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PEDONIN, A SPIRO TETRANORTRITERPENOID INSECT ANTIFEEDANT FROM *HARRISONIA ABYSSINICA*

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Key Word Index—*Harrisonia abyssinica*; Simaroubaceae; antifeedant; tetranortriterpene; limonoid; pedonin.

Abstract—A novel ring-D cleaved spiro tetranortriterpenoid insect antifeedant, pedonin, has been isolated from the roots of *Harrisonia abyssinica*. Pedonin was studied spectroscopically and its structure determined by X-ray analysis.

INTRODUCTION

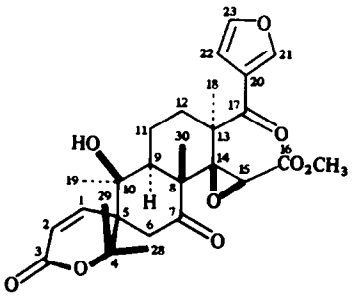
The East African shrub *Harrisonia abyssinica* Oliv (Simaroubaceae) (Kidori, Mkoromando, Mulilyuli, Pedo, Orongonwe, in local dialects) is widely used in local medicine as a remedy for fever, bubonic plague, tuberculosis, haemorrhoid, snake-bite, etc. [1]. Crude extracts of the root bark of the plant have previously been shown to exhibit insect antifeedant, antimicrobial, cytotoxic and plant growth inhibitory activities [2, 3]. Three tetranortriterpenes, obacunone, harrisonin and acetoxyharrisonin, have so far been isolated from the shrub. The first two showed mild antifeedant activities against the larvae of the East African monophagous crop pest *Spodoptera exempta* [2, 4] and the third against the Southern armyworm, *Spodoptera eridania* [3]. Our recent interest in comparative activities of tetranortriterpenes has led us to reexamine the extracts of *H. abyssinica* and we now describe a new antifeedant, pedonin, which we have isolated from the methanolic extracts of the root of the shrub.

RESULTS AND DISCUSSION

The tetranortriterpenoid nature of pedonin (**1**) and its close relationship to obacunone and harrisonin was apparent from spectroscopic evidence. The mass spectrum (FAB) gave a molecular weight of 500. The IR spectrum (ν_{max}^{KBr} cm^{-1}: 3518, 1748, 1722, 1705 and 1670) suggested the presence of a free hydroxyl group and four carbonyl groups, two of which were attributable to an α,β-unsaturated lactone and a six-membered cycloketone groups, respectively. The ^{1}H NMR spectrum showed resonances for five tertiary methyl groups (δ0.97, 1.25, 1.38, 1.58 and 1.63), a disubstituted double bond (6.35 and 6.17, *d*, J = 10 Hz, H-1 and H-2), the characteristics of a β-substituted furan (6.93, 7.38 and 8.59) and a secondary epoxidic proton (3.75, *s*, H-15). A major difference between the ^{1}H NMR spectrum of **1** and those of obacunone and harrisonin was the downward shift (8.59) of one of the furan protons, suggesting the presence of a deshielding anisotropic effect of a C-17 carbonyl group. The ^{13}C NMR spectrum (see Experimental) showed that two of the four carbonyl groups were ketonic and the other two were either ester or lactonic, and confirmed the presence of an α,β-substituted furan and a secondary, tertiary ether unit. This spectral data, however, could not be interpreted in terms of a unique structure, so an X-ray analysis of the compound was undertaken. Figure 1 shows the relative molecular structure obtained. The absolute configurational assignment of pedonin (**1**) is based on comparison with obacunone and other limonoids.

The 2-D homonuclear correlation spectrum (COSY) of **1** in the region 1–4 ppm (Fig. 2) was obtained to allow chemical shift assignments of CH_2 and CH_3 protons to be made. The spin coupling network linked to the C-9 proton suggests that the signals centred at δ 1.86 and 2.05 are due to C-11 α- and β-hydrogens, respectively, and that at 1.38 is due to C-30 methyl hydrogens. The signals of the C-12 hydrogens are located, overlapping with methyl protons, at 1.55 and 1.35, respectively. The uncoupled methyl protons at 0.97 and 1.25 are due to C-28 and C-29 geminal methyl groups. The remaining two resonances at 1.58 and 1.63 may be assigned to C-18 and C-19 methyl protons, respectively.

Pedonin belongs to a growing group of limonoids in which one or more of the rings of the cyclopentenophenanthrene skeleton are cleaved [5]. It resembles oriciopsin [6] in having its ring D oxidatively cleaved to give a β-ketofuran group, and the resulting carbonyl group



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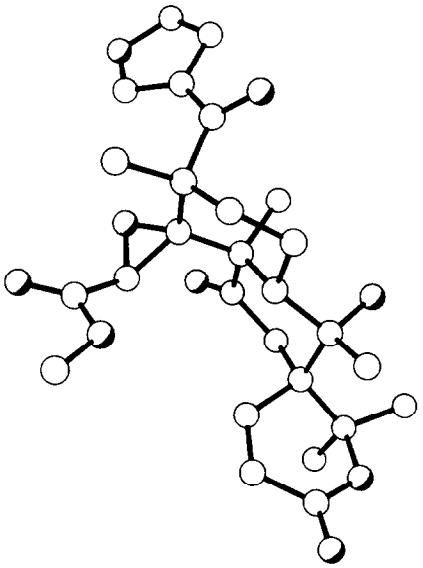

Fig. 1. Relative molecular structure of pedonin by X-ray crystallography.

methylated to a carbomethoxy group. However, to our knowledge it represents the first example of a tetranortriterpenoid in which oxidation of C-5 of the biogenetic parent compound is followed by 1,2 skeletal rearrangement to give a spiro structure.

