A New Diterpene from *Hypoestes serpens*

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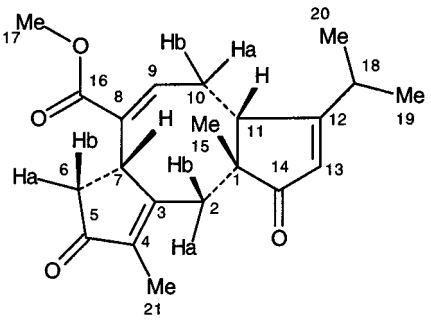
Received March 29, 2000

From a defatted chloroform extract of the aerial parts of *Hypoestes serpens* a new diterpene exhibiting relaxant activity on isolated rat aorta was obtained. The structure of this compound, named serpendione (**1**), was fully established by the interpretation of its spectral data.

Hypoestes serpens (Vahl) R. Br. (Acanthaceae) is a herbaceous plant growing in the central and south highland of Madagascar where, according to our ethnobotanical field work, it is used in traditional medicine to lower blood pressure and maintain it at a normal value for several months after only two weeks of treatment. The defatted chloroform extract from aerial parts showed strong relaxant activity in an assay using isolated rat aorta. Bioassay-guided chromatographic fractionation of this extract resulted in the isolation of a new diterpene, which was given trivial name serpendione (**1**). The present paper describes the structure elucidation of this compound. No previous investigations have been reported on this species.



Serpendione 1

Repeated Si gel column chromatography led to the isolation of **1**, the structure of which was fully established by the interpretation of its spectral data. The molecular formula of $C_{21}H_{26}O_4$ was determined by HRCIMS. An absorption band at 1670 cm^{-1} in the IR spectrum indicated the presence of an α,β-unsaturated ketone, and the absorption maximum at 232 nm in the UV spectrum was consistent with the presence of a conjugated enone chromophore.[1] The presence of a ketone functionality was further supported by the observation of signals at δ 207.8 and 210.6 ppm in the ^{13}C NMR spectrum. As evident from the ^1H NMR spectrum, the structure of serpendione includes one isopropyl, one methyl attached to a quaternary carbon, and one methyl ester. A doublet of three protons at δ 1.59 ppm (J = 2.6 Hz) was attributed to a second methyl group. Therefore, from NMR data, serpendione appeared as a fusicoccane (dicyclopenta[*a*,*d*]cyclooctane) derivative.[2] Full characterization of the structure was accomplished by examination of the COSY, HMQC, HMBC, and NOESY spectral data (Table 1). Thus the isopropyl−cyclopentenone unit was identified by the observation of a series of $^1H-^{13}C$ long-range couplings: methine proton H-18 showed cross-peaks with the trisubstituted olefinic carbons C-12 and C-13 and the aliphatic methine carbon C-11. Furthermore, H-11 (δ 2.77) exhibited long-range couplings with the methine carbon of the isopropyl group (C-18), quaternary carbon C-1 (δ 52.3), and methyl group C-15 (δ 31.1). The cyclopentenone structural unit was further substantiated by the observation of heteronuclear long-range couplings displayed by the olefinic methine proton, H-13 (Table 1). The *gem*-disubstituted allyl subunit C-8, C-9, C-10 attached to the cyclopentenone ring was easily identified from $^1H-^1H$ connectivities. Furthermore, cross-peaks between H-11 and C-9 (δ 140.9), C-15 (δ 31.1) and between methylene protons H-2 and C-11(δ 53.9) revealed the link between the cyclopentenone unit and another ring determined to be a carbomethoxy−cyclooctene on the basis of the heteronuclear long-range correlations

Table 1. ^1H NMR, ^{13}C NMR, HMBC, and NOESY Spectral Data of Serpendione (**1**)

atom	δ_H, mult, J (Hz)	δ_C	HMBC	NOESY
1		52.3		
2a	3.49 dd (13.7, 0.7)	35.6	C_1, C_3, C_4, C_7, C_{11}, C_{14}	2b,21
2b	2.16 d (13.7)		C_1, C_3, C_4, C_7, C_{11}, C_{14}, C_{15}	7, 15
3		172.2		
4		137.3		
5		207.8		
6a	2.09 dd (18.4, 4.2)	43.0	C_5, C_7, C_8	6b
6b	2.90 ddd (18.4, 6.8, 1.2)		C_3, C_4, C_5, C_7	7
7	3.94 m	44.1		
8		134.6		
9	7.05 dd (9.5, 8.5)	140.9	C_7, C_8, C_{10}, C_{11}, C_{16}	10, 11
10a	2.22 m (9.5, 8.2)	29.5	C_1, C_8, C_9, C_{11}, C_{12}	
10b	2.25 m (8.5, 7.0)		C_1, C_8, C_9, C_{11}, C_{12}	
11	2.77 ddd (8.2, 7.0, 1.2)	53.9	C_1, C_9, C_{12}, C_{13}, C_{14}, C_{15}, C_{18}	15
12		187.6		
13	5.94 dd (1.3, 1.2)	124.8	C_1, C_{11}, C_{12}, C_{14}, C_{18}	19
14		210.6		
15	1.14 s	31.1	C_1, C_2, C_{11}, C_{14}	
16		167.4		
17	3.78 s	51.8	C_{16}	
18	2.54 dqq (7.0, 6.6, 1.2)	29.2	C_{11}, C_{12}, C_{13}, C_{19}, C_{20}	10, 19, 20
19	1.19 d (6.6)	20.6	C_{12}, C_{18}, C_{20}	
20	1.12 d (7.0)	21.3	C_{12}, C_{18}, C_{19}	
21	1.59 d (2.6)	9.3	C_3, C_4, C_5	

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10.1021/np0001474 CCC: $20.00
Published on Web 01/18/2001

observed between H-9 and carbons C-11 (δ 53.9), C-7 (δ 44.1), and C-16 (δ 167.4).

