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Antialactone: A New γ-Lactone from *Antiaris africana*, and its Absolute Configuration Determined from TDDFT CD Calculations

Vouffo Bertin[a], Hidayat Hussain[b], Simeon F. Kouam[c], Etienne Dongo[a,*], Gennaro Pescitelli[d], Piero Salvadori[d], Tibor Kurtán[e] and Karsten Krohn[b,*]

[a]*Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P. O. Box 812, Yaounde, Cameroon*

[b]*Department of Chemistry, Universität Paderborn, Warburger Straße 100, 33098 Paderborn, Germany*

[c]*Department of Chemistry, Higher Teachers' Training College, University of Yaounde I, BP 47, Yaounde, Cameroon*

[d]*Università di Pisa, Dipartimento di Chimica e Chimica Industriale, via Risorgimento 35, 56126 Pisa, Italy*

[e]*Department of Organic Chemistry, University of Debrecen, PO Box 20, H-4010 Debrecen, Hungary*

k.krohn@uni-paderborn.de

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Four compounds were isolated from the stem bark of *Antiaris africana*. One of them, a γ-lactone named antialactone (**1a**), is reported for the first time as a natural product. The structures were determined by comprehensive analyses of their 1D and 2D NMR spectra and EI MS data. The absolute configuration of antialactone acetate (**1b**) was established by TDDFT CD calculations and comparison with measured CD spectra. The remaining three known compounds were identified, by comparing their spectroscopic data with those reported in the literature, as lichenxanthone, *β*-sitosterol, and betulinic acid.

Keywords: *Antiaris africana*, Moraceae, xanthone, triterpenes, γ-lactone.

Antiaris africana (Moraceae) is a tree, usually about 15-20 meters high, but sometimes up to 40 meters, which produces a white latex [1,2]. The plant is used in the treatment of chest pain, syphilis, throat infection, leprosy and also as a purgative agent [2,3]. Previous phytochemical investigations of the plant revealed the presence of several cardiac glycosides, triterpenes, steroids, and betaines [3–5]. In the present study of the stem bark, a novel γ-lactone, named antialactone (**1a**), has been isolated (Figure 1), in addition to three known compounds, lichenxanthone, *β*-sitosterol and betulinic acid.

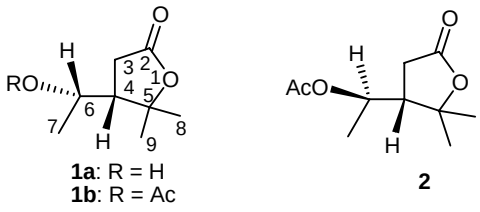

Figure 1: Antialactone (**1a**), antialactone acetate (**1b**), and diastereomer of antialactone (**2**).

Antialactone (**1a**) was isolated as a colorless powder. The molecular formula was determined to be $C_8H_{14}O_3$ by HREIMS. The IR spectrum showed absorption bands for hydroxyl (3445 cm^{-1}) and γ-lactone (1759 cm^{-1}) groups. This was in good agreement with the observation of one exchangeable signal for a hydroxyl group at δ 4.71 (1H, d, J = 5.0 Hz, OH) in the ^{1}H NMR spectrum and a signal for the γ-lactone carbonyl at δ 175.4 (C-2) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum further showed signals due to one secondary methyl group [δ =1.06 (3H, d, J = 6.5 Hz, H-7)], two tertiary methyl groups [δ =1.44 (3H, s, H-8) and 1.30 (3H, s, H-9)], a diastereotopic methylene group [δ 2.35 (1H, dd,

J = 17.0, 8.0 Hz, H-3a) and 2.45 (1H, dd, *J* = 17.0, 12.0 Hz, H-3b)], and two methine groups [δ 3.65 (1H, ddq, *J* = 10.0, 6.5, 5.0 Hz, H-6) and 2.15 (1H, ddd, *J* = 12.0, 10.0, 8.0 Hz, H-4)]. This was in accord with the observation of three methyl, one methylene, two methine, and two quaternary carbon resonances in the ^{13}C NMR and DEPT spectra. The structure was assembled by analysis of the correlation spectra (^{1}H–^{1}H COSY, HMQC) and HMBC experiments (Figure 2). The synthesis of the racemic mixture of compound **1a**, along with its diastereoisomer (**2**) was reported by Ogura *et al*. [6].

