Structural Revision of Harrisonin and 12 β-Acetoxyharrisonin, Two Limonoids from *Harrisonia abyssinica*

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Received April 16, 1997⊗

The structures of the known limonoids harrisonin (**1**) and 12β-acetoxyharrisonin (**2**), isolated from the root bark of *Harrisonia abyssinica*, were reanalyzed by modern 1D and 2D NMR spectroscopic methods, and the molecular structure and relative configuration of 12β-acetoxyharrisonin were determined by single-crystal X-ray diffraction. The spectral findings obtained require revision of the structures of these compounds from **1** and **2** to **3** and **4**, respectively. The new molecular structures **3** and **4** display a novel limonoid skeleton with a unique five-membered ring B.

The East African shrub *Harrisonia abyssinica* Oliv. (Simaroubaceae) ("Mkidori", "Mpapura doko", "Muliyuli", "Pedo", in local dialects) grows widely along the coast of East Africa and in Western Kenya. It is used in Kenya and Tanzania as a remedy for the treatment of fever, bubonic plague, tuberculosis, hemorrhoids, snake bite, and so forth.[1] Crude extracts of the root bark of this plant were shown to exhibit insect antifeedant, antimicrobial, cytotoxic, and plant-growth inhibitory activities.[2,3] Earlier studies on the limonoid constituents of *H. abyssinica* led to the isolation of harrisonin, 12β-acetoxyharrisonin, obacunone (**5**), atalantolide (**6**), pedonin, and 5-dehydrooriciopsin.[2−7] Of these, harrisonin and its 12β-acetoxy derivative attracted considerable attention due to their potent insect antifeedant activities against the Southern army worm (*Spodoptera eridania*)[2,3] and the African army worm (*S. frugiperda*).[8] The respective structures **1** and **2** were proposed for harrisonin[2] and 12β-acetoxyharrisonin,[3] with the structural arguments being based on their relationship to obacunone (**5**). Some inconsistencies, however, remained concerning the anomalous ORD curves of **1** and **2**, which showed significant differences when compared with those of compound **5**. Recently, Byrne *et al.*[9] proposed that **1** and the limonoid perforatin (**7**) could be derived from the same precursor and argued that the stereochemistry of **1** at C-5 may be incorrect. The above inconsistencies, together with our interest in the *Striga* seed-germination activity of compounds from *H. abyssinica*, prompted us to carry out an in-depth NMR study of obacunone (**5**),[7] harrisonin, and 12β-acetoxyharrisonin.[10] Assignments of their ^{1}H-NMR signals involved ^{1}H−^{1}H COSY and 2D NOESY measurements and the ^{13}C-NMR signals were assigned on the basis of ^{1}H−^{13}C HMQC[11] spectra, long-range ^{1}H−^{13}C HMBC,[12] and DEPT experiments. We report below the unambiguous structure determination of harrisonin and 12β-acetoxyharrisonin, which necessitated structural revisions. The structure of 12β-acetoxyharrisonin was confirmed by single-crystal X-ray diffraction.

The assignments of the 1D and 2D ^{1}H- and ^{13}C-NMR spectra of harrisonin and 12β-acetoxyharrisonin are listed in Tables 1 and 2. Most of the ^{1}H- and ^{13}C NMR data obtained are in accord with literature values.[2,3] However, long-range ^{1}H−^{13}C HMBC correlations revealed connectivities that could not be accommodated by the previously proposed structures for harrisonin (**1**) and 12β-acetoxyharrisonin (**2**). A detailed analysis of the ^{1}H−^{1}H COSY and ^{1}H−^{13}C HMQC correlations of harrisonin allowed us to identify the presence of five partial structures (Figure 1). The connectivity of each partial structure and the location of the quaternary carbons at C-5, C-6, and C-7 were obtained from the HMBC data (Table 3 and Figure 2). In harrisonin, the two methyl singlets at δ 1.18 (C-29) and 1.37 (C-28) (segment **A**) gave strong correlations with the quaternary carbons at δ 80.9 and 216.9, corresponding to an oxygen-bearing carbon and a ketone carbonyl carbon (C-5 and C-6 in **1**). This observation however, was not consistent with structure **1**, inasmuch as a correlation of the C-6 carbonyl group would only be possible via a ^4J coupling and not only would involve the geminal methyl groups (C-28 and C-29 of segment **A**) but also

