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Constituents from *Tithonia diversifolia*. Stereochemical Revision of 2α-Hydroxytirotundin

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Dedicated to Professor Pedro Joseph Nathan

Abstract. A chemical study of the aerial parts of *Tithonia diversifolia* led to the isolation and stereostructural characterization of tagitinins A (**1**), C (**2**) and F (**3**), and a mixture of sterols. Tagitinin A (**1**) underwent spontaneous dehydration to **4** during the course of its ^{1}H NMR measurement in $CDCl_3$. The stereochemical analysis of 2α-hydroxytirotundin (**5**), an isomer of **1** previously reported by Kinghorn *et al*, led to the correction of its 3*S*,10*S* configuration to the 3*S*,10*R* stereochemistry. In addition, bioactivity evaluation of isolates showed them to exhibit moderate anti-inflammatory and cytotoxic activity.
Key words. Tagitinins, 2α-hydroxytirotundin
Compositae, *Tithonia diversifolia*, sesquiterpene lactones, furanheliangolides, stereochemical revision.

Resumen. Un estudio químico de las partes aéreas de *Tithonia diversifolia* permitió el aislamiento y la caracterización estructural de las tagitininas A (**1**), C (**2**) y F (**3**), y una mezcla de esteroles. La tagitinina A (**1**) experimentó una deshidratación para formar **4** durante la adquisición de la RMN en $CDCl_3$. El análisis estereoquímico de 2α-hidroxitirotundina (**5**), un isómero de **1** previamente informado por Kinghorn *et al*, condujo a la corrección de su configuración 3*S*,10*S* por la estereoquímica 3*S*,10*R*. Adicionalmente, la bioevaluación de los compuestos aislados mostró que estos poseen actividades antiinflamatoria y citotóxica moderadas.
Palabras clave: Tagitininas**,** 2α-hidroxytirotundin
Compositae, *Tithonia diversifolia*, lactonas sesquiterpénicas, furanoheliangólidas, revisión estereoquímica

Introduction

Tithonia diversifolia, also known as "Mexican arnica", has been used in Mexican traditional medicine to treat inflammatory ailments [1, 2]. Its ethnomedical use has been discussed illustrating its antimalarial, cytotoxic, and anti-inflammatory properties [1-6]. Previous chemical studies on *T. diversifolia* led to the characterization of germacranes and eudesmanes [6-12], including the stereochemical correction of tagitinin A (**1**), by means of NMR, X-ray diffraction, and computational analyses [13].

The structural elucidation of 2α-hydroxytirotundin (**5**), an isomer of **1**, was reported by Kinghorn *et al* [6], establishing the conformation and the relative configuration of **5** on the basis of its spectroscopic data. However, the published NMR data analysis of **5** revealed stereochemical inconsistencies with its reported 3*S*,10*S* configuration. Considering the *Twist-Chair-Boat* conformational preference established for the *cis*-fused tetrahydrofuran family of 3,10-epoxy-germacrolide-6,7-*trans*-lactones, as shown by us for **1** [13], it was decided to reanalyze the stereochemistry of **5**. Herein are reported three known sesquiterpenoid lactones (**1**-**3**), the unexpected dehydration from **1** to **4** in the NMR tube, the stereochemical revision of 2α-hydroxytirotundin (**5**), and the anti-inflammatory and cytotoxic activities of isolates.

