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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Tagitinin 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 1E,4D,6D,7E,8E que difere da orientação 1D em C(1) proposta originalmente na literatura e que foi determinada pelo método de Horeau. Análise do espectro de 1H-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 das 3,10-epoxi-germacrolidas 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 transformadas para 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 1E,4D,6D,7E,8E relative configuration which differed at C(1) from the 1D-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† 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 Brewster1 and Horeau.4 It was used to determine the (R/S)-absolute configuration and subsequent α,β-orientation* 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 2(3H)-furanone [e.g. zexbrevin7 (1)] or to a cis-fused tetrahydrofuran moiety [e.g. tagitinin A* (2)] (both drawn with (6R,7S)-stereochemistry). The skeletons of these
compounds are based upon 1-isopropyl-4,8-dimethylcyclocondane (germacrane). In this method, a chiral secondary-alcohol will react faster with one of the enantiotropic C\(_4\)H\(_7\)C=H(PPh)=C(=O)O—carbonyl groups in excess optically inactive 2-phenylbutyric anhydride [a mixture of d,l and meso-diastereomers] than with its enantiotropic 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.

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 \(4\alpha,1\alpha\)-dimethyl groups, but now known by X-ray diffraction analysis\(^10\) to be \(4\beta,11\beta\) 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 (–)- stereochemistry of the free 8-OH was ascertained 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 (8S). Using a (6R,7S)-6,7-trans-lactone skeleton and the (8S) result, the authors\(^7\) reported an \(\alpha\)-orientation for the 8-hydroxyl group. However, the X-ray crystallographically determined structures of tetrahydrozexbrevin (5)\(^10\) and 9\(\alpha\)-acetoxyzexbrevin,\(^11\) and phototetrahydrozexbrevin A\(^12,13\) were later reported, and the orientation of the 8-isobutyroxy moiety was then found to be ‘\(\beta\)’ for all three compounds. Horeau’s rule failed to predict the correct \(\alpha\)-orientation in this case.

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-isobutyroxy moieties to be 4\(\alpha\) and 8\(\beta\), respectively, and reported \(^3\)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 (1S).\(^8\) The excess recovered (–)-\(\alpha\)-phenylbutyric acid was reported to exhibit \([\alpha]\)\(_D\)\(^27\) –6.55\(^\circ\) (benzene), representing an optical yield of 41.8\%.\(^8\) Based upon a (6R,7S)-skeleton for 6b, they proposed an \(\alpha\)-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 \(\beta\)-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

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.\(^8\) \textit{T. diversifolia} (also known as “Mexican Arnica”) has been used in Mexican traditional medicine to treat inflammatory ailments. Its ethnopharmacology has been discussed recently.\(^1\) Compound 2 was subjected to X-ray diffraction analysis, but the absolute configuration\(^1\) of the chiral crystal was unable to be determined.\(^4\) The resulting molecular configuration determinations were arbitrarily chosen.\(^2\) The resulting molecular structure (depicted as Ball and Stick\(^3\) model 7) within these crystals showed that the originally proposed \(\alpha\)-oriented 1-hydroxy group was indeed inverted to a \(\beta\)-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 carbonyl 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 \((1S)\)-stereochemistry by Horeau’s rule based and \(1\beta\)-relative configuration by X-ray crystallography, as well as \((8S)\)-stereochemistry/\(8\beta\)-relative configuration for 1 are not actually mutually exclusive, but that the germacrolide skeleton simply has the opposite \((6S,7R)\)-6,7-\textit{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 \((6R,7S)\)-absolute configuration for other germacrolide 6,7-\textit{trans}-lactone natural products are listed in the Cambridge Crystallographic Data Base\(^2\) (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.\(^2\) Thus, it is very reasonable that the absolute configuration of the 6,7-\textit{trans}-lactone fragment in the tagitinin A and zexbrevin 3,10-epoxy-germacrolide skeletons should also be \((6R,7S)\), leaving us with the conclusion that Horeau’s rule failed in both cases.

