PERGAMON

Phytochemistry 52 (1999) 127–133

PHYTOCHEMISTRY

11β,12β-Diacetoxyharrisonin, a tetranortriterpenoid from *Harrisonia abyssinica*

Mohamed S. Rajab[a,*], Frank R. Fronczek[b], Dulcie A. Mulholland[c], Joseph K. Rugutt[b]

[a]*Department of Chemistry, Moi University, P.O. Box 1125, Eldoret, Kenya*
[b]*Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803-1804, USA*
[c]*Department of Chemistry and Applied Chemistry, University of Natal, Private Bag X10, Dalbridge 4014, Durban, South Africa.*

Received 15 January 1999; accepted 3 February 1999

Abstract

A new tetranortriterpenoid, 11β,12β-diacetoxyharrisonin, together with the known atalantolide, obacunone, harrisonin, 12β-acetoxyharrisonin, pedonin, cneorin R and perforaquassin A have been isolated from the root bark of *Harrisonia abyssinica*. The structure of the new compound which is based on a rearranged tetranortriterpenoid with a five membered ring-B and a seco ring-A and D was assigned on the basis of extensive NMR studies and X-ray diffraction analysis. In addition, the structure and relative configuration of the known perforaquassin A was confirmed by X-ray diffraction analysis.

Keywords: Harrisonia abyssinica; Simaroubaceae; Isolation; Tetranortriterpenoid; Limonoid; Quassinoid; 11β,12β-diacetoxyharrisonin; Perforaquassin A; X-ray analysis

1. Introduction

The East African shrub *Harrisonia abyssinica* (Oliv) (Simaroubaceae) 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, haemorrhoids and snake bite (Kokwaro, 1976). Crude extracts of the root bark of this plant were shown to exhibit insect antifeedant, antimicrobial, cytotoxic and plant growth inhibitory activities (Kubo et al., 1976; Liu, Kubo, & Nakanishi, 1982; Hassanali et al., 1987). Six limonoids have previously been isolated from *H. abyssinica*; of these, obacunone, harrisonin, 12β-acetoxyharrisonin and pedonin have been isolated from Kenyan samples (Kubo et al., 1976; Liu et al., 1982; Hassanali et al., 1987) whereas obacunone, atalantolide (**3**), 5-dehydrooriciopsin and the chromones alloptaeroxylin, peucinin and *O*-methylalloptaeroxylin were found in Nigerian samples (Okorie, 1982; Balde, Vanhaeler, & Daloze, 1988; Rugutt, Fisher, Nauman, Schmidt, & Berner, 1996). The large variance in the chemical constituents of the East and West African samples of *H. abyssinica* (Okorie, 1982) and the absence before this study of a quassinoid made it chemotaxonomically interesting to search for quassinoids in this plant. This, together with our interest in compounds from *H. abyssinica* with effects on germination of *Striga* (Rugutt, 1996), prompted us to re-examine the extracts of this plant. In the course of these studies, we recently revised the structures of harrisonin and 12β-acetoxyharrisonin (Rajab, Rugutt, Fronczek, & Fischer, 1997). Further investigations of the methanol extract of the root bark of this plant has led to the isolation of a new limonoid, 11β,12β-diacetoxyharrisonin (**1**). This compound, whose skeleton is based on a rearranged basic 4,4,8-trimethyl-17-furanyl steroidal skeleton, has a rare five-membered ring B, which has been observed before during the structural reappraisal stu-

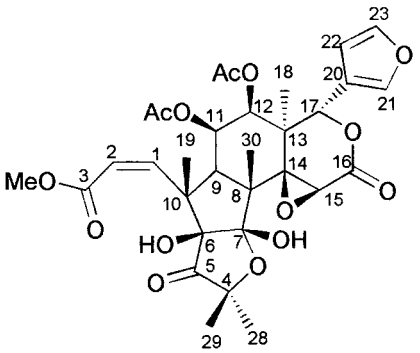
* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00096-5