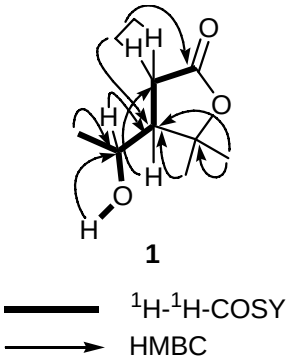

Figure 2: ^{1}H-^{1}H-COSY and HMBC correlations for antialactone (**1a**).

Acetylation of compound **1a** was carried out in the hope of confirming its relative stereochemistry at the centers C-4 and C-6 by comparison of the spectroscopic data with those of the acetylated racemic diastereomers **1b** and **2**. In fact, the ^{1}H NMR spectrum of the acetylated natural product was nearly superimposable with that reported for the synthetic compound **1b**. In particular, the diagnostic chemical shifts for 4-H (δ 2.50) and 6-H (δ 4.92) and the coupling constant $J_{4,6}$ = 10.0 Hz were in perfect agreement, but deviated significantly from the data reported for the diastereoisomer **2** [6]. Thus, with the relative configuration of the acetate **1b** secured, the configuration of **1a** had to be assigned as either (4*S*,6*S*) or (4*R*,6*R*), as shown in Figure 1. The compound was named antialactone after the species from which it was obtained, *Antiaris africana*.

Antialactone (**1a**) showed a negative Cotton effect (CE) n-π* transition [219 nm (–0.26)] in acetonitrile, which was blue-shifted with larger intensity in its acetylated derivative **1b** [210 nm (–0.85)]. In view of the more intense CD, the acetylated derivative **1b** was considered for CD calculations [7] to be compared with the experimental spectrum for the assignment of absolute configuration [8]. First, a conformational search was run on the molecular structure of **1b** using molecular mechanics (MMFF),

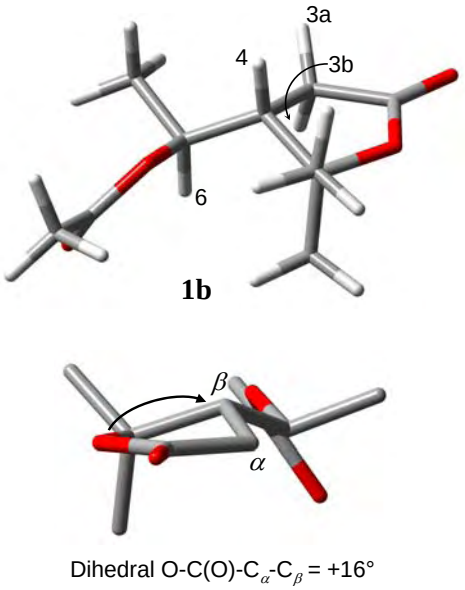

Figure 3: Top: lowest energy B3LYP/6-31G(d) structure of (4*S*,6*S*)-**1b**. Bottom: view from O=C direction emphasizing the positive chirality of the lactone ring.

followed by DFT geometry optimizations. A strongly favored minimum, with a Boltzmann population of 92% at room temperature was obtained, depicted in Figure 3, having the lactone ring in a C$_\beta$-envelope conformation with the C-4 (C$_\beta$) substituent in a pseudo-equatorial position. The computed conformation is in good agreement with the observed diagnostic $^3J_{H\text{-}H}$ couplings, when compared with the values predicted with a Karplus-type equation. Thus, the preferred *anti*-orientation between H-4 and H-6 leads to the large $J_{H4\text{-}H6}$ = 10 Hz (computed: 9.9 Hz); H-4, lying in a pseudo-axial position on the lactone ring, couples with H-3a and H-3b (respectively in a *gauche* and *anti* orientation to H-4) to a very different extent, leading to $J_{H4\text{-}H3a}$ = 8 Hz and $J_{H4\text{-}H3b}$ = 12 Hz (computed: 7.7 and 11.2 Hz, respectively). For a (4*S*,6*S*) absolute configuration, the equatorial orientation of the C-4 substituent imparts a positive chirality to the lactone ring, i.e., a positive O1-C2(O)-C3-C4 dihedral (see Figure 3).