The O(1), and C(3-10) atoms of 2 define an oxacyclononane ring (8) having a \textit{twist-chair-boat}\(^3\) (TCB) conformation. Superimposition\(^5\) of all the ring atoms of 2 on corresponding atoms in Density Functional Theory B3LYP/6-31g(d)\(^2\) calculated TCB conformation oxacyclononane (8) and cyclononane\(^4\) (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 \textit{endo} protons on C(2) and C(7), and as a result, brings the oxygen in 8 slightly closer to C(2) \([2.838 \text{ Å} \text{ and } 2.992 \text{ Å} \text{ O···C(7)}\) in experimentally determined tagitinin A \((7)\) and in calculated model-8, respectively versus 3.352 Å \(\text{C(2)···C(7)}\) in model-9]. The \(14^\circ\) H(6a)···C(6)···C(7)···H(7a) and \(-3^\circ\) H(3a)···C(3)···C(10)···H(10a) torsion angles in 8 enable both a \textit{trans}-6,7-lactone closure and 3,10-ethano bridging to proceed without strain. \(O\text{-ethyl}-tirotundin\(^7\) (10), a 1-deoxy-\(O(2)\)-ethylated analogue of 2, has been isolated from \textit{T. rotundifolia}. While coordinates of 10 are not to be found in the CCDB\(^8\) nor in the article itself,\(^2\) 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 crystallographically determined molecular structures of tagitinin A ((1R,3R,4S,6R,7S,9R,10R)-7) and the 1-deoxy-O(2)-ethylated analogue ((3R,4S,6R,7S,9R,10S)-3-O-ethyl-tirotundin-10), versus corresponding angles in B3LYP calculated Twist-Chair-Boat (TCB) conformational models of oxacyclonane (8) and cyclonanone (9)

| Torsion Angle | 7 | 10 | 8 | 9 |
|--------------|---|----|---|---|
| C(10)–C(1)–C(2)–C(3) | −17.8(4) | 21.3(3) | −101.8(3) | −103.6(3) |
| C(10)–C(1)–C(2)–C(4) | −89.3(3) | 121.3(3) | −89.3(3) | 121.3(3) |
| C(10)–O(1)–C(3)–C(2) | −21.3(3) | 173.8(3) | −21.3(3) | 173.8(3) |
| C(10)–O(1)–C(3)–C(4) | 105.6(3) | −64.0 | 105.6(3) | −64.0 |
| C(10)–O(1)–C(3)–C(5) | −59.8(4) | −52.8 | −59.8(4) | −52.8 |
| C(10)–O(1)–C(3)–C(7) | −10.6(3) | 8.2 | −10.6(3) | 8.2 |
| C(10)–O(1)–C(3)–C(9) | −56.4(4) | −57.6 | −56.4(4) | −57.6 |
| C(10)–O(1)–C(3)–C(11) | −5.9 | −51.0 | −5.9 | −51.0 |
| C(10)–O(1)–C(3)–C(13) | −59.8(4) | −52.8 | −59.8(4) | −52.8 |
| C(10)–O(1)–C(3)–C(15) | 173.8(3) | 173.4 | 173.8(3) | 173.4 |
| C(10)–O(1)–C(3)–C(17) | 59.2(3) | 60.7 | 59.2(3) | 60.7 |
| C(10)–O(1)–C(3)–C(19) | −3.8(5) | −1.2 | −3.8(5) | −1.2 |
| C(10)–O(1)–C(3)–C(21) | 2.9(7) | 2.9(7) | 2.9(7) | 2.9(7) |

In addition to 10, the structures of woodhousin38 (11a), niveusin C-2',3'-epoxide36 (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-e is that the TCB conformation of 7,10 changes into a skew-chair-boat type 3 (SCB type 3) cis-cyclononene. The existence of cis-cyclononene conformational families has recently