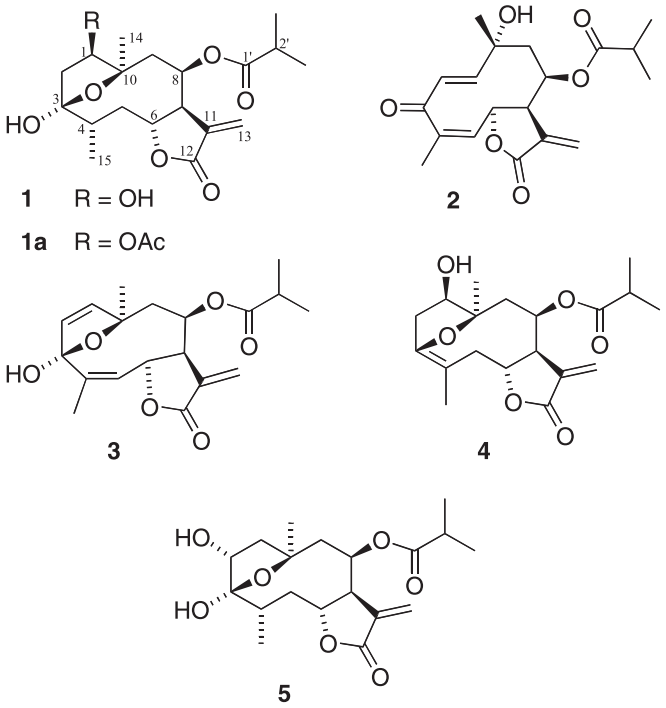

Fig. 1. Sesquiterpene lactones from *Tithonia diversifolia* and derivative **4**.

Results and Discussion

The chemical study of the aerial parts of *T. diversifolia* led to the isolation of three known sesquiterpene lactones, which were characterized as tagitinin A (**1**) [13], C (**2**) [11], and F (**3**) [11] by careful analysis of their spectroscopic data and by comparison with those reported for related metabolites. The acetylation reaction of **1** afforded derivative **1a**, which exhibited identical NMR data to those reported for the natural product 1-acetyltagitinin A (**1a**) [9]. The reported stereochemistry for **1a** [9] should be now corrected according to our earlier revision for **1** [13]. Compound **1** dissolved in $CDCl_3$ underwent spontaneous dehydration to **4** during the course of its NMR data acquisition, which was probably due to the presence of traces of HCl in the deuterated solvent (see experimental section). Therefore, the NMR spectra of **1** were measured in $(CD_3)_2CO$.

In a previous study, the β-configuration of the hydroxyl group at C(1) and the *twist-chair-boat* (TCB) conformation of the oxacyclononane moiety of **1** were established by detailed analyses of its spectroscopic (NOE experiments), crystallographic (X-ray diffraction of a single crystal), and theoretical data (DFT studies of **1** and its analogues) [13]. In this earlier stereochemical study of **1** it was shown that the *cis*-fused tetrahydrofuran family of 3,10-epoxy-germacrolide-6,7-*trans*-lactones, which does not possess a C(4,5) double bond, has the TCB conformation stabilized by the presence of a tetrahedral atom at C(1) [13]. However, 2α-hydroxytirotundin (**5**), a closely related constitutional isomer of **1**, was reported in the literature to have different stereochemistry at C(3) and C(10) and different conformational preference for the oxacyclononane moiety (model **A**, figure **2**) [6]. These inconsistencies provided the impetus for this report in which the stereochemistry of **5** has now been carefully revised.

Kinghorn *et al.* established the relative configuration of **5** on the basis of its ROESY correlations, but a careful analysis of those data revealed stereochemical ambiguities with its 3*S*,10*S*- configuration depicted in model **A** (figure **2**) [6]. The spectroscopic data described for **5** were not consistent with its illustrated conformation wherein ROESY correlations between H-1β and H-4β, between H-6β and H-9, and between H_3-14 and H-1α and H-8α could be observed [6]. Furthermore, the characteristic downfield chemical shift of H-7α (δ 4.19) [6] was not consistent with those reported for the *cis*-fused tetrahydrofuran family of 3,10-epoxy-germacrolide-6,7-*trans*-lactones lacking a double bond at C(4,5) [11]. The examination of the stereochemistry of **5**, by means of Dreiding molecular models, allowed us to establish four possible stereoisomers illustrated in models **A** (3*S*,10*S*), **B** (3*S*,10*R*), **C** (3*R*,10*S*), and **D** (3*R*,10*R*) (Figure **2**). Neither conformer **A** nor conformers **C** and **D** fulfill all ROESY correlations reported for **5**. The 3*R*,10*R* configuration showed in model **D** could not support the ROESY correlation between H-4β and H-6β, because H-4β is pointing away from H-6 probably due to the steric effect of the β-hydroxyl group at C(3) and the strain of this conformer. Instead, the TCB conformation represented in model **B** is in agreement with the reported correlations between H-4β and H-6β and between H-6β and H-9 in which H-9β would be pointing into the interior towards its transannular H-4β and H-6β neighbors. These correlations are in disagreement with the *skew-chair-chair* (SCC) conformation illustrated in models **A** and **C** wherein H-9β would be pointing away from these protons. Additionally, the TCB conformation showed in model **B** supports the downfield chemical shift observed for H-7α (δ 4.19), which is located in the proximity of the 3,10-oxiranyl oxygen. Therefore, the 3*S*,10*R* configuration shown in model **B** depicts the correct configuration and conformation of **5**.

