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New Tetrahydroisoquinolinones from *Hyeronima oblonga* (Euphorbiaceae)

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Abstract: The bioassay-guided fractionation of the CH_2Cl_2 extract from the wood of *Hyeronima oblonga* (Euphorbiaceae) afforded two new alkaloids with a tetrahydroisoquinolinone nucleus described here for the first time. The compounds 3-methyl-4-methoxy-6-(1-oxoctyl)-5,6,7,8-tetrahydro-1(2H)isoquinolinone (Hyeronine A, **4a**) and 3-methyl-4-methoxy-6-(1-oxohexyl)-5,6,7,8-tetrahydro-1(2H)isoquinolinone (Hyeronine B, **4b**) displayed LC_{50} of 4 ppm in the brine shrimp lethality assay.

Alkaloids with the 1,2,3,4-tetrahydroisoquinoline nucleus are derived from tyrosine via a Mannich-like cyclization after the introduction of an additional carbon atom.[1] In this paper we report the isolation and identification of two new 5,6,7,8-tetrahydroisoquinolinones from a plant extract that disclosed significant activity in the brine shrimp (*Artemia salina* Leach.) lethality assay (BSLA), a surrogate bioassay for cytotoxic activity.[2] Although some compounds containing the 5,6,7,8-tetrahydroquinolinone nucleus have already been prepared by synthesis[3,4,5] no report could be found regarding the isolation or preparation of 5,6,7,8-tetrahydroisoquinolinones, described here for the first time.

In a biological screening of plants from the Brazilian coast rain forest ("Mata Atlântica") we used the BSLA model to detect the presence of bioactive substances in extracts. The crude CH_2Cl_2 extract (2.7 g) obtained from the stem wood of *Hyeronima oblonga* (Tul.) Muell. Arg. var. *obtusata* Muell. Arg. (Euphorbiaceae),[6] displayed strong toxicity against *A. salina* (LC_{50} = 10 ppm). This extract was chromatographed over silica gel column to afford 16 fractions (A-P). Fraction M (68 mg) was the most active, with a LC_{50} of 4 ppm. This fraction was chromatographed on a semi-preparative RP-18 HPLC column to yield 2 mg each of Hyeronine A (**4a**) and B (**4b**).Both present LC_{50} of 4 ppm against *A. salina*.

These substances showed UV λ_{max} absorption at 248 nm with a shoulder at 273 nm indicating a conjugated system compatible with absorptions of pyridinone rings.[7]

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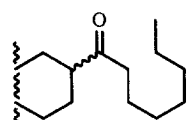
Their alkaloidal nature was reinforced by a positive Dragendorff reaction on silica gel TLC plate. Furthermore, the HR-FABMS of hyeronine A in the positive ion mode showed a base peak $[M+H]^+$ at 320.2008 corresponding to the molecular formula $C_{19}H_{29}NO_3$ (calcd. = 320.2226, Δmmu = 21.8). This molecular formula was in agreement with its ^{13}C, DEPT and ^1H NMR spectra. The ^{13}C NMR spectrum showed the presence of carbonyl signals from amide and saturated ketone. Four signals between δ 130–150, corresponding to insaturated carbons belonging to two double bonds, were also evident. In order to complete the insaturation index, the compound must also have two rings.

The signals of two vicinal methylene groups appear in the ^1H NMR spectrum as two *ddd* signals, one at δ 2.75 (*J* = 18.2, 14.6 and 5.3 Hz) and the other δ 2.57 (*J* = 18.2, 4.4 and 2.5 Hz), one *tt* signal at δ 2.08 (*J* = 14.3 and 4.6 Hz) and a *dddd* centered at δ 2.20 (*J* = 14, 5.3, 2.6 and 2.6 Hz). Analysis of the COSY-45 and HMQC NMR experiments allowed us to deduce the presence of a $-CH_2CHR-$ fragment that was connected to the above vicinal methylenes forming a $-CH_2CHRCH_2CH_2-$ fragment that is part of a ring system.

