenone* (**2**). Crystal data: $C_{20}H_{28}O_3$; M_r = 316.44, orthorhombic, space group $P2_12_12_1(D_2^4)$–No. 19 from the Laue symmetry and systematic absences *h*00 when $h \neq 2n$, 0*k*0 when $k \neq 2n$, 00*l* when $l \neq 2n$; a = 10.406(2) Å, b = 20.034(4) Å, c = 8.487(2)Å, V = 1769(1) $Å^3$, Z = 4, D_{calc} = 1.188 g cm^{-3}, $\mu(CuK_\alpha$ radiation, λ = 1.5418Å) = 5.8 cm^{-1}; crystal dimensions: 0.10 × 0.10 × 0.60 mm.

Preliminary unit-cell dimensions and space group information were derived from oscillation and Weissenberg photographs. One octant of intensity data (2086 reflections) was recorded on an Enraf-Nonius CAD-4 diffractometer [CuK_α radiation, graphite monochromator; ω-2θ scans, θ_{max} = 75°; scanwidth (1.20 + 0.14tan θ)°]. The intensities of four reference reflections, monitored every 2 hr during data collection, showed no significant variation (<1% overall). Refined unit-cell parameters were determined from the diffractometer setting angles for 25 reflections ($35° < \theta < 40°$) widely separated in reciprocal space. The usual Lorentz and polarization corrections were applied to the intensity data; 1681 reflections with $I > 3.0\sigma(I)$ were retained for the structure analysis.

The crystal structure was solved by direct methods (MULTAN11/82). Initial coordinates for all non-hydrogen atoms were obtained from an *E*-map. Positional and thermal parameters (at first isotropic and then anisotropic) of these atoms were adjusted by means of several rounds of full-matrix least-squares calculations {$\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized}. Hydrogen atoms were located in a difference Fourier synthesis, and their positional and isotropic thermal parameters were included as variables during the subsequent least-squares cycles. In the later iterations, an extinction correction *g* was also refined. The parameter refinement converged (max. shift: esd = 0.03) at R = 0.042 (R_w = 0.059, GOF = 1.62, g = 5.1 (4) × 10^{-6}) where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma|F_o|^2]^{1/2}$, $GOF = [\Sigma w(|F_o| - F_c|)^2/(N_{observations} - N_{parameters})^{1/2}$. Attempts to establish the absolute stereochemistry by use of anomalous scattering effects were inconclusive. A final difference Fourier synthesis contained no unusual features ($\Delta\rho$: max. 0.29, min. −0.12). Atomic parameters, bond lengths, bond angles, and torsion angles have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

Crystallographic calculations were performed on PDP11/44 and Micro VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from ref. [16].

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