Table 1. ^{1}H-NMR Spectral Data of Compounds **3** and **4** (300 MHz $CDCl_3$)[a]

	compound	
proton	**3**	**4**
H-1	5.95 (1H, d, 12.5)	5.90 (1H, d, 12.5)
H-2	5.76 (1H, d, 12.5)	5.79 (1H, d, 12.5)
H-9	3.98 (1H, dd, 14, 5)	2.97 (1H, dd, 14, 5)
H-11α	1.92 (1H, m)	2.35 (1H, ddd, 14, 5, 7)
H-11β[b]	1.74 (1H, m)	1.76 (1H, ddd, 14, 14, 1)
H-12α[b]	1.74 (1H, m)	4.75 (1H, dd, 7, 1)
H-12β	1.68(1H, m)	
H-15	4.29 (1H, s)	4.25 (1H, s)
H-17	5.67 (1H, s)	6.01 (1H, s)
H-18	1.25 (3H, s)	1.28 (3H, s)
H-19	1.50 (3H, s)	1.46 (3H, s)
H-21	7.40 (1H, m)	7.34 (1H, m)
H-22	6.33 (1H, m)	6.16 (1H, m)
H-23	7.42 (1H, m)	7.40 (1H, m)
H-28	1.37 (3H, s)	1.37 (3H, s)
H-29	1.18 (3H, s)	1.17 (3H, s)
H-30	1.15 (3H, s)	1.21 (3H, s)
OAc		2.20 (3H, s)
OMe	3.79 (3H, s)	3.78 (3H, s)
6-OH[c]	5.06 (1H, s)	5.11 (1H, s)
7-OH[c]	3.62 (1H, s)	3.65 (1H, s)

[a] Spectra were recorded in $CDCl_3$, at 300 MHz with TMS as internal standard. Proton integration, multiplicities and coupling constants J Hz (in parentheses). [b] Overlapped signal. [c] Exchanged with D_2O.

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⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1997.

S0163-3864(97)00201-2 CCC: $14.00

Table 2. ^{13}C-NMR Spectral Data of Compounds **3** and **4** (75 MHz, $CDCl_3$)[a]

carbon	compound 3	compound 4
1	153.9 d[b]	153.0 d[b]
2	123.1 d	123.4 d
3	166.7 s	166.6 d
4	80.9 s	81.0 s
5	216.9 s	216.5 s
6	88.6 s	88.3 s
7	108.2 s	108.2 s
8	49.9 s	49.3 s
9	46.8 d	45.9 d
10	49.7 s	49.6 s
11	15.2 t	26.0 t
12	26.3 t	72.5 d
13	39.5 s	42.4 s
14	68.5 s	66.7 s
15	57.3 d	55.6 d
16	167.8 s	167.1 s
17	78.4 d	75.1 d
18	18.3 q	16.9 q
19	17.3 q	17.1 q
20	121.0 s	119.9 s
21	141.1 d	141.2 d
22	109.9 d	109.0 d
23	143.0 d	143.6 d
28	24.1 q	23.9 q
29	27.4 q	27.3 q
30	14.7 q	14.2 q
$COCH_3$		169.9 s
$COCH_3$		21.3 q
OCH_3	52.0 q	52.0 q

[a] Chemical shifts are relative to the solvent signal ($CDCl_3$). [b] Multiplicities were established from HMQC and DEPT spectra. s = singlet, d = doublet, t = triplet, and q = quartet.

Table 3. 2D Long-Range ^{1}H−^{13}C Correlations in the HMBC Spectra of Compound **3** and **4**

proton	correlated carbon
1	2, 3, 6, 9, 10, 19
2	3, 1, 10
9	14, 10
15	14, 8
17	14, 13, 20, 21, 22
18	12, 13, 14, 17
19	1, 6, 9, 10
28, 29	5, 4
30	7, 8, 14, 9
OMe	3
6-OH	6, 7, 5, 10
7-OH	6, 7, 8

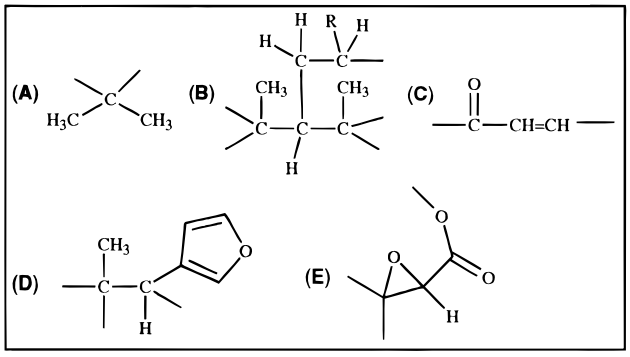


Figure 1. Partial structures for **3** (R=H) and 4 (R= −OAc).

should correlate with the methyl signals due to C-19 and C-30 of segment **B.** The olefinic protons at C-1 and C-2 of segment **C** and the OMe group correlated with the quaternary carbon signal at δ 166.7, corresponding to the C-3 carbonyl group, thus placing the OMe group as part of an α,β-unsaturated ester appendage attached

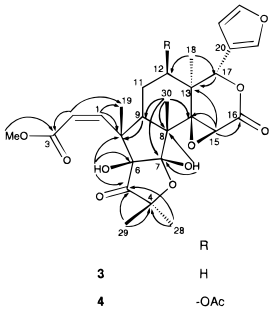


Figure 2. Diagnostic ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum for **3** and **4**.