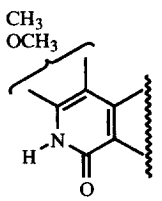
The DEPT-135 sub-spectrum indicated the presence of nine methylene groups. Five of them generated an intense multiplet centered at δ 1.28. In the COSY-45 spectrum they showed couplings with a terminal methyl (δ 0.87) and with a deshielded methylene (δ 1.43), indicating that R in the above fragment is a $-C(O)(CH_2)_6CH_3$ group (**1**). The HR-FABMS corroborates this proposition by the presence of peaks with m/z 220 and 192 Daltons, corresponding to the loss of C_7H_{15} and $C_8H_{15}O$ from the molecular ion, respectively.



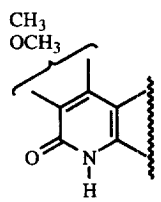
1

The remaining atoms of the molecule correspond to $C_7H_7NO_2$, a fragment containing four insaturations. NMR experiments indicated the presence of four non-hydrogenated carbons forming two double bonds and one amide carbonyl, accounting for 3 insaturations. The insaturation left was attributed to a ring. Signals characteristic of a methoxy and a methyl group, both attached to the above double bonds were also evident from the NMR experiments. Together with an amide NH the molecular formula is now complete. The fragments **2a** and **2b** represent the possible arrangements for these atoms in a ring system. The NOESY experiment showed strong spatial couplings between the protons of the methyl group with both the amide proton and the methoxy protons, indicating that it is in between them. These data point to a pyridinone structure (**2a**) with the substituent arrangement shown in **3**.

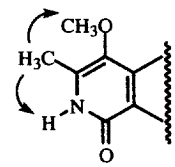
The connection between the fragments **1** and **3** can generate the structures **4a** or **5**. The former is preferred because the vicinity of an amide carbonyl could explain the deshielding of the C-8 methylene protons that appear at δ 2.75 (H_{ax}) and 2.57 (H_{eq}). Thus, according to our analysis **4a** represents the structure of hyeronine A.[8]



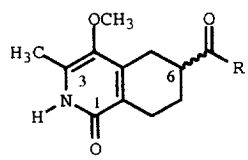
2a

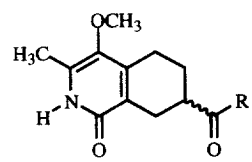


2b



3





4a, R = C_7H_{15}, hyeronine A

4b, R = C_5H_{11}, hyeronine B

5

Hyeronine B afforded spectra very similar to those of hyeronine A. The differences included: the HR-FABMS in the positive ion mode registered the $[M+H]^+$ peak with *m/z* 292.2000, corresponding to the molecular formula $C_{17}H_{26}NO_3$ (calcd.= 292.1992, Δ = -0.8 mmu); the ^{1}H-NMR, ^{13}C NMR and DEPT-135 data indicated the absence of signals from two methylene of the alkanoyl side chain, thus confirming the mass difference (28 daltons) observed in the HRMS. These data[9] attest that hyeronine B has an hexanoyl group attached to C-6 instead of the octanoyl present in hyeronine A.

Hyeronines A and B are minor components in the *H. oblonga* extract and their isolation were pursued due to their bioactivity in the brine shrimp lethality assay. Efforts to isolate larger amounts of these substances are underway and will allow us to perform a more detailed evaluation of their biological activities and to determine their absolute configurations.

