

Assufulvenal, a Novel Bis-sesquiterpene from *Joannesia princeps*^{†,‡}

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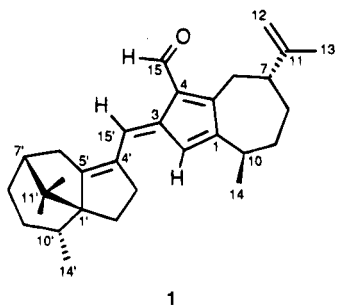
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From the root bark of *Joannesia princeps* assufulvenal (**1**), an unusual fulvenic plant constituent with a bis-sesquiterpene skeleton has been isolated. Its structure was elucidated by spectroscopic methods and corroborated by X-ray crystallographic analysis.

Joannesia princeps Vell. (Euphorbiaceae) is indigenous to subtropical and coastal regions of Brazil.² It grows into a large tree even on poor soil and is named "anda assu" by the native people.² The plant is cultivated in Africa and Asia.³ Its seeds contain an oil that is applied in folk medicine as a strong purgative⁴ and also has been used for industrial purposes.⁵ The stem bark and the leaves are reported to be fish toxins.⁶ Preliminary studies revealed extracts of the root bark to exhibit significant antibacterial, antifungal, and cytotoxic activity. Cyperenal and cyperenol have been detected as the major bioactive principles.⁷

In the course of the chromatographic separation of the petroleum ether extract from the root bark, an intensely red minor component was isolated, whose spectroscopic investigation revealed the unusual structure of a bis-sesquiterpene containing the feature of a fulvene aldehyde moiety. Consequently, the novel plant constituent was named assufulvenal (**1**).



Compound **1** formed red orthorhombic crystals (mp 225–230 °C); it was optically active ($[\alpha]_D^{21} -171^\circ$) and exhibited a strong long-wave maximum at 377 nm in the UV/vis spectrum.

The EIMS was dominated by the molecular ion at m/z 416 (HREIMS: $C_{30}H_{40}O$). The ^{13}C -NMR spectrum showed 30 resonance signals (Table 1) from which 14 could be correlated to C-1 through C-14 of cyperenal,⁸ thus establishing the patchoul-4-ene moiety **a** as a structural element of **1** (Figure 1). This result was

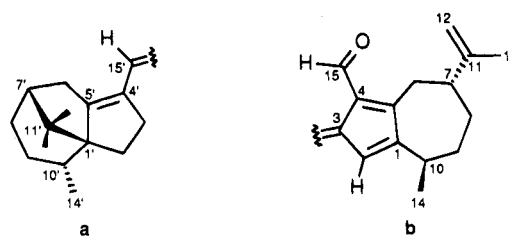


Figure 1. Spectroscopically established structural elements **a** and **b**.

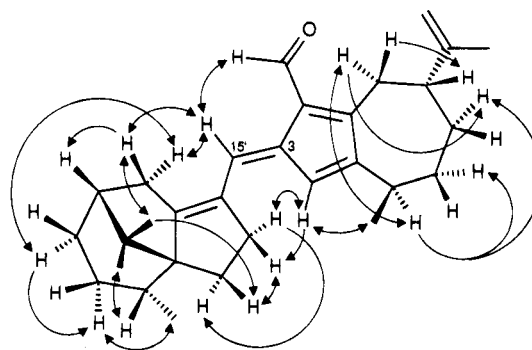


Figure 2. Important enhancements observed in NOE studies of **1**.

corroborated by the 1H -NMR spectrum.

Further NMR studies and particularly HMBC (Table 1) determined partial structure **b** for the remaining 15 carbon atoms with a guaiatriene carrying an aldehyde group at C-4. HMBC, in addition, revealed the connection between partial structures **a** and **b**.

NOE measurements established the *E*-configuration of the C-3,C-15'-double bond, as well as the relative configurations within the ring systems of **a** and **b** (Figure 2).

X-ray studies corroborated the deduced structure and revealed the relative configuration between **a** and **b** as depicted in formula **1** (Figure 3).

Assufulvenal (**1**) constitutes a new type of C_{30} -terpenoid characterized by the intramolecular linkage of a guaiane- and a patchoulane-type sesquiterpene moiety with the connecting bond forming part of a fulvene system.