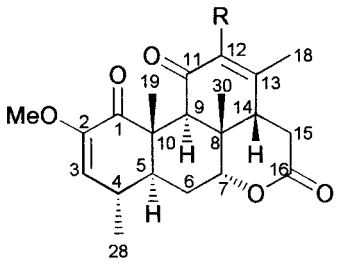
dies of the harrisonins (Rajab et al., 1997). In addition, perfaraquassin A (**2**) and six known limonoids were isolated. Assignments of their ^{1}H-NMR signals involved ^{1}H-^{1}H COSY and 2D NOESY measurements and the ^{13}C NMR signals were assigned on the basis of ^{1}H-^{13}C HMQC (Bax & Subramanian, 1986) spectra, long-range ^{1}H-^{13}C HMBC (Bax & Summer, 1986) and DEPT experiments. The structure of both **1** and the **2** were confirmed by single crystal X-ray analysis.

2. Results and discussion

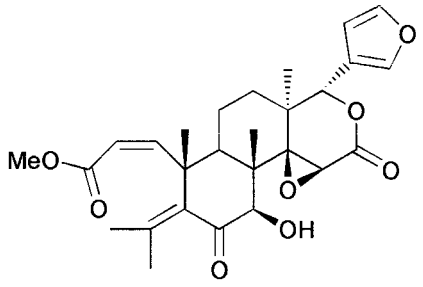
Extraction of the air-dried root bark of *H. abyssinica* with MeOH yielded a thick red oil which was subjected to silica gel chromatography using a hexane-EtOAc gradient (see Section 3) yielded several fractions which after repeated silica gel column chromatography, EtOAc:hexane (2:5) and recrystallisation in Me_2CO afforded seven tetranortriterpenoids and a quassinoid. By means of spectroscopic data and comparison (IR, ^{1}H and ^{13}C NMR spectra) with literature values, six of the compounds were identified as the known atalantolide (**3**), obacunone, harrisonin, 12β-acetoxyharrisonin, pedonin, cneorin R and perfaraquassin A (Kubo et al., 1976; Mondon, 1979; Liu et al., 1982; Hassanali et al., 1987; Epe & Kamuichi et al., 1996; Rugutt et al., 1996). The tetranortriterpenoid nature of 11β,12β-diacetoxyharrisonin (**1**) and its relation to atalantolide (**3**) (Okorie, 1982) and the harrisonins (Kubo et al., 1976; Liu et al., 1982; Rajab et al., 1997) was apparent from spectroscopic evidence. The mass spectrum (FAB) of (**1**) gave a molecular for-



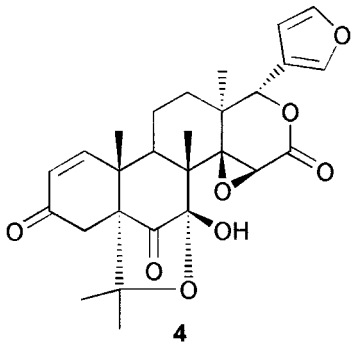
1



2



3



4

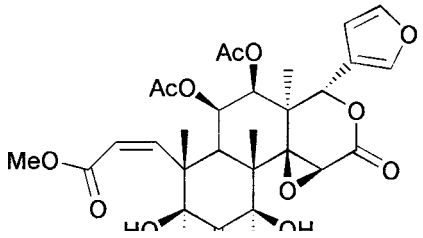


Table 1
^{1}H NMR, ^{13}C NMR and HMBC data for 11β,12β-diacetoxyharrisonin (**1**)[a]

