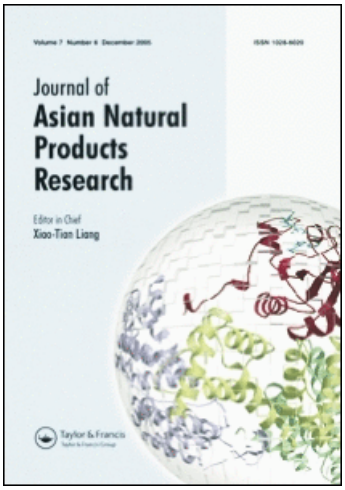

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 09 September 2010

To cite this Article Al-Rehaily, Adnan J. , Al-Yahya, M. A. , Mirza, Humayun H. and Ahmed, Bahar(2002) 'Verticillarone: A new seco- Fusicoccane Diterpenoid Ketonepoxide from Hypoestes verticillaris', Journal of Asian Natural Products Research, 4: 2, 117 — 122
To link to this Article: DOI: 10.1080/10286020290027399
URL: http://dx.doi.org/10.1080/10286020290027399

Journal of Asian Natural Products Research, 2002 VOL. 4 (2), pp. 117–122

VERTICILLARONE: A NEW *SECO*- FUSICOCCANE DITERPENOID KETONEPOXIDE FROM *HYPOESTES VERTICILLARIS*

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(Received 16 August 2001; Revised 20 September 2001; In final form 11 October 2001)

The aerial parts of *Hypoestes verticillaris* (L.F.) Sol. (Acanthaceae) has afforded a new *seco*-fusicoccane diterpenoid ketonepoxide, which was characterized as 1(10) *seco*-fusicocc-3 (4)-ene-5, 11, 14-trione-8 (9), 1 (7)-diepoxide (**1**) on the basis of spectral analysis and named as verticillarone, along with earlier reported diterpenoid, i.e. 13-hydroxy-7-oxo-labda-8, 14-diene (**2**).

Keywords: *Hypoestes verticillaris*; Verticillarone; S*eco*-fusicoccane diterpenoid ketonepoxide; *Jungermannia appressifolia*; 13-Hydroxy-7-oxo-labda-8; 14-diene

INTRODUCTION

Hypoestes verticillaris (L.F.) Sol. (Acanthaceae) commonly known as *Ghazarul-Aqar* in Arabic [1] is wildly distributed in Africa and Asia including Saudi Arabia, especially in the western region and is used in the treatment of chest and heart diseases, gonorrhea [2] and cancer [3]. The extract of the plant has also been reported to possess antineoplastic activity against KB cell line from a human nasopharynx carcinoma at a confirmed level (ED_{50} 8.8–0.001 μg/ml) of cell growth inhibition as well as against P-388 murine lymphocytic leukemia (PS system) (T/C 131–155% at 400 mg/kg) [4]. Previous phytochemical reports have shown the presence of antineoplastic phenanthroindolizidine alkaloids namely hypoestestatin and 14-hydroxy hypoestestatin [4] from East African shrub. We now report herein a new fusicoccane type diterpenoid, which has been characterized as 1(10) *seco*-fusicocc-3 (4)-ene-5, 11, 14-trione-8 (9), 1 (7)-diepoxide (**1**) on the basis of spectral analysis and named as verticillarone, along with earlier reported labdane-type diterpenoid, i.e. 13-hydroxy-7-oxo-labda-8, 14-diene (**2**) isolated from *Jungermannia appressifolia* [5].

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ISSN 1028-6020 print/ISSN 1477-2213 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10286020290027399

RESULTS AND DISCUSSION

Compound **1** named verticillarone was obtained as colourless needles and has the molecular composition $C_{20}H_{26}O_5$ as established on the basis of HRMS (M^+ 346.4174), elemental analysis, ^{13}C NMR and DEPT spectra. The IR spectrum indicated the presence of ketonic group (1740), α, β-unsaturated ketone (1700), double bond (1600) and oxirane ring (1250, 850, 760 cm^{-1}). The ^{13}C NMR and DEPT spectra [6] showed 20 carbon atoms for the molecule consisting of five methyls, four methylenes, three methines, five quaternary and three carbonyl carbon atoms (in total $C_{20}H_{26}$). The sequential assignments of protons and carbon atoms were made with the help of $^1H-^1H$ COSY and HMQC experiments starting with easily distinguishable carbinyl proton at δ4.075, which was assigned at position 9 (δ_C 78.41) and further correlated with HMBC spectrum (Fig. 1, Table II). The ^{13}C NMR spectrum exhibited signals at δ168.51 (quaternary carbon) and 137. 33 (quaternary carbon) for a double bond attributable at position $\Delta^{3(4)}$ of ring-A on the basis of long-range couplings in HMBC spectrum, which exhibited correlations of C-3 with H-2, H-6 and H-16; and of C-4 with H-6 and H-16 (Fig. 1).