Further interpretation of the ^{1}H NMR of **1** assisted by the COSY data showed that the three-proton doublet (δ 1.59 ppm, $J = 2.6$ Hz) could be attributed to CH_3-21, which displayed a long-range allylic coupling with H-7 (δ 3.94). Last, the observation of cross-peaks between CH_3-21 and the olefinic carbons C-3 (δ 172.2) and C-4 (δ 137.3), as well as the carbonyl C-5 (δ 207.8), characterized a second α,β-unsaturated ketone.

Full interpretation of the NOESY data led us to the assignment of its relative stereochemistry: a strong cross-peak observed between H-11 and H-15 indicated a cis-junction for the B and C rings, while cross-peaks between H-2a and CH_3-21 and between H-2b, CH_3-15, and H-7 supported the β-orientation of H-7. Serpendione (**1**), which has the dicyclopenta[*a*,*d*]cyclooctane skeleton previously found in other *Hypoestes* species,[3,4] appeared as a new fusisoccane derivative related to dehydrohypoestone[2] but possessing C-7β H and C-15β CH_3 stereochemistry.

The crude alcohol extract of *H. serpens* showed good relaxant activity against noradrenaline-induced contraction in a dose-dependent manner, with 7.50, 19.55, and >100% reduction at 0.5, 1, and 1.5 mg/mL of test extract, respectively ($ED_{50} = 1.19$ mg/mL). Serpendione (**1**), at a concentration of 0.05 mg/mL, strongly depressed the maximal responses to the contractile agent, with a percentage inhibition of 92.2%. These results validate the use of *H. serpens* as an herbal remedy for the treatment of hypertension. However, the structure of serpendione contains α,β-unsaturated ketones, which, in this case, might produce undesirable associated biological effects.[5,6] These results await further investigation.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.61 MHz, respectively, on a Bruker AMX-400 spectrometer at 300 K with a Bruker gradient unit and an inverse gradient triple-resonance probe-head with a self-shielded gradient coil. The ^{1}H and ^{13}C chemical shifts are expressed in parts per million (ppm) relative to TMS, coupling constants (*J*) are given in hertz.

IR was recorded on a Nicolet Impact 400D spectrometer and HRMS on a JEOL MS700 apparatus. Column chromatography was carried out on 200−400 mesh Si gel 60 (Merck).

Plant Material. Plant material was collected around Antananarivo in March 1997, and identified as *H. sepens* by Armand Rakotozafy by comparison with an authentic specimen deposited at the Department of Botany, Parc Botanique et Zoologique de Tsimbazaza. A voucher specimen has been deposited at the Institut Malgache de Recherches Appliquées of Antananarivo.

Extraction and Isolation of Serpendione. Dried and powdered aerial parts (500 g) were exhaustively extracted by repeated maceration with alcohol. The alcohol extract was then partitioned between H_2O and $CHCl_3$, and the chloroform fraction was further partitioned between MeOH−H_2O (9:1) and hexane to afford 16.5 g of residue. Of this residue, 10 g was submitted to repeated Si gel column chromatography using hexane and increasing amounts of $CHCl_3$, then $CHCl_3$ and increasing amounts of MeOH. Serpendione (**1**) was eluted with 2% $CHCl_3$−MeOH (169 mg, gum): $[\alpha]_D = +109.7°$ (*c* 0.58, $CHCl_3$); EIMS *m*/*z* 342 $[M]^+$ (90), 310 (63), 282 (100), 267 (38), 239 (47), 138 (80), 91 (100); HRCIMS *m*/*z* 343.1917 $[M + H]^+$; calcd for $C_{21}H_{27}O_4$, 343.1909; UV(EtOH) λ_{max} (log ϵ) 205 (4.14), 232 (4.12) nm; IR (film) $\nu_{max} = 1670$ cm.

Assays on Rat Isolated Aorta. Rats weighing 250−300 g were killed by cervical dislocation and exsanguination. The aorta was immediately cut through as near the heart as possible, then transferred to a dish containing Kreb's solution and cut spirally to produce a continuous strip. It was then placed in a 20-organ bath containing Kreb's solution maintained at 37 °C and aerated continuously with carbogen (95% O_2, 5% CO_2). The length of the isolated aorta was subjected to a tension of 2 g. After a stabilization period of 2 h, crude extract of *H. serpens* was assayed on the contractions induced by noradrenaline. To this end, strength of contractions induced by noradrenaline was recorded isometrically using a force-displacement transducer (Ugo Basile Appelex). A contractile response to 10^{-5} M noradrenaline was first determined twice with rinsing prior to addition of the contractile agent. When the contraction had reached a constant value, rising concentrations of crude extract of *H. serpens* (0.5, 1, and 1.5 mg/mL) were cumulatively added without intervening rinses. In separate experiments, 0.05 mg/mL of serpendione (**1**) was added. Reduction of contraction was expressed as percentage of the maximal contraction induced by noradrenaline. At the end of each experiment, the response capability of the isolated aorta was verified. Experiments were repeated on three independent occasions.

Acknowledgment. We thank Professor Adolphe Randriantsoa for monitoring the bioassays on isolated organs.

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NP0001474