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Article

The Solid-State and Solution-State Reassigned Structures of Tagitinin A, a 3,10-Epoxy-Germacrolide from *Tithonia diversifolia*, and the Interconversion of 3,10-Epoxy-Germacrolide Conformational Families *via* a Ring-Atom Flip Mechanism

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Tagitinina A(2), uma 3,10-epoxi-germacrolida-6,7-*trans*-lactona conhecida e isolada de *Tithonia diversifolia* foi estudada através de difração de raios-X de monocristal. Verificou-se que a mesma apresenta a configuração relativa 1β,4α,6α,7β,8β que difere da orientação 1α em C(1) proposta originalmente na literatura e que foi determinada pelo método de Horeau. Análise do espectro de ^{1}H-RMN de 2 em solução de *d6*-acetona mostra que a molécula mantém a conformação *twist-chair-boat* (TCB) observada cristalograficamente para o anel de 9 membros. As conformações *twist-chair-boat/skew-chair-boat do tipo 3* para anéis de 9 membros saturados e insaturados dentro das 3,10-epoxi-germacrolídas podem ser convertidas à conformação *skew-chair-chair* (SCC) através de mecanismo de inversão de C(9) do anel. Como resultado dessa mudança conformacional, a orientação de C(1) e de C(8) da unidade oxicarbonila são transformados de *diequatorial* para *diaxial*. A estereoquímica relatada para lactonas do tipo 3,10-epoxi-germacrolida e resultados de modelagem utilizando-se DFT B3LYP/6-31g(d) indicam que os átomos C(1) tetraédricos estabilizam conformações TCB/SCB do tipo 3 enquanto que aqueles com geometria trigonal estabilizam a conformação SCC.

Tagitinin A (**2**), a known 3,10-epoxy-germacrolide-6,7-*trans*-lactone isolated from *Tithonia diversifolia*, was investigated by single crystal X-ray diffraction analysis. It was found to have a 1β,4α,6α,7β,8β relative configuration which differed at C(1) from the 1α-orientation originally reported in the literature which was determined by Horeau's Rule. Analysis of the 1H NMR spectrum of **2** shows the molecule to maintain its crystallographically observed *twist-chair-boat* (TCB) nine-membered ring conformation in acetone-*d6* solution. The *twist-chair-boat/skew-chair-boat type 3* conformations of saturated/unsaturated nine-membered rings within 3,10-epoxy-germacrolides can be interconverted to the *skew-chair-chair* (SCC) conformation by means of a C(9) ring atom flip mechanism. As a result of this conformational change, the orientation of the C(1) atom and the C(8)-oxycarbonyl moiety are transformed from *diequatorial* to *diaxial*. The reported stereochemistry of 3,10-epoxy-germacrolide lactone structures, and the DFT B3LYP/6-31g(d) modeling findings in this work indicate that tetrahedral C(1) atoms stabilize the TCB/SCB type 3 conformations, while their trigonal counterparts stabilize the SCC conformation.

Keywords: tagitinin A, Horeau's rule, conformational interconversion, molecular modeling

Introduction

Horeau's rule[1] to determine the absolute configuration of chirotopic stereogenic secondary alcohols is considered to be a well-known and proven method,[2] and has been reviewed by Brewster[3] and Horeau.[4] It was used to determine the (*R*/*S*)-absolute configuration and subsequent α,β-orientation[5,6] of secondary hydroxyl groups in 3,10-epoxy germacrolide 6,7-*trans*-lactone sesquiterpene natural products whose 3,10-oxiranyl oxygen affords a —C(3)-O-C(10)— fragment common to either a 3(2H)-furanone [*e.g.* zexbrevin[7] (**1**)] or to a *cis*-fused tetrahydrofurano moiety [*e.g.* tagitinin A[8] (**2**)] (both drawn with (6*R*,7*S*)-stereochemistry). The skeletons of these

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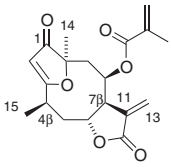
Dedicated to Prof. Kurt Mislow on the occassion of his 80th birthday and to Prof. Alfonso Romo de Vivar, for his 50 years of research at the Instituto de Química, UNAM.

"...חכמתו מתקיימת..." "*...his wisdom shall endure...*" Pirke Aboth, chapter III, verse 12.

compounds are based upon 1-isopropyl-4,8-dimethylcyclodecane (germacrane).[9] In this method, a chiral secondary-alcohol will react *faster* with one of the enantiotopic $C_2H_5C^*H(Ph)C(=O)O$— carbonyl groups in *excess* optically inactive 2-phenylbutyric anhydride [a mixture of *d,l* and *meso*-diastereomers] than with its enantiotopic counterpart in a type of kinetic resolution. Isolation of the *excess unreacted* 2-phenylbutyric acid enables correlation of its sign of optical rotation with the spatial disposition of small, medium, and large local environments around the secondary carbinol carbon.



