

11 α -HYDROXY MUZIGADIOLIDE, A NOVEL DRIMANE SESQUITERPENE FROM THE STEM BARK OF *WARBURGIA UGANDENSIS*

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ABSTRACT. The stem bark of *Warburgia ugandensis* provided a new drimane sesquiterpene 11 α -hydroxy muzigadiolide whose structure was elucidated on the basis of spectral measurements.

INTRODUCTION

The tree *Warburgia ugandensis* Sprague (Canellaceae) is one of the better known medicinal plants in East Africa. It is widely distributed in Kenya highlands and spreads west into Uganda [1]. The bark of this plant is widely used in local folk medicine to alleviate toothache, rheumatism, general body pains, diarrhoea, and malaria. In addition, the leaves are sometimes used as a spice for food [2]. Previous phytochemical investigations of the *Warburgia* species *W. ugandensis* and *W. stuhlmanii* revealed a number of sesquiterpenes based on the drimane skeleton [3-7]. Some of these compounds have attracted a lot of interest because of their potent insect antifeedant activity [3], plant growth regulatory activity [8], cytotoxic activity [9], very hot taste to humans [10], piscicidal [11] and molluscicidal activity [12,13], antifungal activity [14], and activity against mitochondrial oxidative phosphorylation [15].

Our interest in the structure-activity relationship of sesquiterpene dialdehydes necessitated the re-examination of the bark of *W. ugandensis*. This study, yielded four sesquiterpenes, two of which were characterised as the previously recorded muzigadiol (2) and ugandensidial (3) [7], the third as pereniporin B (4) previously isolated from *Perenniporia medullaepanisi* [16, 17] and the fourth as the new drimane sesquiterpene 11 α -hydroxy muzigadiolide (1) (Figure 1). Like its congeners, compound (1) may exhibit interesting bioactivities. This together with the recent report by Kioy *et al.* on the isolation of a closely related derivative, muzigadiolide (5) from *W. stuhlmanii* [7] prompted us to report our results.

RESULTS AND DISCUSSION

Muzigadiol (2), ugandensidial (3) and pereniporin B (4) were characterised by comparison of their ¹H NMR, IR and mass spectral data with those of authentic samples. The CI mass spectrum ([M+H]⁺ m/z 265.1442) of the new drimane and the carbon and proton count from the ¹³C NMR established the molecular formula for 1 as C₁₅H₂₀O₂. It is a crystalline compound (m.p. 182-183 °C) with strong IR absorption at 3400 and 1730 cm⁻¹, implying the presence of a hydroxyl(s) and an α,β -unsaturated γ -lactone system. Further IR bands at 1420, 890 and 1630 cm⁻¹ indicated the presence of an exocyclic double bond and a hemiacetal. The sesquiterpenoid nature of 11 α -hydroxy muzigadiolide (1) and its close relationship to