The five methyl functionalities were found to be attached at positions 1 (δ_H 1.322, *s*; δ_C 27.122), 4 (1.741, *t*, $J = 1.5$ Hz; 8.32), 8 (0.874, *s*; 19.59) and 18 (0.966, *d*, $J = 2.0$ Hz; 16.50 and (0.980, *d*, $J = 2.0$ Hz; 17.08) with the help of $^1H-^1H$ COSY and HMBC spectra. The ^{13}C NMR spectrum displayed three signals at δ207.71, 210.57 and 213.42 due to three carbonyl groups located at C-5, C-14 and C-11 respectively as observed from long-range couplings in HMBC spectrum. The ^1H- and ^{13}C-NMR spectra exhibited signal at δ_H 4.075 (1H, *d*, $J = 8.0$ Hz) and δ_C 78.41 due to one carbinyl proton, which exhibited long range coupling in HMBC with C-8, C-10 and C-11 and thus could be placed at position-9. The coupling interaction with axial proton at position-10 indicated the β-orientation (equatorial) of methine proton and α-orientation of oxygen atom engaged in the oxirane ring-D [7].

The ^{13}C-NMR spectrum exhibited three signals at δ 80.38, 85.02 and 83.60 due to three quaternary carbons attached to oxygen atoms of the oxirane rings. The absorption bands in IR spectrum at 1250, 850 and 760 cm^{-1} also supported the presence of oxirane rings. The compound was not affected by acetylation with Ac_2O/pyridine indicating the absence of free hydroxyl group in the molecule and hence it was concluded that the two oxygen atoms were engaged in oxirane rings. Long-range couplings in HMBC showed that the peak at δ_C 83.60 was attributed to C-8 linked with oxygen atom of oxirane ring-D; while the peaks at 80.38 and 85.02 were respectively attributed to C-1 and C-7 indicating the linkage of the second oxygen atom of the oxirane ring-C with C-7 and C-1 (Fig. 1). The chemical shifts and locations of the remaining methylene and methine groups were also assigned by long-range correlations in HMBC (Fig. 1). In addition, no correlation was observed between H-2 with C-10 indicating the absence of a linkage between C-1 and C-10.

The mass spectrum of the compound exhibited prominent peaks at *m/z* 318, 304, 179, 163, 150, 135, 122, 107, 96, 93, 69 and 55 due to different fragments supporting the proposed structure of the compound. The spectral data of the compound were also compared with fusicoccane type diterpenoids isolated from *Hypoestes forskalei* [8,9], which showed a close resemblance indicating it to be a fusicoccane type diterpenoid.

Thus on the basis of above chemical and spectral analysis the structure of the compound was elucidated as 1(10) *seco*-fusicocc-3 (4)-ene-5, 11, 14-trione-8 (9), 1 (7)-diepoxide and named verticillarone (**1**).

The compound (**2**) was characterized as 13-hydroxy-7-oxo-labda-8, 14-diene and the spectral data were compared with the reported values of the compound isolated from *Jungermannia appressifolia* [5].