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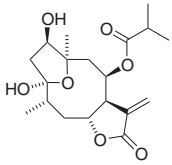

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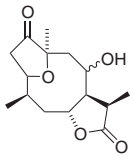

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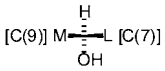
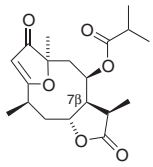


4

Zexbrevin (**1**) has been isolated from the *Zexmenia brevifolia* plant, and its structure and stereochemistry were reported by Romo de Vivar *et al.*[7] It was converted in a number of steps to 8-desmethylacryl-hexahydroxy-zexbrevin [originally drawn with 4α,11α-dimethyl groups, but now known by X-ray diffraction analysis[10] to be 4β,11β and subsequently illustrated as **3**] and the stereochemistry of the free 8-OH was ascertained according to Horeau's procedure as follows. The excess recovered (–)-α-phenylbutyric acid was reported to exhibit $[\alpha]_D^{27}$ –12°, representing an optical yield of 53.3%. According to the rule, the small, medium, and large local environments around C(8) are represented as in **4**, and the absolute configuration was assigned as (8*S*). Using a (6*R*,7*S*)-6,7-*trans*-lactone skeleton and the (8*S*) result, the authors[7] reported an α-orientation for the 8-hydroxyl group. However, the X-ray crystallographically determined structures of tetrahydrozexbrevin (**5**)[10] and 9α-acetoxyzexbrevin,[11] and phototetrahydrozexbrevin A[12,13] were later reported, and the orientation of the 8-isobutyryloxy moiety was then found to be 'β' for all three compounds. Horeau's rule failed to predict the correct α-orientation in this case.



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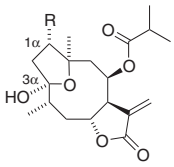
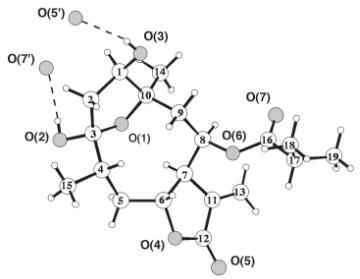


6a R = H
6b R = OH

Tagitinin A (**2**) was originally isolated by Pal *et al.*[14] from *Tithonia diversifolia.* Due to its similarity to tirotundin (**6a**) and its similar chemical behavior, Pal *et al.*[14] proposed structure **6b** without specification of stereochemistry at C(1), C(4), and C(8). Herz and de Jong[8] undertook a more extensive study of tagitinin A and related compounds. They determined the stereochemistry of the 4-methyl and 8-isobutyryloxy moieties to be 4α and 8β, respectively, and reported ^{1}H and ^{13}C NMR chemical shifts [measured at 270 and 67.9 MHz, respectively, $CDCl_3$], plus some of the J_{HH} coupling constants.[8] They also utilized Horeau's method to determine the stereochemistry of the chirotopic stereogenic secondary hydroxyl fragment at C(1), and found it to be (1*S*).[8] The excess recovered (–)-α-phenylbutyric acid was reported to exhibit $[\alpha]_D^{27}$ –6.55° (benzene), representing an optical yield of 41.8%.[8] Based upon a (6*R*,7*S*)-skeleton for **6b**, they proposed an α-orientation for the 1-hydroxyl group.[8] Structure **6b** depicts the stereochemistry of tagitinin A as illustrated in the report of Herz *et al.*[8] The failure of Horeau's rule with the deacylzexbrevin derivative **3** prompted us to reinvestigate the tagitinin A stereochemistry at C(1). This paper reports the solid-state *twist-chair-boat* (TCB) conformation of the nine-membered ring in **2** (as determined by single crystal X-ray diffraction analysis) and the reassignment of a β-orientation for the C(1)-hydroxyl. The solution-state stereochemistry (utilizing NMR techniques) is also described herein. Furthermore, the classification of 3(2H)-furanone [*e.g.* **1**] or *cis*-fused tetrahydrofurano [*e.g.* **2**] 3,10-epoxy-germacrolides into respective oxacyclononane *skew-chair-chair* (SCC) and *twist-chair-boat/skew-chair-boat* (TCB/SCB) conformational families,[15] and their theoretical interconversion *via* a C(9) atom-flip mechanism is also discussed herein.

Results and Discussion



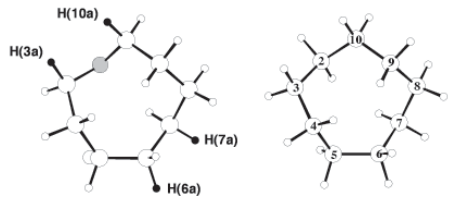
7

Solid-state stereochemistry of tagitinin A

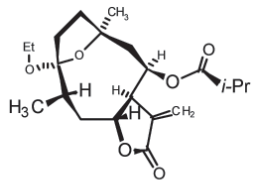
Tagitinin A (**2**) was isolated from *T. diversifolia*, and its chemical and physical properties were found to be identical

to those described in the literature.[8] *T. diversifolia* (also known as "Mexican arnica") has been used in Mexican traditional medicine to treat inflammatory ailments. Its ethnopharmacology has been discussed recently.[16] Compound **2** was subjected to X-ray diffraction analysis, but the absolute configuration[17] of the chiral crystal was unable to be determined.[18] The resulting molecular structure (depicted as Ball and Stick[19] model **7**) within these crystals showed that the originally proposed α-oriented 1-hydroxy group was indeed inverted to a β-orientation. From now on, model **7** will refer to the solid-state structure of crystalline **2**. No unusual bond lengths or bond angles were measured. The hydrogens were placed at calculated positions, and refined as riding atoms on their respective attached atom, with the exception of those ligated to O(2) and O(3) which were located and refined anisotropically. Intermolecular H(O2)···O(7') and H(O3)...O(5') hydrogen-bonds are present in the unit-cell, where O(7') and O(5') are 8-oxycarbonyl and lactonyl carbonyl oxygen atoms related by the respective [–x+1.5, –y, z–0.5] and [–x+0.5, –y+0.5, –z] symmetry transformations.