Position H/C		C(δ)	H(δ)	H,C Long-range HBMC correlation
1	CH[d]	151.1	6.06 (1H, d, J = 12)	2, 3, 6, 9, 10, 19
2	CH	123.4	5.75 (1H, d, J = 12)	3, 1, 10
3	c	166.7		
4	c	81.1		
5	c	216.0		
6	c	87.2		
7	c	107.9		
8	c	49.7		
9	CH	47.8	2.98 (1H, d, J = 7)	14, 10
10	c	50.4		
11	CH	63.2	5.83 (1H, dd, J = 7)	
12	CH	71.1	5.15 (1H, d, J = 7)	
13	c	42.2		
14	c	65.9		
15	CH	54.5	4.24 (1H, s)	14, 8
16	c	166.8		
17	CH	75.3	5.97 (1H, s)	14, 13, 20, 21, 23
18	CH_3	15.5	1.25 (3H, s)	12, 13, 14, 17
19	CH_3	15.9	1.56 (3H, s)	1, 6, 9, 10
20	c	119.5		
21	CH	141.4	7.32 (1H, m)	
22	CH	109.2	6.23 (1H, m)	
23	CH	143.6	7.38 (1H, m)	
28	CH_3	23.9[c]	1.14 (3H, s)	5, 4
29	CH_3	23.9[c]	1.35 (3H, s)[c]	5, 4
30	CH_3	27.3	1.35 (3H, s)[c]	7, 8, 14, 9
11-Oac	CH_3	21.0	1.96 (3H, s)	
12-Oac	CH_3	20.9	2.24 (3H, s)	
11-Oac	c	169.5		
12-Oac	c	169.9		
-Ome	CH_3	52.1	3.76 (3H, s)	3
6-OH			5.20[b] (br)	6, 7, 5, 10
7-OH			3.76[b]	6, 7, 8

[a] Spectra were recorded in $CDCl_3$ with TMS as internal standard. δ ppm and J(parenthesis) in Hz, 300 MHz (^{1}H) and 75 MHz (^{13}C), 25°.
[d] Multiplicities were established from HMQC and DEPT spectra.
[c] Overlapped signal.
[b] Exchanges with D_2O.

mula of $C_{31}H_{37}O_{14}$. The IR spectrum suggested the presence of a free hydroxyl group (3463 cm^{-1}), carbonyl groups (1749 and 1634 cm^{-1}). The presence of ketone; lactone and ester groups was indicated by ^{13}C NMR absorption at δ 216.0; 166.7 and 166.8; 169.9 and 169.5, respectively (Table 1). The two three proton singlets at δ 1.96 and 2.24 in the ^{1}H NMR spectrum (Table 1) allowed assignments of the ester groups as acetates. In addition, the spectrum showed peaks for five tertiary methyl groups (δ 1.14, 1.25, 1.35, 1.35 and 1.56), a disubstituted double bond (δ 6.06 and 5.75, d, J = 12 Hz, H-1 and H-2), the characteristics of a β-substituted furan (δ 6.23, 7.32 and 7.38) and a secondary epoxidic proton (δ 4.24, s, H-15). The ^{1}H NMR spectra revealed that the carbon skeleton of (**1**) bore similarities to the co-occurring 12β-acetoxy harrisonin (Liu et al., 1982; Rajab et al., 1997). A major difference is the presence of an extra acetate function, indicated by the appearance of a peak in the ^{1}H NMR at δ 1.96 for an acetate methyl group and at δ 5.83 for the acetoxy methine proton. The appearance of both the 12-acetate methine proton (δ 5.15, J = 7 Hz) and the C-9 proton (δ 2.98, J = 7 Hz) as doublets implied that the extra acetate must be at C-11. Examination of molecular models showed the 11α-H to have the required geometry for the observed small coupling constant (about 7 Hz) with 12α-H and 9α-H. Also, the chemical shifts of the C-8 and C-10 angular methyl groups which appeared rather downfield (δ 1.35 and 1.56, respectively) can be accounted for by the field effect of the proximate extra β-oriented acetate function at C-11. 2D-Homonuclear J-correlation (COSY) spectrum clarified the coupling protons on the linked carbons C-1 and C-2; C-9, C-11 and C-12. In addition the spin coupling network linked to the C-9 proton suggests that the signal centred at δ 1.35 is due to the C-30 methyl hydrogens and that linked to the C-17 protons, centred at δ 1.25 is due to C-18 methyl hydro-