Anet has developed a very useful general analysis for subsequent assignment of substituent orientation (isoclinal, axial, equatorial) in rings of any size.39 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. Dale31 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 C₇-symmetry oxacyclonane and cyclonanone models 8,9 are located at “corner” positions. Ligation of an ethano bridge to C(3,10) twists this region of the oxacyclonane 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 oxacyclonane 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 C₇-symmetry cyclonanone 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-16,13,35-37 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).
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 1H and 13C 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(8β), H(9α), H(9β), H(13endo), H(13exo) and C(14)H. 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 1H NMR spectrum [1.06 ppm, C(14)H] was simulated using Gnmr 4.1,14 due to second order effects for signals arising from H(4β) [Δν = 15.5 Hz] and from H(9ε) [Δν = 42.5 Hz]. The multiplicity of protons ligated to 13C nuclei was determined by DEPT-135 and DEPT-90 experiments. 1H and 13C signals were correlated using a 2D-NMR HETCOR spectrum {1.06 ppm, C(14)H} determined by DEPT-135 and DEPT-90 experiments. 1H observed in the COSY-90 2D spectrum. The 1H NMR pathways for each of these spin-systems were readily assigned. Homonuclear coupling patterns for each of these spin-systems were readily observed in the COSY-90 2D spectrum. The 1H NMR spectrum [1.06 ppm, C(14)H] was simulated using Gnmr 4.1,14 due to second order effects for signals arising from H(4β) [Δν = 15.5 Hz] and from H(9ε) [Δν = 42.5 Hz]. The multiplicity of protons ligated to 13C nuclei was determined by DEPT-135 and DEPT-90 experiments. 1H and 13C signals were correlated using a 2D-NMR HETCOR spectrum. The H(13endo,13exo) signals overlap at 298°, but are readily differentiated by their coupling patterns. At 223°, H(13endo) and H(8β) appear at δ 5.66 and δ 5.55, respectively. The resolution at low temperature was used to assign H(13endo,13exo) by means of a NOESY spectrum measured at that temperature. In the 2D spectrum, H(8β)

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(13endo) [closer to H(8ε)] and H(13exo) [closer to lactone carbonyl O(5)], respectively.

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 tetrahydroxyzexbrevin A (12) structure from X-ray diffraction analysis15] is that the former set has the C(1) atom and C(8)-oxycarbonyl moiety exhibiting diequatorial orientations 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

| δa (ppm) | Jαα (Hz) | H—C—C—H (°) | δc (ppm) |
|----------|----------|---------------|----------|
| H(1α)    | 4.18 [4.23] | 9.3 | 21.5 | C(1)α | 79.2 [78.5] |
| H(2α)    | 2.35 [2.44] | 1α—2α | 7.2 | 141.0 | C(2)α | 47.8 [46.9] |
| H(3β)    | 2.05 [2.1] | 1α—H(O3) | 5.0 | C(3)β | 106.4 [105.7] |
| H(4β)    | 2.16 [2.1] | 2α—2β | −13.7 | . | C(4) | 45.2 [44.4] |
| H(5α)    | 2.13 [2.1] | 4β—5α | 8.1 | 147.2 | C(5) | 39.2 [37.8] |
| H(6β)    | 1.66 [2.1] | 4β—5β | 0.0 | 96.6 | C(6) | 82.2 [81.9] |
| H(8α)    | 4.56 [4.55] | 4β—C(15)H3 | 7.0 [6.5] | . | C(7) | 48.7 [47.8] |
| H(8α)    | 4.05 [3.39] | 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)    | 1.91 [1.95] | 5α—6β | 10.9 [9] | 161.7 | C(10) | 82.0 [81.7] |
| H(9β)    | 1.826 [1.81] | 5β—6β | 1.3 [3] | 82.1 | C(11) | 139.1 [137.0] |
| H(13endo) | 6.10 [6.25] | 6β—7α | 6.4 [7] | 140.7 | C(12) | 169.4 [169.8] |
| H(13exo) | 5.59 [5.53] | 7α—8α | 3.1 [1.5] | 56.7 | C(13) | 120.8 [121.7] |
| C(14)H | 1.35 [1.43] | 7α—13endo | 3.3 [3.5] | . | C(14) | 25.3 [25.0] |
| C(15)H | 1.06 [1.11] | 7α—13exo | 3.2 [3] | . | C(15) | 19.0 [19.2] |
| CHCH3 | 2.44 [2.44] | 8α—9α | 5.3 [5] | 58.9 | C(16) | 176.2 [176.5] |
| CH = CH | 1.03 [1.07] | 8α—9γ | 11.7 [8] | 175.1 | C(17) | 34.7 [34.1] |
| CHCH3 | 1.01 [1.04] | 9α—9β | −14.3 [13] | . | C(18) | 19.5 [18.8] |
| H(O2) | 4.77 | CH—CH | 7.2 [7] | . | C(19) | 19.0 [18.4] |
| H(O3) | 4.34 | CH—CH | 7.0 [7] | . | . | . |