Pedonin shows potent antifeeding activity against the African crop pests *Eldana saccharina* and *Maruca testulalis*, although it is inactive against the armyworm *Spodoptera exempta*. Details of these tests will be reported elsewhere.

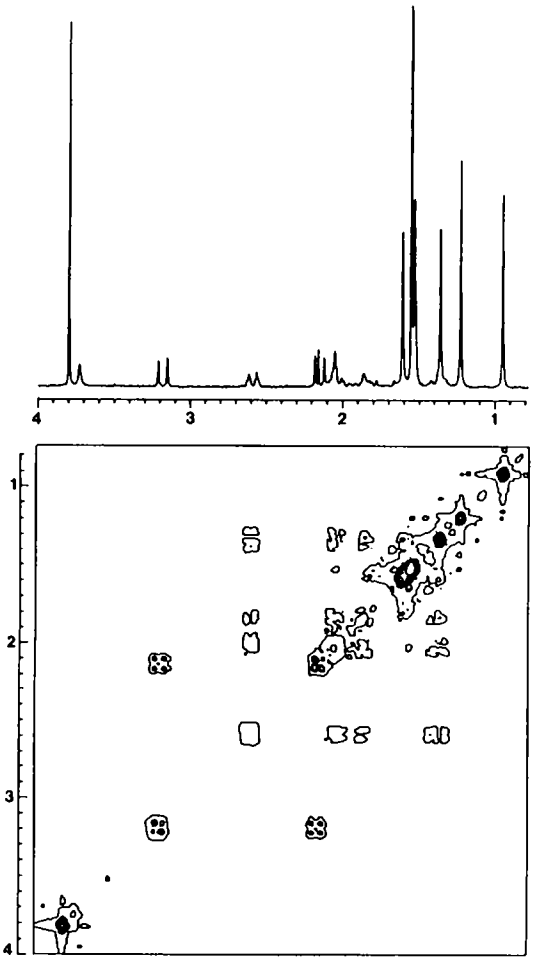

Fig. 2. 2-D proton homonuclear correlation plot for pedonin (1) in the region 1–4 ppm.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were obtained on a Nicolet NT200; COSY spectra were obtained on Varian XL300. FAB mass spectral measurement was carried out on a Finnegan MAT73. Crystal structure determination was done on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) and using ω-scans.

Plant material. The roots of *H. abyssinica* were collected in western Kenya in October, 1983. The plant was identified by S. G. Mathenge and O. M. Mwangangi of the Department of Botany Herbarium, University of Nairobi, Kenya.

Extraction and isolation. H. abyssinica roots (1.5 kg) were chopped into small pieces and allowed to stand for 3 days in MeOH (3.2 l.). After filtration, the MeOH was evaporated and the residue partitioned between H_2O (100 ml) and $CHCl_3$ (3 × 75 ml). The combined $CHCl_3$ phases were dried over dry Na_2SO_4 and evaporated to yield 7.0 g of oil. A portion of the oil (4.7 g) was carefully chromatographed on silica gel (50 × 3.5 cm; 70–130 mesh) using a petrol–Me_2CO (4:1)/Me_2CO gradient. The four limonoids eluted in the order harrisonin, acetoxyharrisonin, obacunone and lastly pedonin (**1**). Compound **1** was recrystallized from aq. Me_2CO to give colourless needles (60 mg, mp 259–261°). MS (FAB) *m/z* 501 $[MH]^+$; IR ν_{max}^{KBr} cm^{-1}: 3518 (OH), 1748, 1722, 1705 and 1670 (C=O); UV λ_{max}^{EtOH} nm: 202, 218 (sh) and 257; ^{1}H NMR (200 MHz, $CDCl_3$): δ0.97, 1.25, 1.38, 1.58, 1.63 (each 3H, *s*), 1.3–2.1 (4H *m*, H-11 and 12), 2.17 (1H, *d*, *J* = 14.7, H-6β), 2.60 (1H, *dm*, *J* = 13 for the doublet, H-9), 3.21 (1H, *d*, *J* = 14.7, H-6α), 3.75 (1H, *s*, H-15), 3.82 (*s*, OCH_3), 6.17 (1H, *d*, *J* = 10, H-2), 6.35 (1H, *d*, *J* = 10, H-1), 6.93 (1H, *d*, *J* = 1.8, H-22), 7.38 (*dd*, *J* = 1.8, 1.2, H-23) and 8.59 (1H, *d*, *J* = 1.2, H-21); ^{13}C NMR (75.429 MHz): δ207.6 (C-7), 196.7 (C-17), 168.8 (C-16), 162.9 (C-3), 149.5 (C-1), 146.9 (C-21), 142.9 (C-23), 125.7 (C-20), 122.3 (C-2), 110.3(C-22), 86.7(C-4), 78.3 (C-10), 69.7 (C-14), 56.6 (C-15), 53.4 (C-5), 52.5 (OCH_3), 52.3 (C-13), 51.0 (C-8), 50.2 (C-9), 42.2 (C-6), 35.1 and 23.9 (C-11 and 12), 27.5, 26.2, 24.1, 19.4 and 17.7 (CH_3). The ^{13}C NMR assignments are based upon a comparison with the values for oriciopsin [6], harrisonin and obacunone [2].