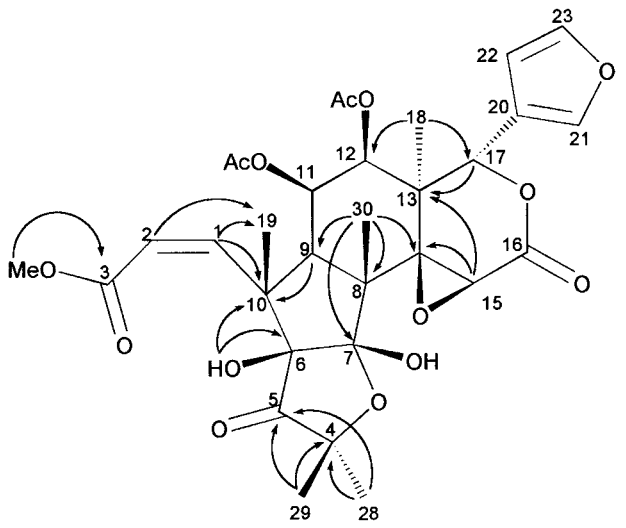


Fig. 1. Diagnostic $^{1}H–^{13}C$ long-range correlations observed in the HMBC spectrum for **1**.

gens. The localization of the quaternary carbons C-5, C-6 and C-7 was deduced from the HMBC data (Fig. 1 and Table 1). The two methyl singlets at δ 1.35 (C-29) and 1.14 (C-28) displayed long-range $^{1}H–^{13}C$ HMBC correlation with the quaternary carbons at δ 87.2 and 216.0, corresponding to an oxygen-bearing carbon (C-6) and a ketonic carbon (C-5). The olefinic protons at C-1 and C-2 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 to C-10. The methyl group absorbing at δ 1.35 and the C-9 proton absorbing at δ 2.98 correlated with the quaternary carbon at δ 107.9 corresponding to C-7. The OH proton at δ 5.20 (C-6 OH) correlated with the quaternary carbons at δ 87.2 (C-6), 216.0 (C-5), 107.9 (C-7) and 50.4 (C-10), and the OH proton at δ 5.20 (C-OH) correlated with the quaternary carbons at δ 87.2 (C-6), 107.9 (C-7) and 49.7 (C-8). These observations suggested that the two quaternary centers absorbing at δ 87.2 and 107.9 be connected to C-10 and C-8. The carbonyl carbon absorbing at δ 216.0 (C-5) must also be connected to the quaternary carbon absorbing at δ 87.2 (C-6). In addition, this