Evidently, within the diastereomeric transition-states of the kinetically controlled Horeau reaction, the "medium" *versus* "large" bulk or steric volume expressed by a particular sub-unit attached to the chirotopic stereogenic secondary carbinol carbon atom may not always be discernible by inspection of simple models. This may rationalize the failure of Horeau's rule to correctly predict both the 1-hydroxyl orientation in **7** and the 8-hydroxyl disposition in **3**. Alternatively, one perhaps could argue that the two experimental findings for **7** [(1*S*)-stereochemistry by Horeau's rule based and 1β-relative configuration by X-ray crystallography, as well as (8*S*)-stereochemistry/8β-relative configuration for **1**] are not actually mutually exclusive, but that the germacrolide skeleton simply has the opposite (6*S*,7*R*)-6,7-*trans*-lactone stereochemistry since neither the absolute configuration of **7** nor those of the zexbrevin-type compounds (*e.g.* **3,5**) were ever determined by X-ray crystallography. However, this assertion is untenable since numerous X-ray crystallographic determinations of (6*R*,7*S*)-absolute configuration for other germacrolide 6,7-*trans*-lactone natural products are listed in the Cambridge Crystallographic Data Base[20] (CCDB), and one can assume that the biosynthetic pathways determining chirality are all very similar for this class of compounds. A few of the many recent representative CCDB examples of germacrolide X-ray crystallographic absolute configuration determinations were arbitrarily chosen.[21-24] Thus, it is very reasonable that the absolute configuration of the 6,7-*trans*-lactone fragment in the tagitinin A and zexbrevin 3,10-epoxy-germacrolide skeletons should also be (6*R*,7*S*), leaving us with the conclusion that Horeau's rule failed in both cases.



8 **9**



10

The O(1), and C(3-10) atoms of **2** define an oxacyclononane ring (**8**) having a *twist-chair-boat*[15] (TCB) conformation. Superimposition[25] of all the ring atoms of **2** on corresponding atoms in Density Functional Theory B3LYP/6-31g(d)[26] calculated TCB conformation oxacyclononane (**8**) and cyclononane[15] (**9**) models affords small root mean square (RMS) differences of only 0.168 and 0.109 Å., respectively [see comparison of torsion angles in Table 1]. Exchange of a methylene in model **9** into an ether oxygen in model **8** removes a transannular interaction between *endo* protons on C(2) and C(7), and as a result, brings the oxygen in **8** slightly closer to C(2) [2.838 Å and 2.992 Å O···C(7) in experimentally determined tagitinin A (**7**) and in calculated model-**8**, respectively versus 3.352 Å C(2)···C(7) in model-**9**]. The 14° H(6a)–C(6)···C(7)–H(7a) and –3° H(3a)–C(3)···C(10)–H(10a) torsion angles in **8** enable both a *trans*-6,7-lactone closure and 3,10-ethano bridging to proceed without strain. *O*-ethyl-tirotundin[27] (**10**), a 1-deoxy-*O(2)*-ethylated analogue of **2**, has been isolated from *T. rotundifolia*. While coordinates of **10** are not to be found in the CCDB[20] using *Conquest 1.4*,[28] nor in the article itself,[27] endocyclic torsion angles for the nine-membered ring, the tetrahydrofuran ring, the lactone, as well as other selected torsion angles for **10,** are available from Supplementary Material deposited for the paper. A comparison of these angles for **10** with those from **7** shows the same stereochemistry for both compounds (see Table 1). The root mean square (RMS) difference for the list of *all* 23 angles provided is only 3.0°. A subunit geometry comparison of **7** versus **10** affords RMS differences of 3.9° for the endocyclic angles of the TCB conformation oxacyclononane moiety, 2.7° for the

tetrahydrofuran ring, and 2.2° for the lactone. The iconic representation of **10** (as well as others to follow) is a 2D-dimensional projection of the actual 3D-dimensional structure.[29]

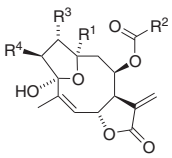
Table 1. Selected torsion angles [°] measured for the X-ray crystallograhically determined molecular structures of tagitinin A [(1*R*,3*R*,4*S*,6*R*,7*S*,8*R*,10*R*)-**7**] and the 1-deoxy-*O(2)*-ethylated analogue [(3*R*,4*S*,6*R*,7*S*,8*R*,10*S*)-3-*O*-ethyl-tirotundin-**10**], versus corresponding angles in B3LYP calculated Twist-Chair-Boat (TCB) conformational models of oxacyclononane (**8**) and cyclononane (**9**)