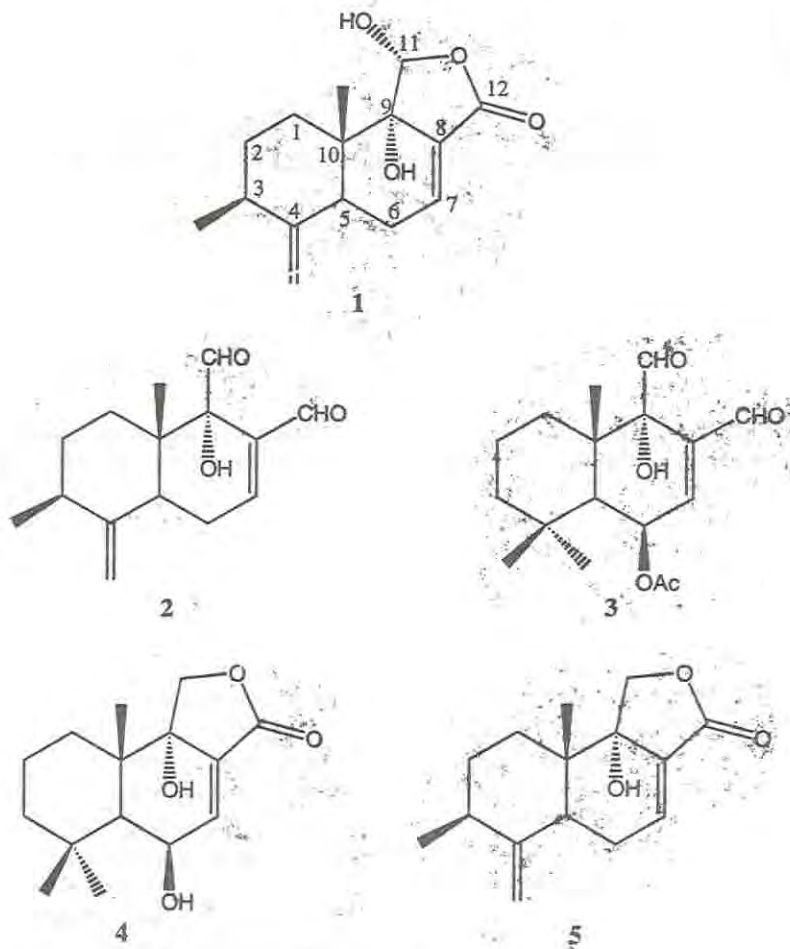


Figure 1. Chemical structures for compounds 1, 2, 3, 4, and 5.

muzigadiolide (5), previously isolated from *W. stuhlmanii* [7], was apparent from spectroscopic evidence. The mass spectrum (EIMS) showed important peaks at 246[M-H₂O]⁺, 235[M-HCO]⁺, 228[M-H₂O-H₂O]⁺ and 210[M-HCO-H₂O]⁺. The latter two fragments together with the broad peak at 5.69 ppm and ¹³C NMR signals at δ 98.6 and 76.5 supported the presence of at least two hydroxyl groups at C-11 and C-9, respectively. ¹³C-NMR spectra together with ¹H-¹³C (HMQC) correlation and DEPT experiments showed the presence of two CH₃, three CH₂, four CH, two quaternary carbons, four olefinic and one carbonyl (Table 1). This was consistent with ¹H NMR signal integration, which suggested the presence of 20 hydrogen atoms in the molecule. 2D-Homonuclear *J*-correlation (COSY) spectrum clarified the coupling protons on the linked carbons (C-1, C-2, C-3 and C-5, C-6, C-7) also, CH₃-10 showed weak *W*-type long range coupling with a signal at 2.15 ppm for H₂-1 (ax) and at 2.62 ppm for H-5. Comparison of the ¹H-NMR spectra of (1), (2) and (5) showed a close similarity. These data suggest that (1) is a re-arranged drim-7-ene sesquiterpene similar to muzigadiolide (5), in which the 11 and 12 methyls were involved in the formation of a lactone ring.

However unlike, muzigadiolide (5), compound (1) gave a broad signal at δ 6.17 representing one of the protons at C-11. This together with a peak at 98.6 ppm in the ^{13}C NMR implied the presence of a hydroxy group at C-11. The orientation of the hydroxyl group is α , this is supported by the observed deshielding of the H-1(eq) having a peak at 1.42 ppm instead of value at a higher field in the range 1.20-1.25 ppm. This assignment was further supported by molecular mechanics (MM3, Alchemy 32) calculations which were performed on the proposed compound (1) with the 11-OH α and when it is β . The compound with the 11-OH α was more stable than the β isomer by 1.05 kcal/mol, implying that compound (1) exists as the 11 α -hydroxy isomer. The second hydroxyl group was placed at C-9 due to the de-shielding of the C-5 proton which displayed a comparable chemical shift as for related compounds (2), (3), (4) and (5) all having a hydroxyl group at C-9. The presence of an enal band in the IR spectrum and appearance of H-7 downfield at 7.12 implied that C-12 is a keto group and part of a lactone. The HMBC spectrum of (1) and the cross-relaxation correlated 2D- ^1H NOE (NOESY) revealed the gross connectivity and conformation for compound (1) (Figure 1).

Table 1. NMR data for 11 α -hydroxy muzigadiolide (1)^a.