Crystal data. $C_{27}H_{32}O_9$, tetragonal, $a = b = 10.325$ (2), $c = 22.506(3)$ Å, $U = 2399$ Å^3, space group $P4_1$ (or enantiomorphic $P4_3$), $Z = 4$, $M_r = 500.5$, $D_c = 1.39$ g cm^{-3}, μ (Cu-K_α) = 8 cm^{-1}, F(OOO) = 1064. 1625 independent observed reflections [$|F_o| > 3\sigma(|F_o|)$, $\theta < 58°$] were measured on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) and using ω-scans. The structure was solved by direct methods and the non-hydrogen atoms refined anisotropically. The protons on both the spiro epoxide and the hydroxyl group were located from a ΔF map and refined isotropically. All other proton positions were idealized (C–H = 0.96 Å), assigned isotropic thermal parameters, U(H) = 1.2 U_{eq} (C) and allowed to

ride on their parent carbon atoms. The orientations of all the methyl groups were determined from a ΔF map, and the groups refined as rigid bodies. It was not possible to determine the absolute configuration from the data. Refinement converged to give $R = 0.026$, $R_w = 0.028$, $[W^{-1} = \sigma^2(F) + 0.00025\,F^2]$. Maximum residual electron density was 0.05 eÅ^{-3} and maximum shift/error in final refinement were 0.02 and 0.06, respectively.

There is an intermolecular O–H . . . O (2.93 Å, OHO = 157°) between the hydroxyl oxygen, on C-10 and the methyl ester carbonyl oxygen.

Tables of final atom coordinates, temperature factors, and observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre.

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24-METHYLENE-25-METHYLLATHOSTEROL: A STEROL FROM *SICYOS ANGULATUS*

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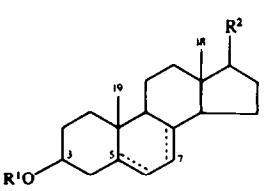
Key Word Index—*Sicyos angulatus*; Cucurbitaceae; sterol; 24-methylene-25-methyllathosterol.

Abstract—A new sterol isolated from the aerial parts of *Sicyos angulatus* has been shown to be 24-methylene-25-methyllathosterol.

We have recently demonstrated the co-occurrence of C-24 epimers of some 24-ethyl-Δ^5- and Δ^7-sterols [1–4], and moreover of 24-ethyl-Δ^8-sterols [4], and 24-methylsterols [4], in plants of the family Cucurbitaceae. Our continuing study on the sterol constituents of cucurbitaceous plants has led to the isolation of a sterol from the aerial parts of *Sicyos angulatus* (bur cucumber), and this paper describes the characterization of the sterol as 24-methylene-25-methyllathosterol (24-methylene-25-methyl-5α-cholest-7-en-3β-ol or 25-methyl-5α-ergosta-7,24(28)-dien-3β-ol, **1a**) which is considered to be a new sterol.

The aerial parts (leaves and stems) of *S. angulatus* (29 kg) were air dried and the lipid (95 g) was extracted with CH_2Cl_2 in a Soxhlet extractor. The unsaponifiable lipid (42.5 g), obtained from the extracted lipid through alkaline hydrolysis (5% KOH in MeOH) under reflux followed by extraction with isopropyl ether, was subjected to column chromatography on silica gel (330 g) (hexane–Et_2O, hexane–EtOAc, and then MeOH as eluant) which provided a sterol fraction (2.6 g, $R_f = 0.19$ on analytical TLC). A portion of the sterol fraction was acetylated, and the steryl acetate (1.1 g) was subjected to argentation TLC to give six bands. The fraction (47 mg) recovered from the fifth band from the solvent front ($R_f = 0.18$ on argentation TLC), consisted of two major components with RR_t 1.98 (**2a**) and 1.61 (**2b**) on GC. This was then subjected to reverse-phase HPLC yielding **2a** (4 mg) and **2b** (4 mg) of which the latter was identified as 24-methylenelathosteryl (24-methylene-5α-cholest-7-en-3β-yl) acetate (**2b**).



1 5α-H, Δ^7, R¹ = H
2 5α-H, Δ^7, R¹ = Ac
3 Δ^5, R¹ = H
4 Δ^5, R¹ = Ac