torsion angle	**7**	**10**[a]	**8**	**9**
C(10)–C(1)–C(2)–C(3)	–17.8(4)	–21.5		
C(10)–O(1)–C(3)–C(2)	21.3(3)	17.8		
C(10)–O(1)–C(3)–C(4)[b]	–101.8(3)	–104.4	–80.4	–70.5
C(2)–C(1)–C(10)–C(9)	–89.3(3)	–85.6		
C(1)–C(2)–C(3)–C(4)	121.3(3)	123.2		
C(1)–C(2)–C(3)–O(1)	–0.8(4)	–3.4		
C(2)–C(3)–C(4)–C(5)	–155.6(3)	–155.4		
O(1)–C(3)–C(4)–C(5)[b]	–37.2(4)	–38.1	–52.2	–50.8
C(3)–C(4)–C(5)–C(6)	92.0(4)	95.1	91.0	103.0
C(12)–O(4)–C(6)–C(7)[c]	9.2(4)	8.2		
C(6)–O(4)–C(12)–C(11)[c]	–3.6(5)	–4.5		
C(4)–C(5)–C(6)–C(7)	–84.1(4)	–84.8	–75.1	–86.3
C(5)–C(6)–C(7)–C(8)	105.6(3)	107.4	106.2	103.0
O(4)–C(6)–C(7)–C(11)[c]	–10.6(3)	–8.3		
C(6)–C(7)–C(8)–C(9)	–56.4(4)	–64.0	–57.6	–51.0
C(6)–C(7)–C(11)–C(12)	8.9(4)	5.9		
C(7)–C(8)–C(9)–C(10)	–59.8(4)	–52.8	–62.6	–70.4
C(8)–C(9)–C(10)–C(1)	173.8(3)	173.4		
C(8)–C(9)–C(10)–O(1)[b]	59.2(3)	60.7	57.3	66.5
C(7)–C(11)–C(12)–O(4)[c]	–3.8(5)	–1.2		
C(13)–C(11)–C(12)–O(5)	–2.9(7)			
H(O2)–O(2)...C(4)–H(4)	29(6)			

[a] Data taken from Supplementary Material deposited for ref. 27, 0.5° average estimated deviation; [b] Atom O(1) in **1**,**8**,**10** corresponds to atom C(1) in **9**; [c] Atom O(4) in **7** corresponds to atom O(3) in **10**.

Anet has developed a very useful general analysis for subsequent assignment of substituent orientation (*isoclinal, axial*, *equatorial*) in rings of any size.[30] Using this method, the O(3) hydroxyl, C(15) methyl, lactone O(4) and C(11) substituents can all be assigned *equatorial* descriptors, while the O(3) hydroxyl bonded to C(1) is *pseudo-equatorial*. In addition, the heterotopic O(2) and C(2) atoms ligated to C(3), the C(14) methyl and C(1) ligated to C(10), as well as the H(8) and oxycarbonyl O(6) bonded to C(8) can all be affixed "*approximately" isoclinal* descriptors. The tetrahydrofuranyl ring has an *envelope* conformation [–0.8° C(1)–C(2)–C(3)–O(1) torsion angle] in which C(10) occupies the *flap* position.

Dale[31] has defined "corner" positions as medium ring atoms which have identically signed *synclinal* (gauche, *ca.* 60°) endocyclic torsion angles on either side. Since corner atoms have two *isoclinal* ligands pointing outwards, *gem*-dimethyl groups are commonly found at corner positions of medium rings. Ring atoms C(3) and C(10) in the TCB C_2-symmetry oxacyclononane and cyclononane models-**8**,**9** are located at "corner" positions. Ligation of an ethano bridge to C(3,10) twists this region of the oxacyclononane ring in **7**, but C(3) and C(10) still retain their character as "corner-like" positions [torsion angles for C(3) are 37° and 102°, while those on either side of C(10) are –89° and –59°]. In accord with this, one finds the only two doubly-substituted oxacyclononane ring atoms in **7** to be C(3) [ligated to O(2) hydroxyl and ethano bridge C(2)] and C(10) [ligated to C(14) methyl and ethano bridge C(1)]. Ordinarily, substituents on these two close proximity doubly-substituted ring atoms [C(3,10)] would have suffered severe steric mutual repulsion, but this is removed in **7** by linking the two groups together as an ethano bridge. In the parent TCB C_2-symmetry cyclononane conformation **9** it is apparent that C(8) is also a "corner" position, since it is homotopic to C(3). However, in the TCB conformation for the *cis*-fused tetrahydrofurano family of 3,10-epoxy-germacrolide lactones it is unlikely that the O(6)-oxycarbonyl [–OC(=O)R] moiety ligated to C(8) in **7** would have an α-orientation (*i.e. syn* to the neighboring C(14) methyl). Such a disposition would afford an unfavorable *1,3-cis-diaxial* type relationship. On the other hand, the O(6)-oxycarbonyl moiety in the *skew-chair-chair* (SCC) conformation 3(2H)-furanone family of 3,10-epoxy-germacrolide lactones can be either α-[32-34] or β-[10-13,35-37] oriented since SCC represents a conformational change involving a flip of ring-atom C(9), and now the C(14) methyl is *equatorial*. Finally, one can predict that a epimeric C(15) methyl diastereomer of **7** would *not* be disposed to retain the TCB conformation since it would then suffer a transannular interaction with the inward pointing *axial* H(9β).



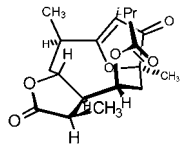
	R^1	R^2	R^3	R^4
11a	CH_3	*i*-Pr	H	OAc
11b	CH_3	epoxy-*trans*-buten-2-yl	OH	H
11c	CH_2OH	(*S*)-*s*Bu	H	H

In addition to **10**, the structures of woodhousin[38] (**11a**), niveusin C-2',3'-epoxide[39] (**11b**) and tithonin[40] (**11c**) are known from X-ray diffraction analysis. These three additional members of the CCDB *cis*-fused tetrahydrofurano family of 3,10-epoxy-germacrolide lactones contain a C(4,5) double-bond. A result of the endocyclic *synperiplanar* torsion angle in **11a-c** is that the TCB conformation of **7**,**10** changes into a *skew-chair-boat type 3* (SCB type 3)[15] *cis*-cyclononene. The existence of *cis*-cyclononene conformational families has recently

been discussed, e.g. SCB types 1-3 differ by having the double-bond located at different positions on the same SCB conformation ring.[15]