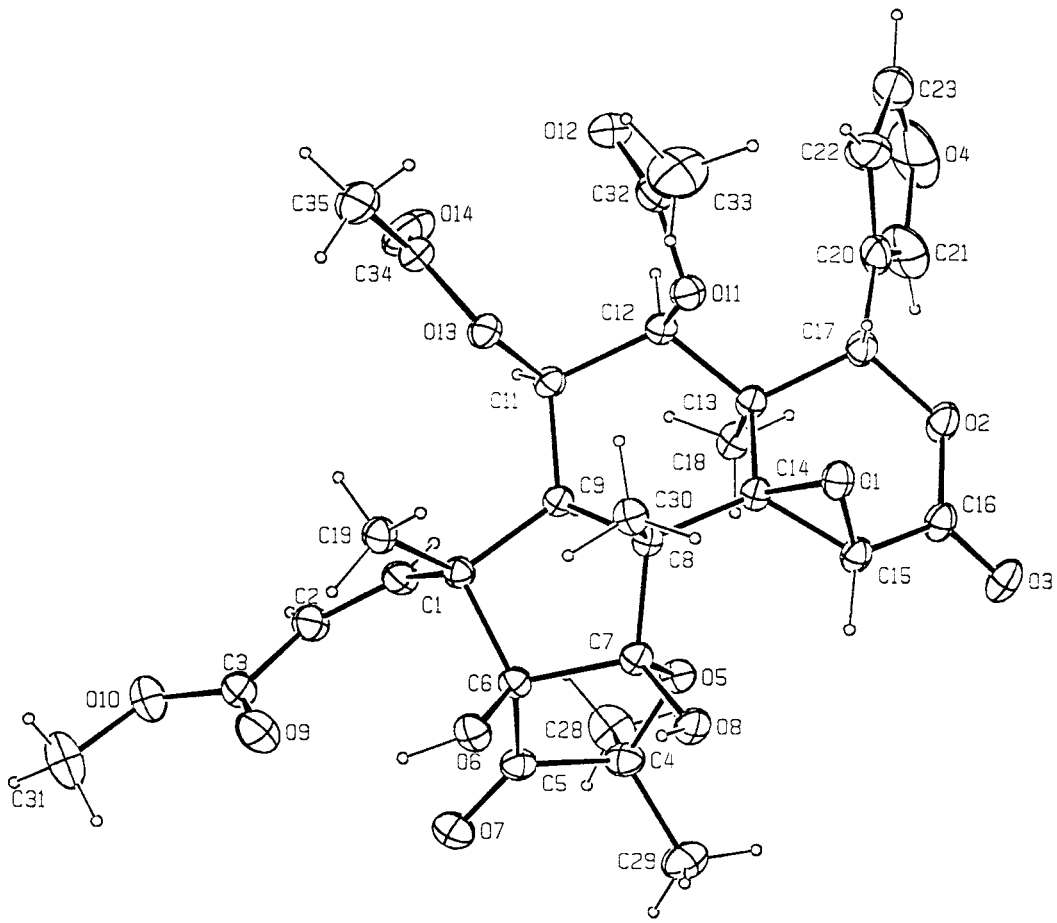


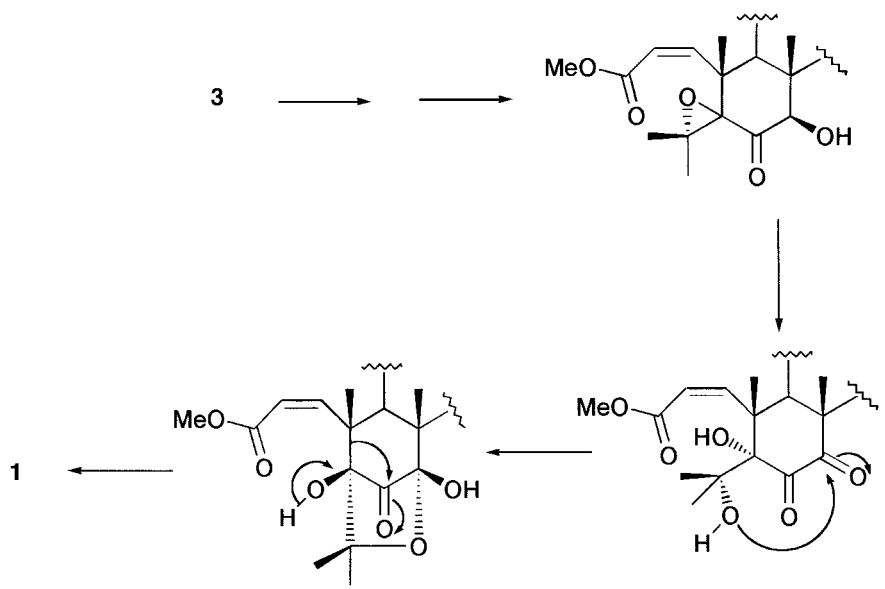
Fig. 2. 11β,12β-Diacetoxyharrisonin.