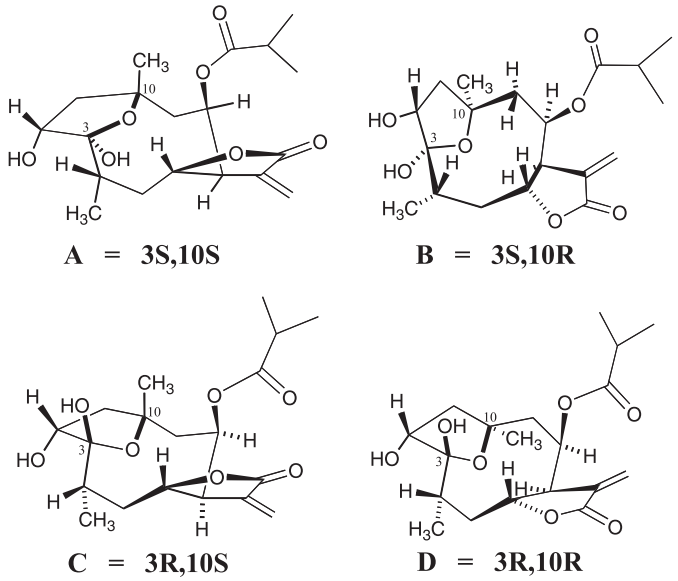

Fig. 2. Four possible stereoisomers of **5**.

Compounds **1** and **2** were evaluated for anti-inflammatory activity against ear edema in mice induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) [14]. Tested compounds showed modest anti-inflammatory activity (24-30 % of inhibition) compared to indomethacin used as a positive control (66.95 % of inhibition); therefore, half inhibitory concentrations were not determined. Furthermore, compounds **1** and **2** were evaluated for cytotoxic activity against the K-562 leukemia and the HCT-15 human colon tumor cell lines [15]. The results indicated that tested compounds possess moderate cytotoxicity (Table 1).

Experimental Section

General Experimental Procedures

Uncorrected melting points were determined on a Fisher John apparatus. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a Shimadzu-UV160 spectrophotometer. Circular dichroism spectra were

Table 1. Cytotoxic activity of **1** and **2**.[a]

Tested compounds	HCT-15	K-562
Tagitinin A (**1**)	18.2 ± 0.37	11.3 ± 2.4
Tagitinin C (**2**)	24.4 ± 2.9	14.9 ± 3.11
Parthenolide	4.41 ± 0.27	3.29 ± 0.12

[a] Half Inhibitory Concentrations in mM ± standard error.

recorded on a Jasco-J720 spectropolarimeter. IR spectra were acquired on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity Plus 500 spectrometer (at 500/125 MHz) and on a Bruker-Avance 300 spectrometer (at 300/75 MHz). EI-mass spectra were measured on a Jeol JMS-AX505HA spectrometer. Column Chromatography (CC) was performed with silica gel 60 (70-230). TLC silica gel 60 F_{254} (Merck) plates were used to follow the fractionation process. Preparative TLC silica gel 60 F_{254} (Merck) plates were used to purify compounds.

Plant Material

Tithonia diversifolia (Hemsl.) A. Gray was collected in San Blas, Nayarit, México on December 2001. A voucher specimen was authenticated by Dr. José Luis Villaseñor and deposited in the National Herbarium, Instituto de Biología, UNAM, with the registry number: MEXU-1014633.