Solution-state stereochemistry of tagitinin A

The ^{1}H and ^{13}C NMR spectral parameters of crystalline **7** dissolved in acetone-*d6* are reported in Table 2. A fourteen-spin system is comprised of H(O2), H(4β), H(5α), H(5β), H(6β), H(7α), H(8α), H(9α), H(9β), H(13*endo*), H(13*exo*) and C(14)$\underline{H}_3$. A four-spin system is composed of H(O3), H(1α), H(2α), and H(2β), while a seven-spin system results from the isopropyl moiety. Homonuclear coupling pathways for each of these spin-systems were readily observed in the COSY-90 2D spectrum. The ^{1}H NMR spectrum {1.06 ppm, C(14)$\underline{H}_3$} was simulated using *Gnmr 4.1*,[41] due to second order effects for signals arising from H(4β,5α) [$\Delta\nu$ = 15.5 Hz] and from H(9α,9β) [$\Delta\nu$ = 42.5 Hz]. The multiplicity of protons ligated to ^{13}C nuclei was determined by DEPT-135 and DEPT-90 experiments. ^{1}H and ^{13}C signals were correlated using a 2D-NMR HETCOR spectrum. The H(13*endo*,8α) signals overlap at 298°, but are readily differentiated by their coupling patterns. At 223°, H(13*endo*) and H(8α) appear at δ 5.66 and δ 5.55, respectively. The resolution at low temperature was used to assign H(13*endo*,13*exo*) by means of a NOESY spectrum measured at that temperature. In the 2D spectrum, H(8α) afforded a markedly higher intensity cross-peak to the 5.66 ppm olefinic proton relative to that observed for the 6.08 ppm geminal neighbor. Therefore, the δ 5.59 and δ 6.10 signals in the 298° spectrum were assigned to H(13*endo*) [closer to H(8α)] and H(13*exo*) [closer to lactone carbonyl O(5)], respectively.



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An important difference between the *cis*-fused tetrahydrofurano family of 3,10-epoxy-germacrolide lactones (**2**, **10**, **11a-c**) and the 3(2H)-furano-type [*e.g.* projection of the tetrahydrozexbrevin A (**12**) structure from X-ray diffraction analysis[10]] is that the former set has the C(1) atom and C(8)-oxycarbonyl moiety exhibiting *diequatorial* orientatons and ligated to a TCB [having a C(4)—C(5) single bond] or SCB type 3 [having a C(4)=C(5) double bond] nine-membered ring, while the latter family has them *diaxially* disposed and attached to a SCC conformation [with either C(4)—C(5) single or double bonds]. This *diaxial* arrangement is found for other 3(2H)-furano-type 3,10-epoxy-germacrolides in the CCDB.[10-13,35-37]

Table 2. ^{1}H and ^{13}C NMR (acetone-*d6*) spectral parameters of tagitinin A, **2**[a]

	δ_H		J_{HH}	$\underline{H}$–C–C–$\underline{H}$[b]		δ_C
H(1α)	4.18 [4.23][c]	1α–2α	9.3	21.5	C(1)[d]	79.2 [78.5]
H(2α)	2.35 [2.44][c,e]	1α–2β	7.2	141.0	C(2)[d]	47.8 [46.9]
H(2β)	2.05 [2.1][c]	1α–H(O3)	5.0	.	C(3)[d]	106.4 [105.7]
H(4β)	2.169 [2.1][c]	2α–2β	–13.7	.	C(4)	45.2 [44.4]
H(5α)	2.138 [2.1][c]	4β–5α	8.1	147.2	C(5)	39.2 [37.8]
H(5β)	1.66 [2.1][c]	4β–5β	0.0	96.6	C(6)	82.2 [81.9]
H(6β)	4.56 [4.55]	4β–C(15)$\underline{H}_3$	7.0 [6.5]	.	C(7)	48.7 [47.8]
H(7α)	4.05 [3.99][c]	4β–H(O2)	1.0	.	C(8)	71.2 [69.9]
H(8α)	5.59 [5.59]	5α–5β	–13.2	.	C(9)	35.6 [34.7]
H(9α)	1.911 [1.95][e]	5α–6β	10.9 [9]	161.7	C(10)	82.0 [81.7]
H(9β)	1.826 [1.81][e]	5β–6β	1.3 [3]	82.1	C(11)	139.1 [137.0]
H(13endo)	6.10 [6.25][e]	6β–7α	6.4 [7]	140.7	C(12)	169.4 [169.8]
H(13exo)	5.59 [5.53][e]	7α–8α	3.1 [1.5]	56.7	C(13)	120.8 [121.7]
C(14)$\underline{H}_3$	1.35 [1.43][f]	7α–13*endo*	3.3 [3.5][e]	.	C(14)	25.3 [25.0]
C(15)$\underline{H}_3$	1.06 [1.11]	7α–13*exo*	3.2 [3][e]	.	C(15)[g]	19.0 [19.2]
C$\underline{H}$(CH$_3$)$_2$	2.44 [2.44][c]	8α–9α	5.3 [5][e]	58.9	C(16)	176.2 [176.5]
CHC$\underline{H}_3$	1.03 [1.07]	8α–9β	11.7[8][e]	175.1	C(17)	34.7 [34.1]
CHC$\underline{H}_3$'	1.01 [1.04]	9α–9β	–14.3 [13]	.	C(18)	19.5 [18.8]
H(O2)	4.77	C$\underline{H}$–C$\underline{H}_3$	7.2 [7]	.	C(19)[g]	19.0 [18.4]
H(O3)	4.34	C$\underline{H}$–C$\underline{H}_3$'	7.0 [7]	.		