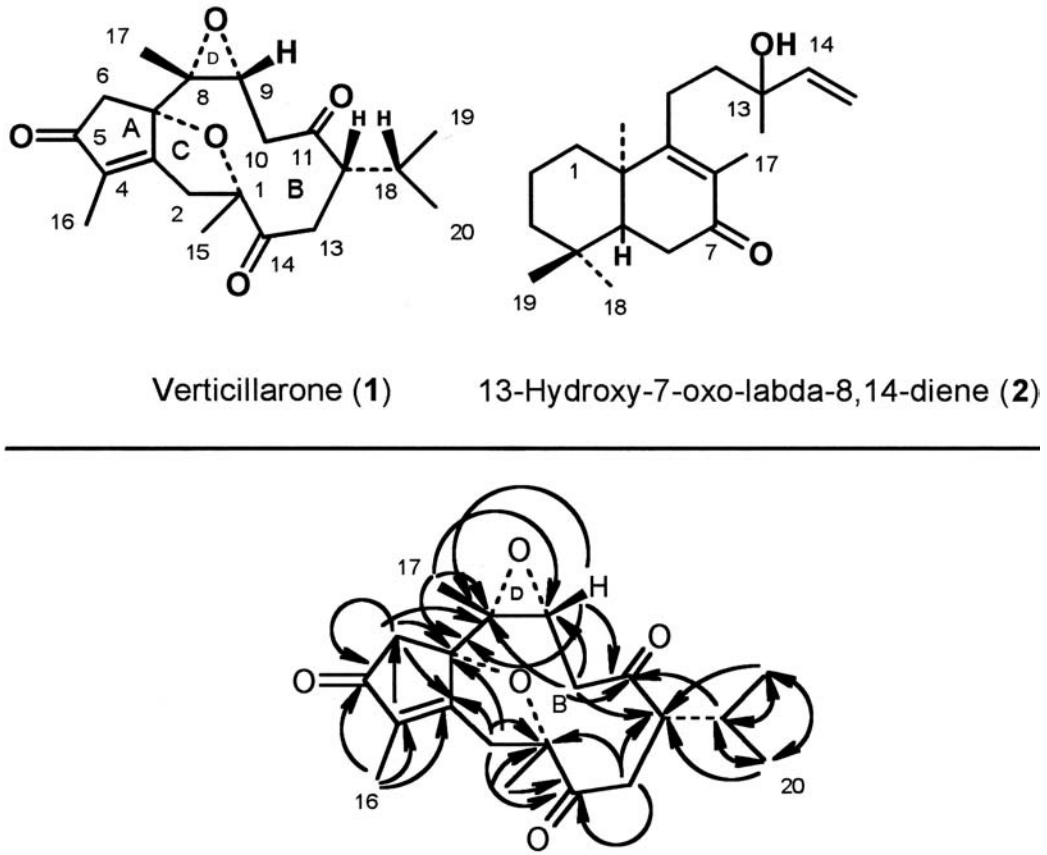

FIGURE 1 Significant heteronuclear multiple bond correleations (HMBC) for verticillarone (1). Arrows point from proton to carbon.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on Metler 9100 Electro thermal apparatus by open capillary method and are uncorrected. The IR spectra were recorded as KBr pellets on PYE UNICAM Spectrophotometer. The mass spectra were recorded on a Finnegan MAT 300 mass spectrometer, and relative intensities have been given in parentheses. The ^{1}H (500 MHz) and ^{13}C & DEPT 90 and 135 NMR (125 MHz) and 2D NMR (COSY, HMBC & HMQC) were recorded on Bruker DRX 500 spectrometer in DMSO-d_6 using TMS as internal standard reference. The elemental analysis was performed on Perkin Elmer CHNSO analyzer, model no. 2400. The column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh) as adsorbent. TLC were performed on silica gel 60 F_{254} Merck plates and sprayed with vanillin-H_2SO_4 and Dragendroff's reagents for visualization of the spots.

Plant Material

The aerial parts of *H. verticillaris* were collected on 2 February 1995 from *Wadi-Haram*, Taif region of western Saudi Arabia and identified by a taxonomist Dr Sultanul-Abedin, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen no. 11174 has been deposited in the herbarium of the center for future reference.

Extraction and Isolation

Air-dried aerial parts (2.5 kg, leaves and stems) were crushed to coarse powder and extracted thrice with 95% alcohol. The alcoholic extract was concentrated and dried under reduced pressure to get a viscous mass (80 gm). It was then defatted with acetonitrile, the filtrate after removal of the fat was concentrated to dryness and subsequently fractionated into petroleum ether (15 gm), chloroform (10 gm) and methanol (13 gm) soluble portions. The chloroform fraction was chromatographed on a column of silica gel and successively eluted with chloroform and ethyl acetate in order of increasing polarity, which afforded compound **1** (90 mg) on elution with chloroform-EtOAc (9:1). Further elution with chloroform-EtOAc (4:1) gave 13-hydroxy-7-oxo-labda-8, 14-diene (**2**) (60 mg).

Verticillarone (1)

Verticillarone (**1**) was obtained as colourless needles from hexane-methanol (1:1), 90 mg, m p 145–146°C; R_f 0.60 ($CHCl_3$: EtOAc, 7:3); IR (KBr) ?$_{max}$ 2950, 2850, 1740 (C=O), 1700 (α, β-unsaturated ketone), 1600, 1460, 1350, 1050 (C–O), 1250, 850, 760 (oxirane ring) cm^{-1}; 1D-NMR data, see Table I; 2D-NMR data, see Table II; HRMS m/z [M^+] 346.4274 (10) (calcd for $C_{20}H_{26}O_5$ 346.4272), EIMS m/z [M^+] 346 (2), 318 (3), 304 (1), 275 (1.5), 257 (1), 229 (1), 205 (1), 179 (12), 163 (2), 150 (8), 137 (10), 135 (5), 122 (3), 121 (4), 107 (7), 96 (5), 97 (10), 93 (8), 83 (34), 69 (11), 55 (7), 43 (100), 41 (30); anal. C 69.45%, H 7.63%, calcd for $C_{20}H_{26}O_5$, C 69.34%, H 7.56%

