Tithoniaquinone A and Tithoniamide B: A New Anthraquinone and a New Ceramide from Leaves of *Tithonia diversifolia*

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From the leaves of *Tithonia diversifolia*, four compounds were isolated and identified. Two of them, the anthraquinone tithoniquinone A (**1**) with an unusual substitution pattern on the anthraquinone skeleton, and the ceramide, named tithoniamide B (**2a**), are reported for the first time as natural products. Their structures were determined by comprehensive analyses of their 1D and 2D NMR and electron impact (EI) mass spectral data. The remaining two known compounds were identified as psoralen and *l*-quebrachitol. Preliminary studies showed that tithoniaquinone A (**1**) is strongly antibacterial and antifungal against Gram-positive *Bacillus megaterium* and *Microbotryum violaceum*, respectively, while psoralen has strong algicidal, fungicidal, and antibacterial activities.

Key words: Anthraquinone, *Tithonia diversiflora*, Ceramide

Introduction

In the course of phytochemical studies of medicinal plants from Cameroon [1 – 3], we investigated *Tithonia diversifolia* (Asteraceae) that is locally called "Satan's flower". It is a gray shrub or small tree growing in west and central Africa [4]. *T. diversifolia* demonstrated good antiplasmodial activity. A sesquiterpene lactone (tagitinin C) isolated from this plant possessed cytotoxic [5] as well as potential cancer chemopreventive [6] and anti-inflammatory activities [7]. Previous studies on this species resulted in the isolation of a number of secondary metabolites which belong to the cadinane, chromene, eudesmane, flavone, germacrane, and sesquiterpene classes [6]. The medicinal uses and the presence of different types of compounds in *T. diversifolia* prompted the present investigation in which we investigated the leaf metabolites. In an earlier study, we had investigated those of their flowers and reported the isolation and structure elucidation of one new isocoumarin dimer and one new ceramide [3]. We now report on the structure elucidation of two new compounds, namely, tithoniaquinone A (**1**), an anthraquinone, and tithoniamide B (**2a**), a ceramide.

Results and Discussion

Dried and powdered leaves of *T. diversifolia* were extracted with methanol. The residue obtained after evaporation of the solvent was fractionated between dichloromethane and water, followed by conventional purification procedures, and resulting in the isolation of four constituents, including one new anthraquinone (**1**) and one new ceramide (**2a**).

Tithoniaquinone A (**1**) was obtained as yellow needles, m. p. 204 ℃ and showed the molecular ion peak in HREIMS at *m/z* 282.0519 corresponding to molecular formula $C_{16}H_{10}O_5$ (calcd. for $C_{16}H_{10}O_5$ 282.0528). The UV spectrum of **1**, which exhibited absorption maxima at 225, 270, 292, and 402 nm, suggested an anthraquinone as the basic structure [8], and it was further supported by IR absorption at 1690, 1660, and 1590 cm^{-1}, indicating an anthraquinone
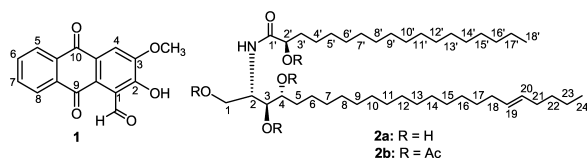
Fig. 1. Structures of tithoniaquinone A (**1**), tithoniamide B (**2a**) and its tetraacetate **2b**.