allowed the placement of the two-hydroxyl groups firmly at C-6 and C-7, as shown in structure (**1**). Other significant HMBC correlations for (**1**) included a correlation of the methyl group absorbing at δ 1.35 (CH_3-30) and the methyl signal at δ 1.25 (CH_3-18) with the quaternary carbon at δ 65.9 (C-14). The 18-Me signal at δ 1.25 with C-12 at δ 71.1 and H-15 at δ 4.24 with C-14 (δ 65.9) and C-8 (δ 49.7). The only remaining connectivity unaccounted for was the oxygen bridge between C-7 and C-4. The chemical shift of C-7 at δ 107.9 characteristic of a hemiketal carbon and the molecular weight and number of degrees of unsaturation for **1** allow for the only logical connectivity between C-7 and C-4 to an ether-type linkage. In addition, these assignments were verified by NOESY techniques; particularly relevant were correlations between H-9 and H-11, H-12 and H-9; H-12 and the α-CH_3 at C-13 confirmed the β-configuration for the C-11 and C-12 acetate groups. From the above observations and comparison with the spectral data of **3** (Okorie, 1982), **4** (Byrne et al., 1991) and other related limonoids (Kubo et al., 1976; Liu et al., 1982; Okorie, 1982; Hassanali et al., 1987; Balde et al., 1988; Barbetti, Grandolini, Fardella, & Chiappini, 1993; Kamuichi et al., 1996; Rugutt et al., 1996; Rajab et al., 1997) it was possible to complete all the major connectivities of **1** and also confirm the similarity of both ring A and D of **1** with that of **3**. This proposal was supported by a single crystal X-ray diffraction analysis of 11β,12β-diacetoxyharrisonin, which yielded the structure shown in **1** (Fig. 2). Compound **1**, thus, joins the group of limonoids that bear a characteristically highly oxidized and much altered carbon framework typical of those isolated from the genus *Harrisonia* (Byrne et al., 1991; Kamuichi et al., 1996). Its biosynthesis could involve an intramolecular rearrangement of the yet unreported compound **5**. Hypothetically, this would require the generation of a carbonyl function from the C-6 hydroxyl group of compound **5**, followed by a 1, 2-shift of the C-5 to C-10-bond to form a new σ-bond between C-10 and C-6 (Scheme 1). This proposal is supported by the fact that **3**, a potential intermediate to **5**, co-occurs with **1**.

Compound **2** was isolated as a colourless crystalline compound, m.p. 190–191° (dec.) the mass spectrum (FAB) of which led to the assignment of the molecular formula $C_{21}H_{26}O_5$. ^1H and ^{13}C NMR data for **2** were in agreement with those reported for perforaquassin A (Kamuichi et al., 1996). While there is no doubt about this structural identity, we obtained crystals of **2** that allowed X-ray analysis to determine the molecular structure and relative configuration of **2** (Fig. 3) and thus confirmed unambiguously its structural homology to perforaquassin A (Kamuichi et al., 1996). The co-occurrence of limonoids and a quassinoid in *H. abyssinica* further supports the biosynthetic link between limonoids and quassinoids and firmly establishes the taxonomic status of *H. abyssinica* in the Simaroubaceae (Byrne et al., 1991).

3. Experimental

Melting points: uncorr. ^1H and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as internal standard. 2D NMR spectra were recorded by using a standard pulse program: in the HMQC and HMBC experiments, $\Delta = 1$ s and J = 145,8 Hz, respectively;



Scheme 1. Suggested biosynthetic pathway to 11β,12β-diacetoxyharrisonin (**1**).