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8. Hyeronine A (**4a**): yellow amorphous powder (2 mg), $[\alpha]^{25}_{D}$ (c 1, $CHCl_3$) +48.57 UV λmax (nm): 210*sh*, 248. ^{1}HNMR (400 MHz, $CDCl_3$, δ): 0.87 (3H, *m*, CH_3-octanoyl), 1.28 (10H, *m*, 5 x CH_2), 1.43 (3H, *m*, -CH_2-CO and H-C5), 1.77 (1H, *m*, H-C5), 2.08 (1H, *dddd*, *J* = 14.3, 14.3, 4.6, 4.6 Hz, H*ax*-C7), 2.20 (1H, *dddd*, *J* = 14.0, 5.3, 2.6, 2.6 Hz, H*eq*-C7), 2.35 (3H, *s*, CH_3-C3), 2.57 (1H, *ddd*, *J* = 18.2, 4.4, 2.5 Hz, H*eq*-C8), 2.75 (1H, *ddd*, *J* = 18.2, 14.6, 5.3 Hz, H*ax*-C8) 3.26 (1H, *m*, H*eq*-C6), 3.94 (3H, *s*, CH_3O-C4), 8.62 (1H, *s*, H-N). ^{13}CNMR (100 MHz, $CDCl_3$, δ): 14.11 (*q*, C-octanoyl), 14.69 (*q*, CH_3-C3), 22.67 (*t*, C-octanoyl), 24.48 (*t*, C-octanoyl), 28.51 (*t*, C-octanoyl), 29.31 (*t*, C-octanoyl), 29.61 (*t*, C-octanoyl), 29.66 (*t*, C-octanoyl), 30.33 (*d*, C6), 30.60 (*t*, C5), 31.88 (*t*, C-octanoyl), 32.25 (*t*, C8), 59.40 (*q*, CH3O-C4), 131.96 (*s*, C3), 137.94 (*s*, C8a), 139.10 (*s*, C4a), 147.63 (*s*, C4), 173.41 (*s*, -C(O)NH-), 194.93 (*s*, O=C-octanoyl). Pos-FABHRMS *m/z* (rel. int.): 320 (100%), 220 (12%), 192 (17%), 206 (24%), 117 (45%).
9. Hyeronine B (**4b**): yellow amorphous powder (2 mg), $[\alpha]^{25}_{D}$ (c 1, $CHCl_3$) +16.36. UV λmax (nm): 210*sh*, 248. ^{1}HNMR (400 MHz, $CDCl_3$, δ): 0.88 (3H, *m*, CH_3-hexanoyl), 1.28 (6H, *m*, 3 x CH_2, hexanoyl), 1.44 (3H, *m*, -CH_2-CO and H-C5), 1.78 (1H, *m*, H*ax*-C5), 2.08 (1H, *dddd*, *J* = 14.3, 14.3, 4.6, 4.6 Hz, H*ax*-C7), 2.20 (1H, *dddd*, *J* = 14.0, 5.3, 2.6, 2.6 Hz, H*eq*-C7), 2.35 (3H, *s*, CH_3-C3), 2.58 (1H, *ddd*, *J* = 18.2, 4.4, 2.5 Hz, H*eq*-C8) 2.75 (1H, *ddd*, *J* = 18.2, 14.6, 5.3 Hz, H*ax*-C8) 3.26 (1H, *m*, H-C6), 3.94 (3H, *s*, CH_3O-C4), 8.62 (1H, *s*, H-N). ^{13}CNMR (100 MHz, $CDCl_3$, δ): 14.10 (*q*, C-hexanoyl), 14.69 (*q*, CH_3-C3), 22.65 (*t*, C-hexanoyl), 24.48 (*t*, C7), 28.46 (*t*, C-hexanoyl), 29.31 (*t*, C-hexanoyl), 30.33 (*d*, C6), 30.59 (*t*, C5), 31.86 (t, C-hexanoyl), 32.25 (*t*, C8), 59.40 (*q*, CH_3O-C4), 131.96 (*s*, C3), 137.93 (*s*, C8a), 139.09 (*s*, C4a), 147.63 (*s*, C4), 173.41 (*s*, -C(O)NH-), 194.93 (*s*, C=O, hexanoyl). Pos-FABHRMS *m/z* (rel. int.): 292 (100%), 291 (3%), 192 (8%).