with one unsubstituted aromatic ring [9]. The compound also showed NMR spectra typical of an 9,10-anthraquinone, with two ^{13}C NMR carbonyl signals ($\delta = 181.9$ and 180.1) for the two non-chelated carbonyl groups and fourteen signals in the aromatic region in addition to a methoxy and an aldehyde signal. The most prominent peaks in the mass spectrum at *m/z* 267 $[M\text{-}CH_3]^+$, 251 $[M\text{-}OMe]^+$, 254 $[M\text{-}CO]^+$, and 253 $[M\text{-}CHO]^+$ also support the above mentioned structure. The ^{1}H NMR spectrum (CDCl$_3$, see Experimental Section) showed two double doublets and two triplets of doublets for four symmetrical AA'BB' type aromatic protons for H-8, H-5, H-6, and H-7 at $\delta = 8.33$ (dd, $J = 1.5, 7.5$ Hz), 8.28 (dd, $J = 1.5, 7.5$ Hz), 7.86 (td, $J = 1.5, 7.5$ Hz), and 7.80 (td, $J = 1.5, 7.5$ Hz), respectively. One singlet at $\delta = 7.71$ for H-4 indicated a trisubstituted C-ring of the anthraquinone. The presence of an aldehyde and one methoxy group in compound **1** is supported further by ^{1}H NMR signals at $\delta = 10.50$ (1 H) and at $\delta = 4.16$ (3 H) and fragment ions *m/z* 267 $[M\text{-}CH_3]^+$, 251 $[M\text{-}OMe]^+$, and 253 $[M\text{-}CHO]^+$ in its EIMS spectrum.

The position of aromatic proton ($\delta = 7.71$) at C-4 was confirmed by its strong HMBC correlations to C-10 ($\delta = 181.9$). Similarly, the methoxy group was found to be attached to C-3 as its protons exhibited HMBC correlations to C-3. To match the molecular formula, the remaining substituent at C-ring had to be a chelated hydroxyl group. This was in good agreement with the chelated signal at $\delta = 12.30$ in the ^{1}H NMR and was also supported by IR absorption at 3450 cm^{-1}. The ^{13}C NMR values of two carbonyl groups at $\delta = 180.1$ and 181.9 indicated the non-chelated nature of these carbonyl groups. Their position was deduced from the HMBC correlations of H-4 and H-5 to C-10 and H-8 to C-9. All data suggest that the aldehyde and OH group must be present at C-1 and C-2, respectively, again confirmed by HMBC interation of the aldehydic proton ($\delta = 10.50$) to C-1, C-2 and C-9a and $\delta = 12.30$ (OH) to C-1, C-2 and C-3. The low field signal for the chelated OH group at $\delta = 12.30$ is formed through hydrogen bond formation

between OH and the neighboring aldehyde carbonyl group at C-1, as also seen by the weak HMBC interaction of $\delta = 12.30$ to the aldehyde carbonyl group ($\delta = 195.4$). Consequently, compound **1** with an unusual substitution pattern on the anthraquinone skeleton, was established to be 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (Fig. 1) and named tithoniaquinone A after the producing organism, *Tithonia diversifolia*.

Tithoniamide B (**2a**) was obtained as a colorless powder, m. p. 135 °C and was assigned the molecular formula $C_{42}H_{83}NO_5$ on the basis of HREIMS. A ceramide skeleton was indicated by the presence of signals in the ^{1}H NMR spectrum: $\delta = 0.85$ (t, $J = 6.8$ Hz, H-24 and H-18') for two terminal methyl groups; protons of long methylene chains [$\delta = 1.25 - 2.10$ (64H)], and an amide proton ($\delta = 8.55$ d, $J = 8.0$ Hz). In agreement with this was an NH-attached methane carbon signal at $\delta = 53.5$ (CH) in the ^{13}C NMR spectrum and a signal at $\delta = 175.7$ for a carbonyl carbon, as well as a strong absorption band at 1640 cm^{-1} for a secondary amide group in the IR spectrum [10].