*1H NMR (500 MHz); 13C NMR (125 MHz); chemical shifts relative to TMS (external). 298 K. acetone-d6; δc and Jαα [Hz] values from spectral simulation using Gnmr 4.1; the standard deviation of the last digit in Jαα values is ca. 0.1 Hz, values in square brackets from ref. 8 (measured in CDCl3); * Vicinal dihedral angle [°] in X-ray crystallographic molecular structure; 7; † Listed as a multiplet (m) in ref. 8; ‡ 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; ‡ Geminal protons not differentially assigned in ref. 8; ‡ Listed as broad in ref. 8; ‡ Two overlapping peaks at about double intensity.
The magnitudes of the \( ^3J(8\alpha\text{–}9\alpha) \) and \( ^3J(8\alpha\text{–}9\beta) \) 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 \( H(8\alpha\text{–}C(8)\text{–}C(9)\text{–}H(9\alpha, \text{exo}) \) and \( H(8\alpha)\text{–}C(8)\text{–}C(9)\text{–}H(9\beta, \text{endo}) \) are respectively \textit{synclinal} [59°] and \textit{antiperiplanar} [175°] in TCB 7, while both are \textit{synclinal} in the SCC conformation [e.g. corresponding angles are 64° and 53°, respectively in structure 12]. The \( ^3J(8\alpha\text{–}9\alpha) \) 5.3 Hz and \( ^3J(8\alpha\text{–}9\beta) \) 11.7 values measured in the spectrum for an acetone-\( d_6 \) 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 SCC 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 \( ^3J(8\alpha\text{–}9\alpha) \) and \( ^3J(8\alpha\text{–}9\beta) \) coupling constants for nineuscin C-2'3'-epoxide (11b, which has a 1\alpha-hydroxyl \textit{cis}-tetrahydrofurano moiety) are respectively 6.8 and 9.5 Hz.\textsuperscript{39} For 1,2-dehydronineuscin C-2'3'-epoxide, a 1,2-dehydrofurano analogue of 11b, both values are reported to be 3.5 Hz.\textsuperscript{39} This is in accord with a conformational change from what is now known as a SCC type 3 for 11b to a SCC conformation where both \( ^3J(8\alpha\text{–}9\alpha) \) and \( ^3J(8\alpha\text{–}9\beta) \) coupling constants are expected to have similar \textit{synclinal} magnitudes.

Irradiation of H(9\beta) \( \delta \ 1.83 \) afforded a 4.2% nuclear Overhauser effect intensity enhancement to H(6\beta) and a 3.2% NOE effect for the signal at \( \delta \ 2.16 \) [overlapping \( \delta \ 2.17 \) H(4\beta) and \( \delta \ 2.14 \) H(5\alpha)]. Similarly, \( \delta \ 2.16 \) gave 7.8% and 4.7% NOE effects to the respective H(6\beta) and H(9\beta) resonances. Finally, an 1.8% NOE to H(9\beta) and a 2.3% NOE to the overlapping \( \delta \ 2.17 \) H(4\beta) and \( \delta \ 2.14 \) H(5\alpha) multiplets were measured upon \( \delta \ 4.56 \), H(6\beta)). These NOE results are all consistent with a TCB conformation in which H(9\beta) is pointing into the interior towards its transannular H(4\beta) and H(6\beta) 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 \( ^3J(4\beta\text{–}5\beta) \) and ca. 0 Hz \( ^3J(4\beta\text{–}5\beta) \) values are also consistent with a TCB conformation and not those expected for the SCB. The dihedral angles H(4\beta)–C(4)–C(5)–H(5\alpha) and H(4\beta)–C(4)–C(5)–H(5\beta) are respectively, 147° and 97° in structure 7. Therefore, the vicinal proton-proton coupling constants and NOE experiments are all consistent with an acetone-\( d_6 \) solution-state nine-membered ring conformation that is similar to the TCB found for crystalline 7. However, inspection of the \( ^1\text{C} \) NMR and DEPT spectra shows the presence of low intensity \( \delta \ 79.1, 47.9 \) (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 \( ^1\text{C} \) 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 tetrahydrofurano 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-moeyty, or involving different rotamers about the C(3)—O(2) bond. In this regard, it is noted that a 1.0 Hz long-range \( ^3J(4\beta\text{–}H(O2)) \) coupling is apparent from the H(O2) doublet which transformed into singlet multiplicity upon homonuclear decoupling [2.17 ppm, H(4\beta)]. Similarly, the broadened signals for H(4\beta) 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 \( ^1\text{H} \) NMR spectrum, but none was observed.