Extraction and Isolation

Dried aerial parts (1.2 Kg) of *Tithonia diversifolia* (Hemsl.) A. Gray were extracted successively with hexane, CH_2Cl_2, and MeOH. The CH_2Cl_2 extract was concentrated *in-vacuo* to give a dark-green residue (30 g), which was separated on a silica gel 60 column chromatography (260 g, fractions of 250 mL were collected) and eluted with hexane and increasing concentrations of EtOAc in hexane to afford seven fractions (100, 95:5, 9:1, 4:1, 7:3, 3:2, 1:1, A-G). Fractions B and C were mixed and fractionated by silica gel CC to afford 105 mg of a mixture of stigmasterol:β-sitosterol, 1:1 (9:1, hexane:AcOEt). Fraction D is a mixture of saturated fatty acids, which were not identified. Fraction E was subjected to Silica gel CC eluted with $CHCl_3$ and mixtures of $CHCl_3$:MeOH. Fractions eluted with 96:4 of $CHCl_3$:MeOH were purified by two-dimensional preparative TLC (eluted with $CHCl_3$:MeOH (97:3) and hexane:EtOAc (7:3)) affording 3.9 mg of tagitinin F (**3**). Fraction F was subjected to silica gel CC and eluted with hexane followed by hexane:EtOAc mixtures. Subfractions eluted with 7:3 of hexane:EtOAc afforded a yellow oil. Dissolution in EtOAc afforded tagitinin C (**2**) (32 mg) as a white solid. Fraction G was processed by CC over silica gel and eluted with an isocratic system of CH_2Cl_2:$(CH_3)_2CO$. Some fractions gave a white precipitate, which was crystallized from EtOAc:$(CH_3)_2CO$. Recrystallization from MeOH gave tagitinin A (85 mg) (**1**) as colorless crystals. An unexpected dehydration of **1** to yield **4** was detected during the course of its NMR data acquisition in $CDCl_3$; therefore, the NMR spectra of **1** were acquired in $(CD_3)_2CO$.

Tagitinin A (1). Colorless crystals: mp 172-174°C; $[\alpha]_D^{25}$ – 123.5 (*c* 0.2, MeOH); UV (*c* 2×10^{-5} M, MeOH) λ_{max} (log ε) 211 (4.06) nm; CD: (*c* 2×10^{-5} M, MeOH), $[\theta]_{252}$ – 314, $[\theta]_{210}$ – 3702; IR ($CHCl_3$) ν_{max} 3606, 3495, 2930, 2856, 1756, 1663, 1598, 1447, 1384, 1145, 1090, 1049, 1011, 946 cm^{-1}; ^{1}H ($(CD_3)_2CO$, 500 MHz) and ^{13}C NMR ($(CD_3)_2CO$, 125 MHz) data were identical to those previously reported [13]. EI-MS, *m/z* (rel. int.): 369 $[M + H]^+$ (12), 351 (3), 33 (2), 280 (11), 262 (21), 211 (32), 121 (24), 97 (20), 71 (62), 43 (100), 27 (8), 18 (2), 15 (1)