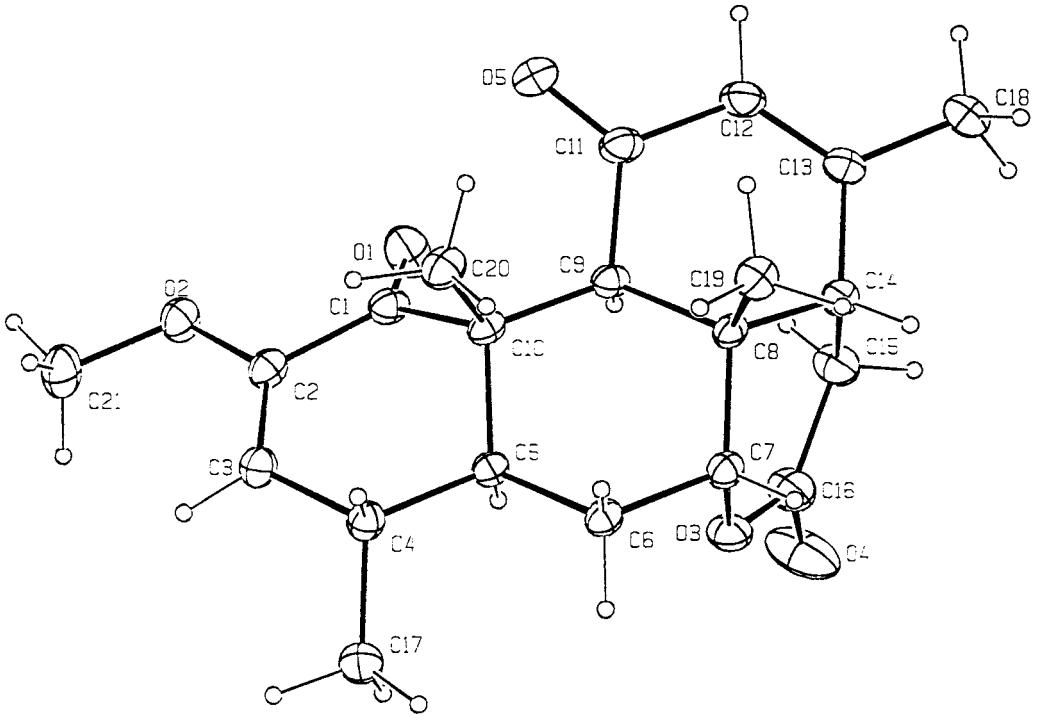


Fig. 3. Perforaquassin A.

the correlation maps consisted of 512×1 K data points per spectrum, each composed of 16 to 64 transients.

3.1. 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 Department.

3.2. 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 $2 \times$. 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 $CHCl_3$ (3×200 ml). The combined $CHCl_3$ phases were dried over Na_2SO_4 and evaporated to yield 22.8 g of thick red oil. A portion of the oil (10 g) was subjected to column chromatography on silica gel using a EtOAc/hexane (1:9) gradient using the same procedure as that reported by Hassanali et al. (1987). Seven limonoids and a quassinoid were isolated. The compounds were identified as atalantolide (**3**) (6 mg), harrisonin (62 mg), 12β-acetoxyharrisonin (50 mg), 11β,12β-diacetoxyharrisonin (**1**) (28 mg), obacunone (150 mg), cneorin R (25 mg), pedonin (100 mg) and lastly perforaquassin A (**2**) (26 mg).

3.3. 11β,12β-diaceloxyharrisonin (*1*)

Colourless crystals (acetone) $R_f = 0.38$, 30% EtOAc/hexane, m.p. 249–250°C IR (KBr): ν_{max} cm^{-1}: 3463 (OH), 1749 (CO), 1634, 1369, 1237, 1030, 754. ^{1}H- and ^{13}C-NMR; Table 1.

3.4. X-ray structure determination

X-ray data for (**1**) and (**2**). Intensity data were collected on Enraf-Nonius CAD4 diffractometers equipped with CuKα radiation ($\lambda = 1.54184$ Å) for (**1**), MoKα radiation ($\lambda = 0.71073$ Å) and an Oxford Cryostream device for (**2**) and graphite monochromators. Crystal data are: (**l**): $C_{31}H_{36}O_{14}$, $M_r = 632.6$, orthorhombic space group $P2_12_12_1$, $a = 11.360(2)$, $b = 11.586(1)$, $c = 24.257(3)$ Å, $V = 3192(1)$ Å^3, $Z = 4$, $d_c = 1.316$ g cm^{-3}, $T = 25$°C. (**2**): $C_{21}H_{26}O_5$, $M_r = 358.4$, orthorhombic space group $P2_12_12_1$, $a = 7.4359(8)$, $b = 10.983(2)$, $c = 22.400(3)$ Å, $V = 1829.4(8)$ Å^3, $Z = 4$, $d_c = 1.301$ g cm^{-3}, $T = 150$ K. Intensity data were measured by $\omega-2\theta$ scans of variable rate. One octant of data was collected within the limits $2.5 < \theta < 75°$ for (**1**) and $2.5 < \theta < 32°$ for