Experimental Section

General Experimental Procedures. The mp was determined on a Kofler hot-stage apparatus and is

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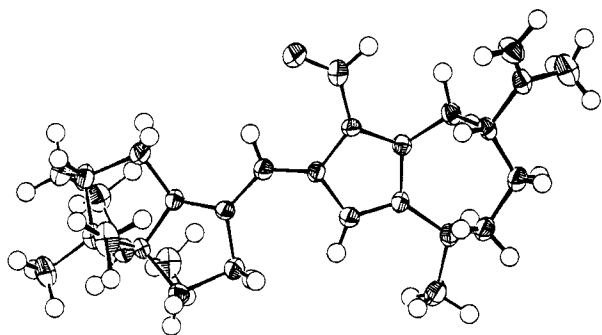
[†] Constituents of Tropical Medicinal Plants. 73. For Part 72, see ref 1.

[‡] Dedicated to Prof. Dr. R. Neidlein, Heidelberg, on the occasion of his 65th birthday.

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Table 1. NMR Spectral Data of Compound **1** (δ (ppm); J (Hz))

position	DEPT	^{13}C	^1H	H-C long-range correlation to ^{13}C signal at:
1		152.9		
2	CH	121.7	6.62 (s)	34.3, 127.3, 136.5, 152.9, 162.1
3		136.5		
4		127.3		
5		162.1		
6	CH ₂	32.4	2.50 (dd; 13.5, 11) 3.39 (d; 13.5)	37.4, 47.8, 127.3, 162.1 37.4, 47.8, 127.3, 150.8, 152.9, 162.1
7	CH	47.8	2.00 ^a	
8	CH ₂	37.4	1.68 ^a 1.97 ^a	
9	CH ₂	37.7	1.28 ^a 1.87 ^a	
10	CH	34.3	2.61 (m)	37.7, 152.9
11		150.8		
12	CH ₂	109.0	4.71 (m) 4.74 (m)	20.4, 47.8, 150.8 20.4, 47.8, 150.8
13	CH ₃	20.4	1.78 (br s)	47.8, 109.0, 150.8
14	CH ₃	20.3	1.31 (d; 6.5)	34.3, 37.7, 152.9
15	CH	185.6	10.08 (s)	127.3, 136.5
1'		67.0		
2'	CH ₂	26.5	1.67 ^a 1.79 ^a	
3'	CH ₂	39.4	2.88 (dd; 14.5, 9.5) 3.02 (m)	132.9, 170.9 132.9
4'		132.9		
5'		170.9		
6'	CH ₂	29.2	2.26 (br d; 19) 2.70 (br dd; 19, 6)	27.3, 42.0, 48.0, 170.9 27.3, 132.9, 170.9
7'	CH	48.0	2.01 ^a	
8'	CH ₂	27.3	1.39 (m) 1.92 ^a	
9'	CH ₂	28.1	1.11 (m) 1.53 (m)	27.3, 36.3 27.3, 36.3, 48.0, 67.0
10'	CH	36.3	2.11 (ddq; 13, 6.5, 6.5)	18.2, 28.1, 42.0, 67.0, 170.9
11'		42.0		
12'	CH ₃	26.1	0.79 (s)	19.5, 42.0, 48.0, 67.0
13'	CH ₃	19.5	1.01 (s)	26.1, 42.0, 48.0, 67.0
14'	CH ₃	18.2	0.84 (d; 6.5)	28.1, 36.3, 67.0
15'	CH	135.2	8.17 (s)	39.4, 121.7, 127.3, 132.9, 136.5, 170.9

^a Signal overlapped.**Figure 3.** CRYSTAN drawing⁹ of **1** (no absolute configuration is implied).

uncorrected. UV/vis and CD measurements were made in MeOH solution; the IR spectrum and the optical rotation were taken in CHCl₃. NMR spectra were conducted in CDCl₃ at 360 MHz (for ^1H NMR) and 62.8 MHz (for ^{13}C NMR); inverse heteronuclear correlations were performed with the sequences of Bax and Summers for HMBC¹⁰ and Bax and Subramanian for HMQC;¹¹ NOEs were recorded by the difference technique. The EIMS was measured at 70 eV on a Finnigan-MAT TSQ 70 instrument; unless they are key ions, only masses with $m/z \geq 100$ and intensities $\geq 10\%$ are stated. The HREIMS was obtained on a Varian-MAT 312 instrument at 70 eV by peak matching.