[a] ^{1}H NMR (500 MHz); ^{13}C NMR (125 MHz); chemical shifts relative to TMS (external), 298 K, acetone-*d6*; δ_H and J_{HH} [Hz] values from spectral simulation using *Gnmr 4.1*,[41] the standard deviation of the last digit in J_{HH} values is *ca.* 0.1 Hz, values in square brackets from ref. 8 (measured in $CDCl_3$); [b] Vicinal dihedral angle [°] in X-ray crystallographic molecular structure **7**; [c] Listed as a multiplet (*m*) in ref. 8; [d] Low intensity δ 79.11, 47.88 *shoulder*, and 106.27 signals assigned to minor species respective C(1), C(2), and C(3); major:minor *ca.* 3:2; [e] Geminal protons not differentially assigned in ref. 8; [f] Listed as 'broad' in ref. 8; [g] Two overlapping peaks at about double intensity.

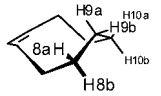
The magnitudes of the $^3J(8\alpha–9\alpha)$ and $^3J(8\alpha–9\beta)$ coupling constants are very characteristic of either TCB/SCB type 3 or SCC 3,10-epoxy-germacrolide nine-membered ring conformations. X-ray diffraction analyses shows that dihedral angles H(8α)–C(8)–C(9)–H(9α, *exo*) and H(8α)–C(8)–C(9)–H(9β, *endo*) are respectively *synclinal* [59°] and *antiperiplanar* [175°] in TCB **7**, while both are *synclinal* in the SCC conformation [*e.g.* corresponding angles are 64° and 53°, respectively in structure **12**]. The $^3J(8\alpha–9\alpha)$ 5.3 Hz and $^3J(8\alpha–9\beta)$ 11.7 values measured in the spectrum for an acetone-*d6* solution of crystalline **7** are consistent with a TCB conformational bias for the C(8)-C(10) fragment in this medium. Unequal magnitude coupling constants are also found in SCB type 3 conformation nine-membered rings which differ from those of the TCB type in that the C(4)—C(5) single bond has been replaced by a C(4)=C(5) double-bond. For example, the $^3J(8\alpha–9\alpha)$ and $^3J(8\alpha–9\beta)$ coupling constants for niveusin C-2'3'-epoxide (**11b**, which has a 1α-hydroxyl *cis*-tetrahydrofurano moiety) are respectively 6.8 and 9.5 Hz.[39] For 1,2-dehydroniveusin C-2'3'-epoxide, a 1,2-dehydrofurano analogue of **11b**, both values are reported to be 3.5 Hz.[39] This is in accord with a conformational change from what is now known as a SCB type 3 for **11b** to a SCC conformation where both $^3J(8\alpha–9\alpha)$ and $^3J(8\alpha–9\beta)$ coupling constants are expected to have similar *synclinal* magnitudes.

Irradiation of H(9β) {δ 1.83} afforded a 4.2% nuclear Overhauser effect intensity enhancement to H(6β) and a 3.2% NOE effect for the signal at δ 2.16 [overlapping δ 2.17 H(4β) and δ 2.14 H(5α)]. Similarly, {δ 2.16} gave 7.8% and 4.7% NOE effects to the respective H(6β) and H(9β) resonances. Finally, an 1.8% NOE to H(9β) and a 2.3% NOE to the overlapping δ 2.17 H(4β) and δ 2.14 H(5α) multiplets were measured upon {δ 4.56, H(6β)}. These NOE results are all consistent with a TCB conformation in which H(9β) is pointing into the interior towards its transannular H(4β) and H(6β) neighbors, while it would be pointing away from these protons in the SCC conformation.

For the C(3)-C(6) fragment, the measured 8.1 Hz $^3J(4\beta–5\alpha)$ and *ca.* 0 Hz $^3J(4\beta–5\beta)$ values are also consistent with a TCB conformation and not those expected for the SCB. The dihedral angles H(4β)–C(4)–C(5)–H(5α) and H(4β)–C(4)–C(5)–H(5β) are respectively, 147° and 97° in structure **7**. Therefore, the vicinal proton-proton coupling constants and NOE experiments are all consistent with an acetone-*d6* solution-state nine-membered ring conformation that is similar to the TCB found for crystalline **7**. However, inspection of the ^{13}C NMR and DEPT spectra shows the presence of low intensity δ 79.1, 47.9 (*shoulder*), and 106.3 methine signals that are *ca.* 0.1 ppm from methine resonances assigned to the respective C(1), C(2), and C(3) [major:minor *ca.* 3:2]. Low intensity ^{13}C NMR signals were not observed from other carbon nuclei in the molecule. Thus, while the nine-membered ring TCB conformation appears to be population biased, some small degree of flexibility appears to exist for the tetrahydrofuran moiety of tagitinin A in solution. The lower magnitudes of the minor component peak intensities are consistent with a slow exchange partner(s) either having a different puckering of the THF-moiety, or involving different rotamers about the C(3)—O(2) bond. In this regard, it is noted that a 1.0 Hz long-range $^4J(4\beta–H(O2))$ coupling is apparent from the H(O2) doublet which transformed into singlet multiplicity upon homonuclear decoupling {2.17 ppm, H(4β)}. Similarly, the broadened signals for H(4β) sharpened upon {4.77 ppm, H(O2)}. Variable temperature experiments [from 313 to 223 K] were then undertaken to search for a slow exchange partner in the ^{1}H NMR spectrum, but none was observed.