TDDFT calculations [9] run on (4*S*,6*S*)-**1b**, in its lowest energy conformation, led to a negative CD band centered at 210-220 nm (Δε – 1.2), whose sign and relative intensity was almost insensitive to the functional (B3LYP, PBE1PBE, BH&HLYP) and basis employed (aug-cc-pVDZ, aug-cc-pVTZ). It is due to the two n-π* ester transitions, both allied to a negative rotational strength for (4*S*,6*S*)-**1b**. CD spectra were also computed with B3LYP/aug-cc-pVDZ for DFT conformations of **1b** higher in energy than the absolute minimum (> 2 kcal/mol); this led to

a negative n-π* CD band for (4*S*,6*S*)-**1b** when the C-4 substituent was equatorial (positive chirality of the lactone ring), and to a positive CD when it was axial (negative chirality). Since the computed intensities are similar to those for the absolute minimum, and the Boltzmann populations at room temperature are negligible (< 3%), these high-energy conformers do not contribute much to the observed CD. Interestingly enough, the computed trend negative CD ↔ positive chirality of the lactone ring is in keeping with the available CD chirality rules for γ-lactones [10]. These are based on the assumption that the chirality of the ring (a perturber belonging to the so-called second sphere) brings about the dominant contribution to the observed CD. We have already stressed elsewhere the significance of modern re-evaluation of semi-empirical CD rules on the basis of a combination of DFT geometry optimizations and TDDFT excited-state calculations [11].

The good match between experimental and computed CD spectra, especially in consideration of the robustness of TDDFT calculations discussed above, allows us to assign the absolute configuration of the two related antialactones as (–)-(4*S*,6*S*)-**1a** and (–)-(4*S*,6*S*)-**1b**.

Lichenxanthone [12], *β*-sitosterol [13], and betulinic acid [14] were identified by comparison with published data.

Experimental

General: ^{1}H, 2D ^{1}H-^{1}H COSY, ^{13}C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts are referenced to internal TMS (δ = 0) and coupling constants (*J*) are reported in Hz. IR spectra were recorded with a NICOLET 510P FT-IR spectrometer, and a Perkin-Elmer 241 polarimeter. CD spectra were recorded on a J-810 spectropolarimeter.

Plant material: The stem bark of *A. africana* was collected in Mont Eloundem (Yaounde), Central Province of the Republic of Cameroon, in May 2006 and identified by Victor Nana, National Herbarium of Cameroon. A voucher specimen (N^{o} 5925 SRFCam) is deposited in the National Herbarium, Yaounde, Cameroon.

Extraction and isolation: The air dried and powdered stem bark (8 kg) of *A. africana* was macerated with methanol at room temperature. The crude extract was concentrated *in vacuo* to obtain a MeOH soluble fraction. The crude MeOH extract (80 g) was subjected to column chromatography on silica gel using *n*-hexane, *n*-hexane-EtOAc, EtOAc-MeOH, and finally pure MeOH as the mobile phase to yield six fractions (A-F). Fraction E was subjected to silica gel column chromatography, eluting with *n*-hexane-EtOAc (6:4) to give **1a** as a colorless powder (8.5 mg). Similarly, repeated column chromatography of fraction B, eluting with *n*-hexane-EtOAc (7:3), furnished lichenxanthone (9 mg) and *β*-sitosterol (10 mg). Further purification of fraction C, using CC with *n*-hexane-EtOAc (6.5:3.5) as the eluant, afforded betulinic acid (8.6 mg)

Antialactone (**1a**)