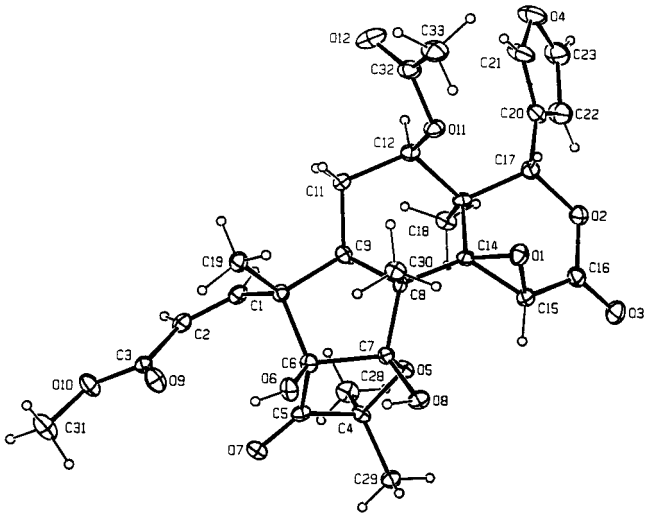
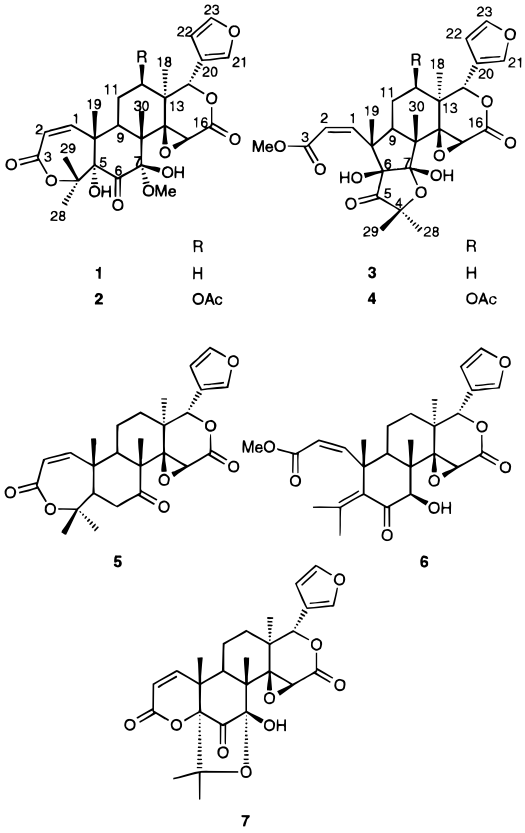


Figure 3. Molecular structure of compound **4**.

to C-10. Therefore, harrisonin does not possess a lactone ring A as in **1**, but should contain an α,β-unsaturated methyl ester similar to that of atalantolide (**6**). In agreement with structure **3**, the OH proton absorption at δ 5.06 (C-6−OH) correlated with the quaternary carbons at δ 216.9 (C-5), 88.6 (C-6), 108.2 (C-7), and 49.7 (C-10), and the OH absorption at δ 3.62 (C-7−OH) correlated with the quaternary carbons at δ 88.6 (C-6), 108.2 (C-7), and 49.9 (C-8). These observations suggested that segment **B** was connected to the two quaternary centers absorbing at δ 88.6 and 108.2 by bonds connecting C-10 and C-6 and C-8/C-7. The carbonyl carbon absorbing at δ 216.9 (C-5) must also be connected to the quaternary carbon absorbing at δ 88.6 (C-6). In addition, this enabled the placement of the two hydroxyl groups firmly at C-6 and C-7, as shown in structure **3**. Other significant HMBC correlations for harrisonin included a correlation of the methyl groups absorbing at δ 1.15 (CH_3-30) in segment **B**, and the methyl signal at δ 1.25 (CH_3-18) in segment **D** with the quaternary carbon at δ 68.5 (C-14). This, together with the heteronuclear correlation of the 18-Me signal at δ 1.25 with C-12 at δ 26.3 and H-15 at δ 4.29 with C-14 (δ 68.5) and C-8 (δ 49.9), confirmed the connectivity of segments **B** and **D** and also the connectivity of both segments **B** and **E** with the quaternary carbon at δ 68.5 (C-14). From the above observations and comparison with the spectral data of **5**,[7] **6**,[5] **7**,[9] and other related limonoids,[13,14] it was possible to complete all the major connectivities of **3** and also confirm the similarities of both rings A and D of **3** with those of **6**. The only remaining connectivity unaccounted for was the oxygen bridge between C-7 and C-4. The chemical shift of C-7