The ^{1}H NMR spectrum in C$_5$D$_5$N (see Experimental Section) exhibited five downfield signals assignable to protons geminal to oxygen-bearing groups. Three resulted from oxymethine groups [$\delta = 4.62$ (dd, $J = 4.0, 8.0$ Hz, H-2'), 4.34 (dd, $J = 4.5, 6.5$ Hz, H-3), and 4.28 (m, H-4)], and two were due to an oxymethylene group [$\delta = 4.51$ (dd, $J = 4.5, 10.5$ Hz, H-1a) and 4.41 (dd, $J = 4.5, 10.5$ Hz, H-1b). The presence of oxygenated carbons at 77.4 (CH), 73.5 (CH), 73.0 (CH), and 62.5 (CH$_2$) in the ^{13}C NMR spectrum, and the formation of a tetraacetate **2b** proved the presence of four hydroxyl groups in compound **2a**, further supported by the absorption band at 3610 cm^{-1} for hydroxyl group(s) in the IR spectrum. Two CH protons observed at $\delta = 5.51$ (dt, $J = 6.00, 14.5$ Hz), 5.48 (dt, $J = 6.00, 14.5$ Hz), and two CH carbons at $\delta = 131.3$ and 131.2 indicated that the compound possessed one double bond.

The geometry of the C$_{19}$/C$_{20}$ alkene bond was *trans*, as evidenced by the large vicinal coupling constant ($J_{19-20} = 14.5$ Hz). The absence of a proton triplet at *ca.* $\delta = 2.2 - 2.5$ and the appearance of a 1 H multiplet at $\delta = 5.11$ in the ^{1}H NMR spectrum suggested the presence of a –CO-CH(OH)-CH$_2$- moiety in **2a** [10]. The remaining methylene protons are buried in the range $\delta = 1.25 - 2.10$ region. In addition to carbon signals for several methylene groups in the range of $\delta = 26.3 - 34.7$, the terminal methylene carbon at
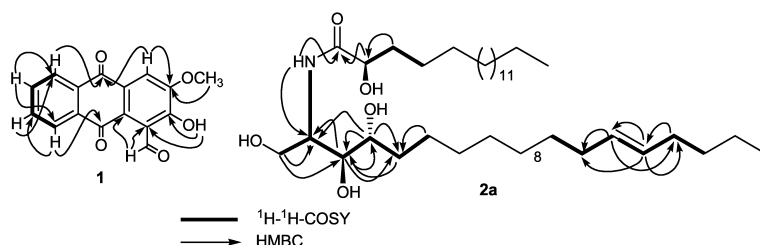
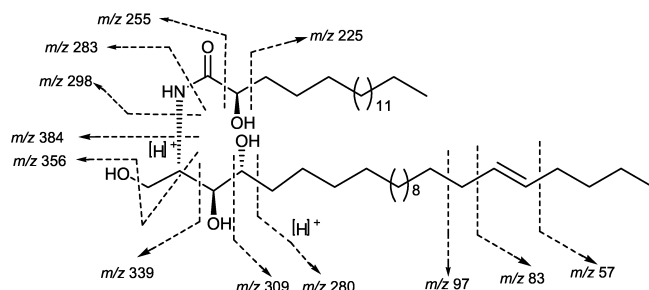Fig. 2. ^{1}H-^{1}H-COSY and HMBC correlations for **1** and **2a**.

Fig. 3. Mass fragmentation pattern of compound **2a**.

$\delta = 23.4$ and the end methyls of aliphatic chains at $\delta = 14.7$ indicated the absence of any branching [10]. The position of the hydroxyl groups were ascertained by the mass fragmentation pattern (Fig. 3), the ^{1}H-^{1}H-COSY, and the HMBC spectra (Fig. 2). Cross peaks in ^{1}H-^{1}H-COSY were observed between an amide proton ($\delta = 8.55$) and the H-2 methine ($\delta = 5.11$), which, in turn, was coupled to three protons at $\delta = 4.51$ (H-1a), $\delta = 4.41$ (H-1b), and $\delta = 4.34$ (H-3). Furthermore, H-3 ($\delta = 4.34$) showed correlations with H-2 ($\delta = 5.11$). No cross peaks were observed of the signal at 4.62 to any downfield proton signals but in the HMBC spectrum it showed strong correlation to C-1' ($\delta = 175.7$). This suggested that the fourth hydroxyl group is present at C-2' of the fatty acid chain. The position of the three hydroxyl groups in the long chain base was further confirmed from the HMBC correlations (Fig. 2) as well as from the mass fragmentation pattern (Fig. 3).