Nine-membered ring conformational interconversion and molecular modeling

Medium rings are large enough to undergo conformational interchange by segmental motion.[15] One of the mechanisms for medium ring conformational interchange is the ring atom-flip[42] (also referred to as "wagging").[43] In this interconversion, one of the ring atoms flips to the other side of the ring, and in so doing, the *axial* and *equatorial* disposition of its exocyclic ligands are interchanged. This exchange of *axial/equatorial* orientations also occurs to exocyclic ligands on ring-atoms located at either side of the flipping atom.[15] Thus, H(8a) is *equatorial* in SCB type 3 (**13**) and *axially* oriented in SCC (**14**) B3LYP/6-31g(d) models of cyclononene.[15] Flipped ring-atoms conformations have been observed by X-ray diffraction analysis: two ring atom-flipped eight-membered ring conformations superimpose upon each other in conformationally dynamically disordered crystals of nefopam methobromide or methiodide quaternary ammonium salts.[44,45]



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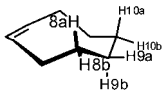

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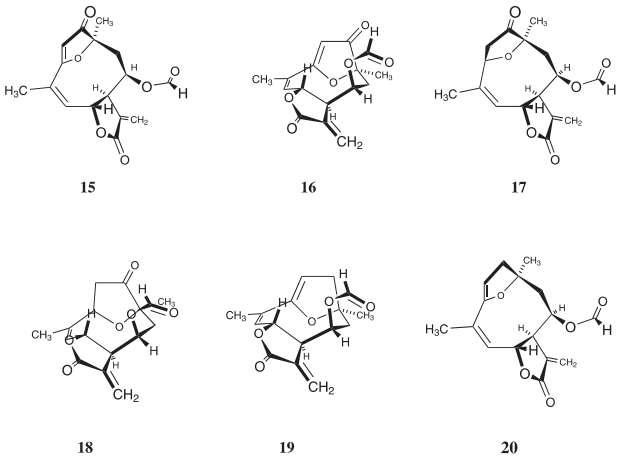
The above stereochemical analysis can be combined with an earlier observation by Gershenzon *et al.*[39] who isolated **11b** (a 1α-hydroxyl *cis*-tetrahydrofurano analogue) and the corresponding 1,2-dehydrofurano germacrolide lactone from the *Viguiera microphylla* plant. The authors

noted that there were significant differences in the chemical shifts and coupling constants of the H(6,7,8,9α,9β) protons measured in their 1H NMR spectra [see ^3J(8α–9α) and ^3J(8α–9β) values for **11b** noted above].[39] These were attributed to the presence of a 1α-hydroxyl group or a 1,2-double bond, and also to conformational differences involving the orientation of the 8β-oxycarbonyl moiety. "The side chain appears to have an *equatorial* orientation in the 1α-hydroxyl compounds and an *axial* orientation in the $\Delta^{1(2)}$ compounds."[39] We note that the hybridization of C(1) seems to be at the root for (T/S)CB versus SCC conformational preference, and subsequent *diequatorial* or *diaxial* disposition for the C(1) atom/8β-oxycarbonyl moiety. As an input structure for DFT B3LYP/6-31g(d) modeling, the **11c** skeleton was converted into a 3(2H)furanone having an 8β-oxyformyl group for simplicity. A trigonal C(1) atom appears to be essential for an SCC conformational preference with an *axially* oriented C(1), while a tetrahedral C(1) affords a preferred SCB type 3 with an *equatorial* C(1). The 3(2H)furanone SCB type 3 conformational model (**15**) was found to be 0.87 kcal higher in energy than the SCC model (**16**). Keeping the C(1) carbonyl intact while changing the C(2)=C(3) double bond to a single bond still afforded an SCB type 3 model (**17**) that was higher in energy [1.70 kcal] versus the SCC 2,3-dihydrofuranone model (**18**). However, when the trigonal C(1) carbonyl was changed to a tetrahedral-type methylene carbon, while now keeping the C(2)=C(3) double bond, the SCC conformation (model **19**) then became higher [2.60 kcal] relative to that for the SCB type 3 diastereomer (model **20**).



Finally, the observation of a ^4J(4β–H(O2)) 1.0 Hz long-range coupling constant for the H(O2) doublet can be rationalized if there is a solution-state conformational bias for the same coplanar "W-type" geometry involving the H(O2), O(2), C(3), C(4), and H(4β) atoms as found in crystalline state **7** [with a 29(6)° approximate *synperiplanar* H(O2)–O(2)···C(4)–H(4β) torsion angle], where H(O2) is hydrogen-bound to the 8-oxycarbonyl oxygen O(7a) of an adjacent symmetry equivalent molecule.

In conclusion, as with 8-desmethylacryl-hexahydroxyzexbrevin (**3**), Horeau's rule also failed to predict the correct α/β-relative configuration for the 1-hydroxyl group in tagitinin A, and its configuration at C(1) must now be reassigned as 1β. Analysis of the ^{1}H NMR spectrum of **2** shows tagitinin A to maintain its crystallographically found TCB conformation and "W-like" H(O2)–O(2)–C(3)–C(4)–H(4β) arrangement in acetone-*d6* solution. Finally, the TCB/SCB type 3 conformations of the saturated/unsaturated nine-membered moieties within 3,10-epoxy-germacrolide rings can be interconverted to SCC by means of a C(9) ring atom-flip mechanism which changes the orientation of the C(1) atom and C(8)-oxycarbonyl moiety from *diequatorial* to *diaxial*. The stereochemistry of 3,10-epoxy-germacrolide lactone structures in the CCDB, and the DFT B3LYP/6-31g(d) modeling results in this work can be interpreted as showing that tetrahedral C(1) atoms stabilize the TCB/SCB type 3 conformations, while their trigonal counterparts stabilize the SCC conformation.