\textbf{Nine-membered ring conformational interconversion and molecular modeling}

Medium rings are large enough to undergo conformational interchange by segmental motion.\textsuperscript{15} One of the mechanisms for medium ring conformational interchange is the ring atom-flip (also referred to as "wagging").\textsuperscript{43} In this interconversion, one of the ring atoms flips to the other side of the ring, and in so doing, the \textit{axial} and \textit{equatorial} disposition of its exocyclic ligands are interchanged. This exchange of \textit{axial/equatorial} orientations also occurs to exocyclic ligands on ring-atoms located at either side of the flipping atom.\textsuperscript{15} Thus, H(8a) is \textit{equatorial} in SCC type 3 (13) and \textit{axially} oriented in SCC (14) B3LYP/6-31g(d) models of cyclononene.\textsuperscript{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.\textsuperscript{44,45}

The above stereochemical analysis can be combined with an earlier observation by Gershenzon et al.\textsuperscript{39} who isolated 11b (a 1\alpha-hydroxyl \textit{cis}-tetrahydrofurano analogue) and the corresponding 1,2-dehydrofurano germacrolide lactone from the \textit{Viguiera microphylla} plant. The authors
noted that there were significant differences in the chemical shifts and coupling constants of the H(6,7,8,9\alpha,9\beta) protons measured in their 1H NMR spectra [see J(8\alpha-9\alpha) and J(8\beta-9\beta) values for 11b noted above]. These were attributed to the presence of a 1\alpha-hydroxyl group or a 1,2-double bond, and also to conformational differences involving the orientation of the 8\beta-oxyformyl moiety. “The side chain appears to have an equatorial orientation in the 1\alpha-hydroxyl compounds and an axial orientation in the \Delta^{1(2)} compounds.” 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/8\beta-oxyformyl moiety. As an input structure for DFT B3LYP/6-31g(d) modeling, the 11c skeleton was converted into a 3(2H)furanone having an 8\beta-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 J(4\beta-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(4\beta) atoms as found in crystalline state [with a 29(6)° approximate synperiplanar H(O2)--O(2)--C(4)--H(4\beta) torsion angle], where H(O2) is hydrogen-bound to the 8-oxyformyl 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 cis-/trans-relative configuration for the 1-hydroxyl group in tagitinin A, and its configuration at C(1) must now be reassigned as 1\beta. Analysis of the 1H 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(4\beta) 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, 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.5 170 °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, and all were found
to have only positive values for vibrational frequencies. Superimposition of molecular structures was performed with the *MacMimic* 3.0 program. Ball and stick-type non-ionic molecular graphics were drawn with the *Ball&Stick* 3.8/3 program. 2D-ionic 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.

**NMR Spectroscopy**

$^1$H and $^1$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-$d_6$ 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 atomic multiplicity of the $^1$C signals. COSY 2D NMR spectroscopy was used to determine the spin-spin coupling, and HETCOR 2D-NMR spectroscopy was used to correlate the $^1$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 provided to R.G. by BGUN.

**Crystallography**

Crystallographic measurements were made on a Bruker Smart Apex automatic diffractometer with a CCD area detector using graphite-monochromated Mo Ka ($\lambda = 0.71073$ Å) radiation. A clear, colorless plate crystal of $C_{19}H_{28}O_{7}$, [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 was used for centering, indexing, and data collection. Unit cell dimensions were obtained by least-squares fit of 3439 carefully centered reflections in the range of 2.27° $\leq$ $\theta$ $\leq$ 30.94°. Cell constants correspond to an orthorhombic system $P_{2_1}2_12_1$, cell with dimensions at 291(2) K of: $a = 9.6580(13)$ Å, $b = 9.9775(13)$ Å, $c = 20.360(3)$ Å, $V = 1961.9(5)$ Å³. 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 $\omega$ 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 $\theta$ value of 24.99° (100% completeness to $\theta$) 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 *SHELXLT97* program. Atomic scattering factors were taken from Volume IV of the *International Tables for X-ray Crystallography*. 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 Å² 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 $I_{net} > 2\sigma(I_{net})$ 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, CDDC, 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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