The length of the fatty acid was determined by the characteristic ions (Fig. 3) at m/z 283 $[CH_3(CH_2)_{15}CH(OH)CO]^+$, 300 $[CH_3(CH_2)_{16}CH(OH)CONH_2+H]^+$ and 355 $[CH_3(CH_2)_{15}CH(OH)C(OH)=NC(=CH_2)CH_2OH]^+$ in the EIMS. The length of the long chain base was also determined by the characteristic ions at m/z 342 $[M-CH_3(CH_2)_{17}(CH)_2(CHOH)_2]^+$, 339 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2]^+$ and 356 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2OH]^+$ in the EIMS [1, 2, 11 – 16]. This also confirmed that a double bond is present in the long chain base. The assignments were further confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC correlations (Fig. 2). Thus, the long chain base and fatty acid of **2a** must be 2-amino-19-tetracosene-1,3,4-triol and 2-hydroxyoctadecanoic acid, respectively.

The ^{1}H NMR spectrum of compound **2a** corresponded to that of the synthetic ceramide (2*S*,2'*R*, 3*S*,4*R*)-2-(2-hydroxytetracosanoylamino)hexadecane-1,3,4-triol, with regard to the signals due to H-1a, H-1b, H-2, H-3, H-4 and H-2' (Table 1) [17]. The above NMR data and comparison of the optical rotation of compound **2a** (+10.23) and the synthetic ceramide (+9.1) [17] as well as with a related naturally occuring ceramide [18], suggested that compound **2a** has the same absolute configuration for the core structure of the 2,3,4,2' part. On the basis of this evidence, the structure of **2a** is suggested to be (2*S*, 2'*R*, 3*S*, 4*R*, 19*E*)-*N*-[2'-hydroxyoctadecanoyl]-2-amino-19-henicosene-1,3,4-triol.

Two known compounds, psoralen [19] and *l*-quebrachitol [20] were identified by comparison with published data.

Tithoniaquinone A (**1**), tithoniamide B (**2a**), psoralen and *l*-quebrachitol were tested for herbicidal, antibacterial, and antifungal activities (Table 2). Tithoniaquinone A (**1**) showed strong antibacterial activity against the Gram-positive bacterium *Bacillus megaterium* and antifungal activity against *Microbotryum violaceum*, while psoralen had strong algicidal, fungicidal and antibacterial activities. Algicidal and herbicidal activities roughly correlate. Tithoniamide B (**2a**) and *l*-quebrachitol were inactive in these tests.

Table 1. ^{1}H NMR data (δ and J values) of compound **2a** and synthetic ceramide in C_5D_5N.

H	Tithoniamide B (**2a**)	Synthetic ceramide[a]
H-1a	4.51 (dd, J = 4.5, 10.5 Hz)	4.52 (dd, J = 4.5, 10.7 Hz)
H-1b	4.41 (dd, J = 4.5, 10.5 Hz)	4.43 (dd, J = 5.0, 10.6 Hz)
H-2	5.11 (m)	5.12 (m)
H-3	4.34 (dd, J = 4.5, 6.5 Hz)	4.36 (dd, J = 4.6, 6.6 Hz)
H-4	4.28 (m)	4.29 (m)
H-2'	4.62 (dd, J = 4.0, 8.0 Hz)	4.63 (dd, J = 4.0, 7.6 Hz)

[a] Data from ref. [17].

Table 2. Biological activity of the pure compounds[a] in an agar diffusion test.

Compound	algicidal Chl	antifungal Mb	antibacterial Bm
Tithoniaquinone A (**1**)	0	10	5
psoralen	9	6	5

[a] *Chlorella fusca* (Chl), *Microbotryum violaceum* (Mb) and *Bacillus megaterium* (Bm). The radius of zone of inhibition was measured in cm.