Position	^{13}C	^1H	HMBC
1	31.0	1.42 (1H, m, H-1 eq) 2.15 ^b (1H, m, H-1 ax)	
2	32.2	1.72 (1H, m, H-2 eq) 1.22 (1H, m, H-2 ax)	H-14 with C-2
3	38.3	2.09 m	H-13, H-14 with C-3
4	130.5		H-14 with C-4
5	40.2	2.62 ^c (1H, m)	H-13, H-15 with C-5
6	27.5	2.34-2.42	
7	141.4	7.12 (1H, m)	
8	130.5		
9	76.5		H-15 with C-9
10	53.7		H-15 with C-10
11	98.6	6.17 (1H, br s)	
12	167.3		H-7 with C-12
13	105.9	4.74 (1H, br s) 4.92 (1H, br s)	
14	18.5	1.09 (3H, d, J = 6.4)	
15	15.2	0.75 (3H, s)	

^aChemical shifts are in ppm from TMS, coupling constants are in parentheses in Hertz, and samples were dissolved in CDCl_3 . ^bC-1 axial hydrogen displays W coupling with C-10 Me. ^cWeak W type coupling between C-1 Me with C-5 H.

EXPERIMENTAL

The ^1H - and ^{13}C -NMR spectra were recorded in CDCl_3 on a Bruker ARX 300 MHz spectrometer. 2D NMR spectra were recorded by using Bruker's standard pulse program: in HMQC and HMBC experiments, $\Delta = 1$ s and $J = 145, 8$ Hz, respectively; the correlation maps consisted of 512×1 K data points per spectrum, each composed of 16 to 64 transients. Mass spectra were obtained on a Hewlett-Packard 5971A GC-MS or a TSQ70 FAB mass

spectrometer. IR spectra were run on a Perkin-Elmer 1760X spectrometer as a film on KBr plates.

Molecular mechanics. Molecular mechanics (MM3) calculations were performed with the Alchemy 32 version 2.0 copyright © 1997, Tripos, inc. St. Louis, Mo (U.S.A).

Plant material. *W. ugandensis* bark was collected in Eldoret, Uasin Gishu District, Kenya, in March 1990. The plant was identified by Prof. M.S. Rajab by comparison with a preserved standard specimen at the National Museum Herbarium in Nairobi, Kenya. A voucher specimen (MU/BOT/61) has been deposited in the Botany Department Herbarium Collection, Chepkoilel Campus, Moi University, Eldoret.

Extraction and isolation of compounds. *W. ugandensis* bark (2.0 Kg) was air dried, powdered and allowed to stand in 4 L MeOH at room temperature for 1 week. The extract was decanted and the residual pulp similarly extracted a second time using 4 L MeOH. The combined extracts were evaporated under vacuum and the residue partitioned between 2 L CHCl₃ and 1 L H₂O. After drying over Na₂SO₄, the CHCl₃ layer was evaporated under vacuum to yield 28 g of a brown oil. An aliquot of this (15 g) was subjected to CC over silica gel eluting with hexane and then hexane containing increasing amounts of EtOAc to give in order of elution 2 (120 mg), 3 (140 mg), 4 (60 mg) and 1 (70 mg).

Identification of compounds. The structures of the known compounds, muzigadiol (2), ugandensidial (3) and pereniporin B (4) were identified by comparison of physical data (mp, ¹H NMR, MS) with those of known standards.

11 α -Hydroxy muzigadiolide (1). Needles from acetone-hexane, mp 182-183 °C, TLC (1:1 acetone/hexane) R_f 0.5. IR ν_{\max} cm⁻¹: 3400, 1730, 1630, 1420, 1240. High resolution CIMS m/z 265.1442 [M+H]⁺, calculated for C₁₅H₂₀O₄, 265.14398. Low resolution EIMS m/z (rel. int): 246 [M-H₂O]⁺ (2), 235 (46), 228 (3), 217 (100), 203 (5), 190 (26), 185 (7), 175 (95), 161 (10), 147 (31), 133 (30), 128 (11), 119 (19), 105 (35), 91 (51).

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