TABLE I 1D-NMR data of verticillarone **1**

Positions	^{1}H-NMR*		^{13}C-NMR	DEPT†
	a	b		
1	–	–	80.38	C
2	3.362 *d* (14.0)	2.79 *d* (14.0)	44.14	CH_2
3	–	–	168.51	C
4	–	–	137.33	C
5	–	–	207.71	C
6	2.421 *d* (18.0)	1.985 *d* (18.0)	34.91	CH_2
7	–	–	85.02	C
8	–	–	83.60	C
9	–	4.075 *dd* (7.5, 7.2)	78.41	CH
10	2.691 *ddd* (11.5, 8.5, 2.5)	2.668 *ddd* (11.5, 8.5, 2.5)	37.74	CH_2
11	–	–	213.42	C
12	–	3.532 *dddd* (5.5, 2.5, 4.5, 1.5)	38.52	CH
13	2.399 *ddd* (11.0, 7.5, 2.5)	1.852 *dd* (12.0, 2.5)	36.59	CH_2
14	–	–	210.57	C
15	1.322 *s*	–	27.12	CH_3
16	1.741 *t* (1.5)	–	8.32	CH_3
17	–	0.874 *s*	19.59	CH_3
18	–	1.768 *septet* (1/2 w 9.0)	38.44	CH
19	0.966 *d* (6.9)	–	16.50	CH_3
20	0.980 *d* (6.9)	–	17.08	CH_3

* Assignments were based on ^{1}H–^{1}H- and ^{1}H–^{13}C-COSY, and HMQC experiments, a and b denotes two diastereotopic methylene protons. Coupling constants in Hertz are given in parentheses; *s*: singlet, *d*: doublet, *t*: triplet.
† DEPT chemical shifts are presented at $\theta=3\pi/4$ when methylene groups reaches negative maximum.

TABLE II 2D-NMR data of verticillarone **1**

Positions	^{1}H–^{1}H COSY correlations	HMQC* (^{1}H–^{13}C COSY)	$^2J_{CH}$	HMBC† $^3J_{CH}$
1	–	80.38 *s*	–	–
2a	H-2b	44.14 *t*	C-3	C-7, C-14
2b	H-2a	44.14 *t*	C-1	C-7, C-14
3	–	168.51 *s*	–	–
4	–	137.33 *s*	–	–
5	–	207.71 *s*	–	–
6a	H-6b	34.91 *t*	C-5	C-3, C-4, C-8
6b	H-6a	34.91 *t*	C-5	C-3, C-4, C-7, C-8
7	–	85.02 *s*	–	–
8	–	83.60 *s*	–	–
9	H-10a	78.41 *d*	C-8	C-11, C-7
10a	H-9, H-10b	37.74 *t*	C-11, C-9	C-8, C-12
10b	H-10a	37.74 *t*	C-11, C-9	C-8
11	–	213.42 *s*	–	–
12	H-13a, H-18	38.52 *d*	–	–
13a	H-13b, H-12	36.59 *t*	C-14, C-12	C-1
13b	H-13a	36.59 *t*	–	–
14	–	210.57 *s*	–	–
15	–	27.12 *q*	C-1	C-14, C-13, C-4
16	–	8.32 *q*	C-4	C-5, C-3
17	–	19.59 *q*	C-8	C-7, C-9
18	H-19, H-20, H-12	38.44 *d*	C-19, C-20	C-11
19	H-18	16.50 *q*	C-18	C-20, C-12
20	H-18	17.08 *q*	C-18	C-19, C-12

* C-multiplicities were established by DEPT experiment.
† The correlations in HMBC have been shown from protons to carbons.

Acetylation of Compound 1

The compound **1** (20 mg) was treated with Ac_2O/Py (1:1), kept over night and refluxed for 6 h, on usual work up it afforded the product, which showed the same R_f value on comparison with compound **1**.

13-Hydroxy-7-oxo-labda-8, 14-diene (2)

Compound **2** was obtained as colourless needles and the data were compared with reported values [5].

Acknowledgements

The authors are thankful to Mr. Abdullah and H. Haroon Mirza for their technical assistance and Dr Charles D. Hufford, Department of Pharmacognosy, School of Pharmacy, University of Mississippi for taking the spectra of compounds.

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