Experimental Section

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. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and Perkin-Elmer 241 polarimeter.

Plant material

The leaves of *Tithonia diversifolia* Hemsl. (Asteraceae) were collected at the University of Yaounde I campus, Central province of the Republic of Cameroon, in December 2004, and identified by Mr. Louis Zapfack (plant taxonomist), Department of Biology and plant Physiology, University of Yaounde I, Cameroon. A voucher specimen (No. 10196) has been deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and isolation

Dried and powdered leaves (3 kg) were extracted with MeOH (20 l) at room temperature for 48 h. The crude extract was suspended in water (1 l) and extracted with *n*-hexane to yield a hexane fraction 80 g, and then extracted with CH_2Cl_2 to yield a dichloromethane fraction (60 g) and similarly with EtOAc to yield an ethyl acetate fraction (3.5 g). The dichloromethane fraction (60 g) was then subjected to column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity) yielding 15 fractions. Fraction F_8 was eluted with a mixture of *n*-hexane-EtOAc (7.5:2.5) yielding tithoniaquinone A (**1**) (10.2 mg), and fractions F_{33-34} [*n*-hexane-EtOAc (5:5)] subjected to a

CC, afforded tithoniamide B (**2**) (11.3 mg). Column fraction F_{17-19} [*n*-hexane-EtOAc (8:2)] and F_{52} [EtOAc-MeOH (7:3)] were similarly subjected to a CC, yielding psoralen (20.5 mg) and *l*-quebrachitol (22.3 mg), respectively.

Tithoniaquinone A (**1**): Yellow crystal, m. p. 204 °C. – UV (CHCl$_3$) λ_{max} (log ε): 225 (3.78), 270 (3.77), 292 (3.65), 402 (3.21). – IR ν_{max} (CHCl$_3$): 3450, 1690, 1660, 1590, 710 cm^{-1}. – ^{1}H NMR (500 MHz, CDCl$_3$): δ = 4.16 (s, 3 H, OMe), 7.71 (s, 1 H, 4-H), 7.80 (td, J = 1.5, 7.5 Hz, 1 H, 7-H), 7.86 (td, J = 1.5, 7.5 Hz, 1 H, 6-H), 8.28 (dd, J = 1.5, 7.5 Hz, 1 H, 5-H), 8.33 (dd, J = 1.5, 7.5 Hz, 1 H, 8-H), 10.50 (s, 1 H, CHO), 12.30 (s, 1 H, OH). – ^{13}C-NMR (125 MHz, CDCl$_3$): δ = 64.7 (OMe), 113.1 (C-4), 117.7 (C-1), 118.0 (C-2), (C-9a), 127.1 (C-1, C-5), 127.4 (C-8), 132.5 (C-10a), 133.6 (C-7), 134.8 (C-6), 134.9 (C-8a), 141.7 (C-4a), 166.6 (C-2), 166.7 (C-3), 180.1 (C-9), 181.9 (C-10), 195.4 (CHO). – HREIMS: *m/z* 282.0519 (calcd. 282.0528 for C$_{16}$H$_{10}$O$_5$). – CIMS (CH$_4$): *m/z* 283.2 [M+1]$^+$. – EIMS (rel. int.): *m/z* 282.1 [M]$^+$ (30), 267 [M-CH$_3$]$^+$, 251 [M-OMe]$^+$, 254 [M-CO]$^+$, 226 [M-2CO]$^+$, 240 (50), 225 (38), 208 (22), 167 (36), 149 (64), 139 (35), 127 (20), 83 (20), 71 (25), 57 (40), 43 (22).