at δ 108.2, characteristic of a hemiketal carbon, and the C-4 chemical shift at δ 80.9 were very similar to those of compound **7**.[9] Also, the molecular weight and number of degrees of unsaturation for **3** allow for the only logical connectivity between C-7 and C-4 by an ether-type linkage. From the above, it was concluded that the structures of harrisonin and 12β-acetoxyharrisonin should be revised from **1** and **2** to **3** and **4,** respectively.



The above-proposed structure **4** was supported by single-crystal X-ray diffraction of 12β-acetoxyharrisonin (Figure 3), which showed the skeletal arrangement and relative stereochemistry in accord with the NMR-derived structure **4**. By spectral correlation, the structure and relative stereochemistry of harrisonin was unambigously confirmed to be **3**. Compounds **3** and **4** represent a new group of limonoids that characteristically bear a highly oxidized and an altered carbon framework typical of constituents isolated from members of the genus *Harrisonia*.[9,15,16] The revised structures **3** and **4** have a unique limonoid skeleton with a biosynthetically modified five-membered ring B.

Experimental Section

General Experimental Procedures. ^{1}H- 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 the HMQC and HMBC experiments, $\Delta = 1$ s and $J = 145$, 8 Hz, respectively; the correlation maps consisted of 512 $\times$ 1 K data points per spectrum, each composed of 16 to 64 transients.

Plant Material. The root bark of *H. abyssinica* was collected in Kisumu, Western Kenya, in July 1995. The plant material was identified at the Department of Botany Herbarium, Moi University, Eldoret, Kenya, and a voucher specimen (no. MU/BOT/73M) is deposited in the same department.

Table 4. Positional Parameters and Their Estimated s.d.s for **4**

atom	x	y	z	B_{eq}(Å^2)
O-1	0.4882(3)	0.0000	0.8264(3)	1.59(4)
O-2	0.8504(3)	0.0564(1)	0.9714(3)	1.79(5)
O-3	0.9251(4)	−0.0077(1)	1.1761(3)	2.31(6)
O-4	0.8921(4)	0.2021(1)	0.6434(3)	2.82(6)
O-5	0.7357(3)	−0.13243(9)	0.7258(3)	1.21(4)
O-6	0.2909(3)	−0.1863(1)	0.4354(3)	1.51(4)
O-7	0.6195(3)	−0.2512(1)	0.4625(3)	1.72(5)
O-8	0.4457(3)	−0.1487(1)	0.7541(3)	1.45(4)
O-9	0.3176(3)	−0.2289(1)	0.1251(3)	1.84(5)
O-10	0.5029(3)	−0.2610(1)	−0.0089(3)	1.91(5)
O-11	0.4053(3)	0.0671(1)	0.4897(3)	1.51(4)
O-12	0.3548(4)	0.1141(1)	0.2432(3)	2.69(6)
C-1	0.6106(4)	−0.1344(1)	0.2420(4)	1.42(6)
C-2	0.6035(4)	−0.1782(1)	0.1427(4)	1.48(6)
C-3	0.4587(5)	−0.2237(1)	0.0902(4)	1.41(6)
C-4	0.8003(4)	−0.1854(1)	0.6850(4)	1.17(5)
C-5	0.6328(4)	−0.2054(1)	0.5249(4)	1.18(5)
C-6	0.4702(4)	−0.1608(1)	0.4701(4)	1.25(6)
C-7	0.5296(4)	−0.1270(1)	0.6434(4)	1.09(5)
C-8	0.4838(4)	−0.0657(1)	0.5913(4)	1.05(5)
C-9	0.5508(4)	−0.0632(1)	0.4376(4)	1.20(6)
C-10	0.4720(4)	−0.1165(1)	0.3277(4)	1.16(6)
C-11	0.5200(5)	−0.0066(1)	0.3494(4)	1.44(6)
C-12	0.5777(4)	0.0419(1)	0.4796(4)	1.33(6)
C-13	0.7025(4)	0.0242(1)	0.6667(4)	1.18(6)
C-14	0.5929(4)	−0.0212(1)	0.7248(4)	1.12(6)
C-15	0.6661(4)	−0.0299(1)	0.9141(4)	1.47(6)
C-16	0.8239(5)	0.0058(1)	1.0312(4)	1.67(7)
C-17	0.7318(4)	0.0737(1)	0.7931(4)	1.41(6)
C-18	0.9005(4)	0.0030(1)	0.6765(4)	1.58(6)
C-19	0.2720(5)	−0.1066(1)	0.1805(4)	1.56(6)
C-20	0.8364(5)	0.1229(1)	0.7587(4)	1.71(7)
C-21	0.7561(5)	0.1642(2)	0.6436(5)	2.37(8)
C-22	1.0369(5)	0.1357(2)	0.8397(5)	2.32(8)
C-23	1.0618(6)	0.1842(2)	0.7670(5)	2.58(9)
C-28	0.9814(4)	−0.1746(2)	0.6494(4)	1.80(7)
C-29	0.8380(4)	−0.2268(1)	0.8320(4)	1.48(6)
C-30	0.2651(4)	−0.0573(1)	0.5409(4)	1.47(6)
C-31	0.3731(5)	−0.3079(2)	−0.0717(5)	2.40(8)
C-32	0.3036(5)	0.1019(2)	0.3590(4)	1.83(7)
C-33	0.1224(5)	0.1215(2)	0.3772(5)	2.22(8)