(**2**). Data reduction included corrections for background, Lorentz, polarization, and (for (**1**)) absorption effects. Absorption corrections ($\mu = 8.4$ cm^{-1}) were based on ψ scans, with minimum relative transmission coefficient 95.1%. Of 3705 unique data, 3410 had $I > 1\sigma(I)$ and were used in the refinement for (**1**). For (**2**), these values were 3602 and 3163. Refinement was by full-matrix least squares, treating nonhydrogen atoms anisotropically, using the Enraf-Nonius MolEN programs (Fair, 1990). Hydrogen atoms were located using difference maps and all were refined isotropically for (**2**), while only those on C15 and the OH groups were refined for (**1**). Convergence was achieved with $R = 0.042$, $R_w = 0.047$ and GOF = 2.123 for (**1**) and with $R = 0.05\ 1$, $R_w = 0.050$ and GOF = 1.829 for (**2**). The crystal structures are illustrated in Figs. 2 and 3. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (see Instructions to Authors (1997). *Phytochemistry*, *44*(1), 1–6).

Acknowledgements

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 MSR. This study was supported in part by a Fulbright Fellowship Grant to MSR.

References

Balde, A. M., Vanhaeler, M., & Daloze, D. (1988). *Phytochemistry*, *24*, 942.

Barbetti, P., Grandolini, G., Fardella, G., & Chiappini, I. (1993). *Phytochemistry*, *24*, 1013.

Bax, A., & Subramanian, S. (1986). *Journal of Magnetic Resonance*, *67*, 565.

Bax, A., & Summer, M. F. (1986). *Journal of the American Chemical Society*, *108*, 2093.

Byrne, L. T., Tri, M. V., Phuong, N. M., Sargent, M. V., Skelton, B. W., & White, A. H. (1991). *Australian Journal of Chemistry*, *44*, 165.

Epe, B., & Mondon, A. (1979). *Tetahedron Letters*, *22*, 2015.

Fair, C. K. (1990). *MolEN. An interactive system for crystal structure analysis*. Delft, The Netherlands: Enraf-Nonius.

Hassanali, A., Bentley, D. M., Slavin, A. M. Z., Williams, D. J., Shepard, R. N., & Chapya, A. W. (1987). *Phytochemistry*, *24*, 523.

Kamuichi, K., Mitsunaga, K., Koike, K., Ouyangi, Y., Ohmoto, T., & Nikado, T. (1996). *Heterocycles*, *43*(3), 653.

Kokwaro, J. O. (1976). *Medicinal plants of East Africa*. Nairobi: East African Literature Bureau (p. 203).

Kubo, I., Tanis, S. P., Lee, Y. W., Miura, I., Nakanishi, K., & Chapya, A. (1976). *Heterocycles*, *5*, 485.

Liu, H. W., Kubo, I., & Nakanishi, K. (1982). *Heterocycles*, *17*, 67.

Okorie, D. A. (1982). *Phytochemistry*, *21*, 2424.

Rajab, M. S., Rugutt, J. K., Fronczek, F. R., & Fischer, N. H. (1997). *Journal of Nat. Prod.*, *60*, 822.

Rugutt, J. K., Fischer, N. H., Nauman, M. A., Schmidt, T. J., & Berner, D. K. (1996). *Spectroscopy Letters*, *29*, 711.

Rugutt, J. K. (1996). *Control of African Striga species by natural products from native plants*. Ph.D. dissertation, Louisiana State University.