Tithoniamide B (**2a**): Colorless powder, m. p. 135 °C; $[\alpha]_D^{20}$ + 10.23 (c = 0.92, CHCl$_3$ + MeOH); IR ν_{max} (CHCl$_3$ + MeOH): 3610, 2940, 2860, 1640, 1297 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 0.85 (t, J = 6.5 Hz, 6 H, 18'-H, 24-H), 1.30 (s, 7-16-H, 4'-15'-H), 1.58 (m, 2 H, H-23), 1.70 (m, 2 H, 3'-H), 1.73 (m, 2 H, 6-H), 1.86 (m, 2 H, 5-H), 1.95 – 2.10 (m, 4H, 18-H, 21-H), 4.28 (m, 1 H, 4-H), 4.34 (dd, J = 4.5, 6.5 Hz, 1 H, 3-H), 4.41 (dd, J = 4.5, 10.5 Hz, 1b-H), 4.51 (dd, J = 4.5, 10.5 Hz, 1 H, 1a-H), 4.62 (dd, J = 4.0, 8.0 Hz, 1H, 2'-H), 5.11 (m, 1H, 2-H), 5.48 (dt, J = 6.0, 14.5 Hz, 1H, 20-H), 5.56 (dt, J = 6.0, 14.5 Hz, 1H, 19-H), 8.55 (d, J = 8.8 Hz, 1 H, NH). – ^{13}C NMR (125 MHz, CDCl$_3$): δ = 14.7 (C-18', C-24), 23.4 (C-22), 26.3 (C-17', C-23), 27.1 (C-16'), 30.1 (C-8-17, C-4'-15'), 32.6, C-6), 33.4 (C-5), 33.8 (C-18, C-21), 34.7 (C-3'), 53.5 (C-2), 62.5 (C-1), 73.0 (C-2'), 73.5 (C-4), 77.4 (C-3), 131.2 (C-20), 131.3 (C-19), 175.7 (C-1'). – HREIMS: *m/z* 681.6252 (calcd. 681.6271 for C$_{42}$H$_{83}$NO$_5$). – CIMS (CH$_4$): *m/z* 682.4 [M+1]$^+$. – EIMS data and important, ^{1}H-^{1}H COSY and HMBC correlations are illustrated in Figs 2 and 3.

Acetylation of tithoniamide B (**2a**): Dry pyridine (0.5 ml) and Ac$_2$O (1.0 ml) were added to compound **2a** (7 mg), and left overnight. After the usual workup, the tetraacetate of **2a** was isolated to afford the tetraacetate **2b** (5 mg), m. p. 69 °C. $[\alpha]_D^{20}$ + 11.4 (c = 0.92, CHCl$_3$ + MeOH). IR ν_{max} (CHCl$_3$): 2970, 1730, 1620, 1270 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 0.86 (t, J = 6.5 Hz, 6 H, 18'-H, 24-H), 1.30 (s, 7-16-H, 4'-15'-H), 1.60 (m, 2 H, H-23), 1.72 (m, 2H, 3'-H), 1.76 (m, 2 H, 6-H), 1.90 (m, 2 H, 5-H), 1.98 – 2.10 (m, 4H, 18-H, 21-H), 4.53 (dd, J = 4.5, 6.5 Hz, 1 H, 3-H), 4.72 (dd, J = 4.5,

10.5 Hz, 1 H, 1a-H), 4.80 (dd, J = 4.5, 10.5 Hz, 1b-H), 4.97 (m, 1 H, 4-H), 5.01 (dd, J = 4.0, 8.0 Hz, 1H, 2'-H), 5.13 (m, 1H, 2-H), 5.46 (dt, J = 6.0, 14.5 Hz, 1H, 20-H), 5.53 (dt, J = 6.0, 14.5 Hz, 1H, 19-H), 8.88 (d, J = 8.8 Hz, 1 H, NH).

Bioactivity tests: Agar diffusion test

The tested compounds were dissolved in acetone at a concentration of 1 mg/ml. 50 μl of the solution were pipetted onto a sterile filter disc, which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the respective test organism [21]. The test organisms were *Bacillus megaterium* (NB medium), *Microbotryum violaceum* (Mb) and *Chlorella fusca* (MPY); the radius of zone of inhibition was measured in cm.

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