Extraction and Isolation. *H. abyssinica* root bark (2 kg) was chopped into small pieces and allowed to stand for 3 days in MeOH (4 L). The solvent was decanted and the root bark extracted a second time. The combined MeOH extracts were filtered and evaporated under vacuum to yield 22.8 g of a red oil, which was partitioned between H_2O (250 mL) and $CHCl_3$ (3 $\times$ 200 mL). The combined $CHCl_3$ phases were dried over Na_2SO_4 and evaporated to yield 18 g of a thick red oil. A portion of the oil (10 g) was subjected to column chromatography using the same procedure as that reported by Hassanali *et al.*[4] Five known limonoids were isolated and characterized by comparing the ^{1}H- and ^{13}C- NMR, IR, and mp with literature values.[2−5,7] The compounds were identified as atalantolide (**6**, 8 mg), harrisonin (**3**, 62 mg), 12β-acetoxyharrisonin (**4**, 50 mg), obacunone (**5**, 150 mg), and pedonin (100 mg).

X-ray Crystallographic Analysis.[17] A colorless lath fragment of dimensions 0.57 $\times$ 0.45 $\times$ 0.12 mm was used for data collection at 100 K on an Enraf-Nonius CAD4 diffractometer equipped with an Oxford Cryosystems cryostat, with Mo Kα radiation ($\lambda = 0.710\,73$ Å), and a graphite monochromator.

Crystal data are: $C_{29}H_{34}O_{12}$, $M_r = 574.6$, monoclinic space group $P2_1$, $a = 7.500(1)$ Å, $b = 23.932(4)$ Å, $c = 8.355(2)$ Å, $\beta = 112.65(1)°$, $V = 1384.1(8)$ Å^3, $Z = 2$, d_c

$= 1.379$ g cm^{-3}. Intensity data were measured by $\omega - 2\theta$ scans of variable rate. One quadrant of data was collected within the limits $1 < \theta < 32°$.

Data reduction included corrections for background, Lorentz, and polarization effects. Absorption corrections ($\mu = 1.0$ cm^{-1}) were unnecessary. Of 4890 unique data, 4036 had $I > 1\sigma(I)$ and were used in the refinement. The structure was solved by direct methods using SIR92[18] and refined by full-matrix least-squares methods, treating non-hydrogen atoms anisotropically, using the Enraf-Nonius MolEN programs.[19] Hydrogen atoms were located using difference maps. Those of the OH groups and that on C-15 were refined isotropically, while others were placed in calculated positions. Convergence was achieved with $R = 0.062$, $R_w = 0.054$, and GOF = 1.613. The molecular structure is illustrated in Figure 3, and its coordinates are tabulated in Table 4.

Acknowledgment. We thank Mr. A. Odero and W. Kigangi of the Department of Chemistry, Moi University, Kenya, for assistance with plant collection and the Department of Chemistry, Louisiana State University, for hosting M.S.R. This study was supported in part by a Fulbright Fellowship Grant to M.S.R. and a Rockefeller Foundation Fellowship grant to J.K.R. Improvements to the LSU X-ray Crystallography Facility were supported by Grant No. LEQSF(1996−97)-ENH-TR-10 administered by the Louisiana Board of Regents.

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NP970201P