Experimental

Isolation of tagitinin A (2)

Dried aerial parts (1 kg) of *Tithonia diversifolia* (Hemsl.) A. Gray (collected in San Blas, Nayarit, México, on December 2001, voucher deposited in the National Herbarium, Instituto de Biología de la UNAM, registry number: MEXU-1014633) were extracted successively with hexane and dichloromethane. The dichloromethane extract was concentrated *in-vacuo* to give a dark-green residue (30 g), which was separated on a silica gel 60 column (260 g, fractions of 250 mL were collected). Hexane was used as the initial mobile phase, and was followed by hexane-ethyl acetate mixtures (95:5, 9:1, 4:1, 7:3, 3:2, 1:1). The residue (4.2 g) from the fractions eluted with a 1:1 solvent mixture was subjected again to silica gel 60 column chromatography (18 g) eluted with dichloromethane-acetone. Fractions eluted with dichloromethane-acetone (9:1) gave a white amorphous solid, which was crystallized from ethyl acetate-isopropyl ether, and then recrystallized from methanol to afford **2** [85 mg, mp 172-174 °C (lit.[8] 170 °C)].

Molecular modeling and graphics

Density Functional Theory B3LYP/6-31g(d) geometry optimized models **8**,**9**,**13**-**20** were produced with the *Gaussian-98W revision A-7* program,[26] and all were found

to have only positive values for vibrational frequencies. Superimposition of molecular structures was performed with the *MacMimic 3.0* program.[25] Ball and stick-type non-iconic molecular graphics were drawn with the *Ball&Stick* 3.8β3 program.[19] 2D-iconic projections of the molecular models and X-ray crystallographic 3D-structures were generated using the combination of *CS-Chem3D Pro 5.0* and *CS-ChemDraw Ultra 5.0* programs.[29]

NMR Spectroscopy

^{1}H and ^{13}C NMR spectroscopy were recorded at 500 and 125 MHz, respectively, at 298 K on a Varian Unity-Plus500 NMR spectrometer. Samples were measured in acetone-*d6* using the deuterio solvent as an internal lock, and tetramethylsilane (TMS) as the internal spectral reference. DEPT (90° and 135° pulse angles) were used to determine the hydrogen multiplicity of the ^{13}C signals. COSY 2D NMR spectroscopy was used to ascertain the spin-spin coupling systems, and HETCOR 2D-NMR spectroscopy was used to correlate the ^{13}C and ^{1}H chemical shifts. NOE experiments were performed using the NOE-Difference technique, as well as by a NOESY 2D spectrum. ^{1}H spectral simulation was performed using the *Gnmr 4.1* program.[41]

Crystallography

Crystallographic measurements were made on a Bruker Smart Apex automatic diffractometer with a CCD area detector using graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. A clear, colorless plate crystal of $C_{19}H_{28}O_7$, **2**, [grown by slow crystallization from methanol] having approximate dimensions 0.40 x 0.20 x 0.18 mm was chosen, mounted on glass fiber, fixed on a goniometer head, and then placed in the X-ray diffractometer. The SMART 5.625 program[46] was used for centering, indexing, and data collection. Unit cell dimensions were obtained by a least-squares fit of 3439 carefully centered reflections in the range of 2.27° $\Omega\theta$ Ω30.94°. Cell constants correspond to an orthorhombic system $P2_12_12_1$ cell with dimensions at 291(2) K of: a = 9.6580(13) Å, b = 9.9775(13) Å, c = 20.360(3) Å, V = 1961.9(5) Å^3. For Z = 4 and FW = 368.41, the calculated density is 1.247 g cm^{-3}. Data were collected at 291(2) K using the ω scan technique. Space group determination was based upon systematic absences, packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure. Data were collected to a maximum θ value of 24.99° (100% completeness to θ) and no significant decay was observed.

The structure was solved by direct methods and refined by full matrix least squares on F^2 using the *SHELXTL97* program.[47] Atomic scattering factors were taken from *Volume IV* of the *International Tables for X-ray Crystallography.*[48] Non-hydrogen atoms were refined anisotropically, while hydrogens were placed at calculated positions, and refined as riding atoms on their respective attached atom, with the exception of those ligated to O(2) and O(3) which were located and refined as non-hydrogen atoms with a U 1.2 Å^2 thermal isotropic factor from the attached O-atom. At convergence, the final discrepancy indices on F were $R(F) = 0.0523$, $R_w(F^2) = 0.0990$ and GOF on $F^2 = 0.902$ for the 3459 reflections with $I_{net} \varnothing 2\sigma(I_{net})$ and 245 parameters refined with 0 restraints and 0 constraints. The largest difference peak and hole was 0.149 and –0.130 e.Å^{-3}.

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Electronic Supplementary Information

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 261295. Copies of the material can be obtained, free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge, CB2 1EZ UK; Tel: +44 1223 336408; Fax: +44 1223 336033; or e-mail: deposit@ccdc.cam.ac.uk).

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