TLC was performed on ready-made SIL-20/UV₂₅₄ nanoplates (Macherey-Nagel) in cyclohexane/EtOAc (19:1). Detection was carried out by its native color, by UV, and with anisaldehyde reagent according to Stahl.¹² For CC, Si gel 60 (Macherey-Nagel) was used. MPLC was conducted on Si gel 60 (Macherey-Nagel) and LiChroprep RP-18 (40–60 μm , Merck) at ca. 2 bar using a Büchi gradient 687 apparatus, with detection by its color in visible light.

Plant Material. Root bark of *Joannesia princeps* Vell. (Euphorbiaceae) (syn. *Anda gomesii* Adr. Juss.³) was collected from Mazaras Botanical Gardens (near Mombasa, Kenya) in May 1995. Botanical identification was by Mr. L. B. Mwasumbi, Curator of the herbarium at the University of Dar-es-Salaam, Tanzania. A voucher specimen is held at the Institut für Pharmazie und Lebensmittelchemie der Universität Erlangen under Code No. 95/03.

Extraction and Isolation. The dried, pulverized root bark of *J. princeps* (1.95 kg) was repeatedly extracted with 7.5 L of petroleum ether at room temperature. Evaporation afforded a total of 5.6 g of extract. Chromatographic separation of the extract (5.5 g) [Si gel, cyclohexane/Me₂CO (increasing amounts of acetone up to 100%)] yielded 11 fractions. Fraction 2 (1.9 g) contained the major constituent cyperenal^{7,8} and a red pigment; further separation was achieved by CC [Si gel, cyclohexane/EtOAc (49:1)] and then MPLC

(LiChroprep RP-18, MeOH). Final purification by CC [Si gel, cyclohexane/EtOAc (49:1)] resulted in the isolation of **1** (4 mg).

Assufulvenal ((-)-*rel*-(10*R*)-3-((10*R*,*E*)-1 β ,11-cycloguai-4-en-15-ylidene)guaia-1,4,11-trien-15-al) (**1**) was obtained as intensely red crystals (CH₂Cl₂/EtOH): mp 225–230 °C; TLC *R_f* 0.43, red-brown with anisaldehyde reagent; $[\alpha]_D^{21}$ -171° (c 0.2); UV/vis λ max (log ϵ) 268 (4.19), 377 (4.41) nm; CD λ max ($\Delta\epsilon$) 250 (-5.16), 280 (+2.12), 337 (+0.92), 398 (-2.12), 435 (+1.00) nm; IR ν max 2926, 1641 cm⁻¹; EIMS *m/z* [M]⁺ 416.3079 (100) (calcd for C₃₀H₄₀O, 416.3079), 373.2533 (12) (calcd for C₂₇H₃₃O, 373.2531), 228 (14), 227.1437 (40) (calcd for C₁₆H₁₉O, 227.1436), 189 (15), 188 (10), 185 (11), 165 (11), 145 (17), 133 (14), 131 (11), 105 (12); ¹H and ¹³C NMR spectral data, see Table 1.

X-ray Crystal Structure Analysis of Assufulvenal (1). Crystal data: C₃₀H₄₀O, mol wt 416, space group *P*2₁2₁2₁, orthorhombic, *a* = 8.6822(9) Å, *b* = 14.206(1) Å, *c* = 19.979(8) Å, *Z* = 4, *D_c* = 1.123 g·cm⁻³, Mo-K α radiation: λ = 0.709 26 Å. Crystal dimensions 0.56 × 0.13 × 0.13 mm. A complete data set with 17 506 reflections was measured. The unique data set consisted of 2002 (3° ≤ θ ≤ 24° and $|F| \geq 1\sigma$) reflections. Program(s) used to solve structure: SHELXS86.¹³ Program(s) used to refine structure: CRYSTAN.⁹ The number of least squares parameters was 372, the weighting scheme was $w = 1/\sigma(F)$. Structure refinement led to *R* = 0.053 and ωR = 0.026.¹⁴

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- (14) Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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