White powder.
MP: 122°C.
$[\alpha]^{25}_{D}$: –13.8° (*c* 0.22, CH_2Cl_2).
CD (MeCN, λ [nm] (Δε), c = 8.9×10^{-4}): 219 (–0.26).
IR ν_{max} ($CHCl_3$): 3445, 1759, 1620, 1292, 950 cm^{-1}.
^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.06 (3H, d, $J_{7,6}$ = 6.5 Hz, H-7), 1.30 (3H, s, H-9), 1.44 (3H, s, H-8), 2.15 (1H, ddd, $J_{4,3b}$ = 12.0, $J_{4,6}$ = 10.0, $J_{4,3a}$ = 8.0 Hz, H-4), 2.35 (1H, dd, $J_{3a,3b}$ = 17.0, $J_{3a,4}$ = 8.0 Hz, H-3a), 2.45 (1H, dd, $J_{3b,3a}$ = 17.0, $J_{3b,4}$ = 12.0 Hz, H-3b), 3.65 (1H, ddq, $J_{6,4}$ = 10.0, $J_{6,7}$ = 6.5, $J_{6,OH}$ = 5.0 Hz, H-6), 4.71 (1H, d, $J_{OH,6}$ = 5.0 Hz, OH).
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (3H, d, $J_{7,6}$ = 6.5 Hz, H-7), 1.40 (3H, s, H-9), 1.57 (3H, s, H-8), 2.30 (1H, dd, $J_{3a,3b}$ = 17.0, $J_{3a,4}$ = 8.0 Hz, H-3a), 2.51–2.43 (2H, m, H-4, H-3b), 3.83 (1H, dq, $J_{6,4}$ = 10.0, $J_{6,7}$ = 6.5 Hz, H-6).
^{13}C-NMR (125 MHz, DMSO-d_6): δ 22.0 (CH_3, C-9), 23.7 (CH_3, C-7), 29.7 (CH_3, C-8), 32.9 (CH_2, C-3), 52.3 (CH, C-4), 66.7 (CH, C-6), 87.2 (C, C-5), 175.4 (C, C-2).
HREIMS: *m/z* 158.0929 (calcd. 158.0939 for $C_8H_{14}O_3$).

Acetylation of antialactone (1a): Compound **1a** (5 mg) was dissolved in a mixture of dry pyridine (0.5 mL) and Ac_2O (1.0 mL) and left overnight. After the usual workup, the monoacetate **1b** was isolated (3.5 mg).

Antialactone acetate (**1b**)

Colorless crystals.
MP: 79-81°C.
$[\alpha]^{25}_{D}$: –6.4 (*c* 0.07, CH_2Cl_2).
CD (MeCN, λ [nm] (Δε), c = 6.4×10^{-4}): 210 (–0.85).
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (3H, d, $J_{7,6}$ = 6.5

Hz, H-7), 1.26 (3H, s, H-9), 1.51 (3H, s, H-8), 2.06 (3H, s, C*H*$_3$CO$_2$), 2.37 (1H, dd, $J_{3b,3a}$ = 17.0, $J_{3b,4}$ = 12.0 Hz, H-3b), 2.50 (1H, m, H-4), 2.55 (1H, dd, $J_{3a,3b}$ = 17.0, $J_{3a,4}$ = 8.0 Hz, H-3a), 4.92 (1H, dq, J = $J_{6,4}$ = 10.0, $J_{6,7}$ = 6.5 Hz, H-6).
^{13}C NMR (125 MHz, $CDCl_3$): δ 21.3 (CH_3, CH_3COO), 22.5 (CH_3, C-9), 23.1 (CH_3, C-7), 29.3 (CH_3, C-8), 33.2 (CH_2, C-3), 50.3 (CH, C-4), 70.5 (CH, C-6), 87.0 (C, C-5), 169.2 (C, CH_3CO_2), 175.2 (C, C-2).

Computational section: Conformational searches were run employing MMFF, with standard parameters and convergence criteria, implemented in Spartan'06, Wave function Inc., Irvine CA. The minima thus found were optimized with DFT at the B3LYP/6-31G(d) level, using Gaussian'03W, Revision D.01, Gaussian Inc., Pittsburgh PA. TDDFT calculations were executed with G'03W, employing the hybrid functionals B3LYP, PBE1PBE and BH&HLYP, and the aug-cc-pVDZ and -pVTZ basis sets [15]. The transitions responsible for the CD bands above 190 nm had energies below the estimated ionization potential (7.58 eV), and involved virtual orbitals with negative eigenvalues. CD spectra were generated using rotational strengths computed with dipole-length gauge formulation to which a Gaussian band-shape was applied with 8,300 cm^{-1} half-height width, corresponding to 40 nm at 220 nm. Rotational strengths computed with dipole-velocity gauge formulation differed from dipole-length values by less than 6% with aug-cc-pVTZ.

J-coupling estimations were executed with the Mestre-J program, MestreLab Research, Santiago de Compostela, using the Haasnoot-de Leeuw-Altona equation for chemical groups [16].

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