Mixture of tagitinin A:Δ^{3,4}-tagitinin A (1:1). **Tagitinin** A (**1**). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 6.27 (1H, d, *J* = 3.6 Hz, H-13a), 5.58 (1H, ddd, *J* = 2.7, 5.4, 8.4 Hz, H-8α), 5.54 (1H, d, *J* = 3.3 Hz, H-13b), 4.57 (1H, ddd, *J* = 1.8, 6.6, 10.2 Hz, H-6β), 4.25 (1H, dd, *J* = 7.5, 9.3 Hz, H-1α), 4.08 (1H, ddd, *J* = 2.7, 6.6, 9.9 Hz, H-7α), 2.44 (1H, m, H-2a), 2.44 (1H, m, H-2'), 2.08 (1H, m, H-4b), 2.08 (1H, m, H-2b), 2.08 (1H, m, H-5β), 2.08 (1H, m, H-5a), 1.96 (1H, dd, *J* = 5.7, 14.0 Hz, H-9b), 1.82 (1H, m, 9.0, 12.9, H-9α), 1.44 (3H, s, CH_3-C10), 1.11 (3H, d, *J* = 6.6 Hz, CH_3-C4), 1.08 (3H, d, *J* = 6.9 Hz, CH_3-C2'), 1.06 (3H, d, *J* = 6.9 Hz, CH_3-C2'); ^{13}C NMR ($CDCl_3$, 75 MHz), δ 176.34 (C-1'), 169.42 (C-12), 137.07 (C-11), 121.57 (C-13), 105.73 (C-3), 81.77 (C-6), 81.46 (C-10), 78.26 (C-1), 70.39 (C-8), 47.84 (C-7), 47.04 (C-2), 44.34 (C-4), 37.79 (C-5), 34.62 (C-9), 34.03 (C-2'), 24.99 (C-14), 19.18 (C-15), 18.72 (C-3'), 18.35 (C-4'). **Δ^{3,4}-Tagitinin A** (**4**). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 6.29 (1H, d, *J* = 3.0 Hz, H-13a), 5.60 (1H, d, *J* = 3.0 Hz, H-13b), 5.50 (1H, ddd, *J* = 2.7, 5.4, 8.4 Hz, H-8α), 4.36 (1H, ddd, *J* = 1.0, 6.0, 10.0 Hz, H-6β), 4.14 (1H, dd, *J* = 5.4, 10.2 Hz, H-1α), 3.96 (1H, ddd, *J* = 3.0, 6.0, 9.0 Hz, H-7α), 2.91 (1H, m, H-2a), 2.84 (1H, m, H-5a), 2.10 (1H, m, H-2'), 2.18 (1H, m, H-5b), 2.17 (1H, m, H-2b), 1.86 (1H, m, H-9a), 1.76 (1H, m, H-9b), 1.70 (3H, s, CH_3-C4), 1.47 (3H, s, CH_3-C10), 1.06 (3H, d, *J* = 6.9 Hz, CH_3-C3'), 1.04 (3H, d, *J* = 6.9 Hz, CH_3-C4'), some data were previously reported [11]; ^{13}C NMR ($CDCl_3$, 75 MHz), δ 176.34 (C-1'), 169.42 (C-12), 147.96 (C-3), 136.88 (C-11), 122.12 (C-13), 107.45 (C-4), 81.40 (C-1), 78.63 (C-10), 78.11 (C-6), 69.68 (C-8), 49.75 (C-7), 39.11 (C-2), 34.31 (C-9), 30.80 (C-5), 21.45 (C-14), 19.17 (C-15), 34.03 (C-2'), 18.65 (C-3'), 18.25 (C-4').

Acetyltagitinin A (1a). 15 mg of **1** were esterified with acetic anhydride in anhydrous pyridine to afford **1a** (12 mg) as colorless needles: mp: 214-216°C; ^{1}H NMR ($CDCl_3$, 500 MHz), δ 6.25 (1H, d, *J* = 3.5 Hz, H-13a), 5.60 (1H, m, H-8α), 5.53 (1H, d, *J* = 2.5 Hz, H-13b), 5.06 (1H, dd, *J* = 6.5, 9.5 Hz, H-1α), 4.56 (1H, dddd, *J* = 1.5, 2.0, 7.0, 11.0 Hz, H-6β), 4.01 (1H, m, H-7α), 2.57 (1H, dd, *J* = 9.5, 14.5 Hz, H-2α), 2.46 (1H, sept, *J* = 7.0 Hz, H-2'), 2.11 (1H, dd, *J* = 6.5, 14.5 Hz, H-5α), 2.11 (1H, dd, *J* = 6.5, 14.5 Hz, H-2β), 2.09 (1H, m, H-4b), 2.08

(3H, s, CH_3-CO_2C-1), 1.88(1H, dd, *J* = 2.0, 13.5 Hz, H-5β), 1.85(1H, dd, *J* = 8.5, 14.0 Hz, H-9β), 1.83 (1H, dd, *J* = 6.5, 14.0 Hz, H-9α), 1.48 (3H, s, CH_3-C-10), 1.12 (3H, d, *J* = 6.5 Hz, CH_3-C-4), 1.09 (3H, d, *J* = 7.0 Hz, CH_3-C-2'), 1.06 (3H, d, *J* = 7.0 Hz, CH_3-C-2'); ^{13}C NMR ($CDCl_3$, 125 MHz), was previously reported [9].

Anti-inflammatory Assay

The anti-inflammatory activity of **1** and **2** was evaluated against mice ear edema induced by TPA as described previously [14].

Cytotoxicity Assay

The cytotoxic activities of compounds **1** and **2** against HCT-15 and K-562 cell lines were evaluated according to a previously described procedure [15].

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