Isolation of Nor-secofriedelanes from the Sedative Extracts of Galphimia glauca

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Preparative-scale recycling HPLC was used for the complete resolution of a complex mixture of nor-secofriedelanes into five major peaks (I—V) from the sedative methanolic extracts prepared from the aerial parts of Galphimia glauca. Argentation chromatography was used to show peaks I, II, IV, and V to be mixtures of isomers around the E-ring double bond, represented by the endocyclic C-20, C-21 double-bond isomers, galphimines A (3), B (1), D (4), and E (2), and the C-20, C-29 exocyclic forms, galphimines F—I (5–8). Galphimine C (9), isolated from peak III, corresponded to the C-19, C-20 double-bond isomer of the previously known major sedative constituent galphimine B. The characterization of all the new triterpenes (3–9) was performed primarily by high-field NMR spectroscopy. Comparison between experimental and calculated 1H vicinal coupling constants and the analysis of molecular mechanics structures revealed that the ring B of these compounds exists in a boatlike conformation. The absolute configuration for the stereogenic carbinol center at C-4 was established by the application of the Mosher ester derivatization technique carried out in NMR tubes.

Galphimine B (1), a nor-seco triterpene and the main sedative component of Galphimia glauca (Cav.) Kuntze (Malpighiaceae), was isolated from the aerial parts of this plant, which is traditionally utilized in Mexico for the treatment of central nervous system disorders.1,2 The first report on the isolation of this active principle was published about a decade ago when a product was obtained by fractional crystallization from a complex mixture of nor-secofriedelanes. The structure of compound 1 has been resolved by X-ray diffraction studies,3 but no reference to any physical constants (mp and [α]D) nor to spectral data (NMR and MS) has been made. Recently, a second investigation conducted on G. glauca tissue cultures led to not only the isolation of the above-mentioned major constituent but also a second related compound, 6-acetoxygalphimine B (2).4 The critical factor in achieving total purification of both samples was the development of a suitable HPLC procedure as a simple routine analytical process able to detect and quantify the in vitro production of the sedative galphimines 1 and 2 in cell suspension cultures.5

For further biotechnological in vitro studies, a more complete knowledge of the friedelane profile present in the wild plant is necessary, since pure samples of the triterpenes involved are needed for use as chromatographic standards to monitor their biosynthetic production. Therefore, an important prerequisite to accomplish their isolation is the standardization of analytical HPLC procedures, which can be scaled up easily for preparative purposes. This was achieved by using reversed-phase chromatography operating in a recycling mode. The present study describes the isolation of five major HPLC peaks from the original sedative MeOH extract that was prepared from the aerial parts. Four of the eluted peaks represent diastereomeric mixtures of Δ20 and Δ20(29) isomers. Through the use of argentation chromatography,6 for both silica gel TLC and column chromatography, their complete resolution is described. In addition, a fifth peak corresponding to the Δ19 isomer of galphimine B is also mentioned in this paper.

Results and Discussion

The mixture of galphimines present in the sedative triterpene fraction of G. glauca aerial parts has remained unresolved after a decade of extensive pharmacological investigation in Mexico.1,2 Due to our present biotechnological approach focus on the in vitro production of the main active constituent galphimine B (1),3,5 the separation and characterization of the additional major components of the mixture has become a high-priority phytochemical issue. These pure samples are needed for use as HPLC standard compounds for the quantification of the in vitro triterpene production by cell and hairy root suspension cultures. In addition, the identification of novel compounds will be the starting point for the elucidation of the complete metabolic pathway, which would allow the future implementation of molecular biology-based methods for manipulation of the friedelane biosynthesis. It is hoped that this approach will lead to an increase in the in vitro production of the sedative principles of G. glauca.

The fractionation by reversed-phase HPLC of the nor-secofriedelane mixture present in the MeOH extract of G. glauca resulted in the collection of five subfractions (peaks I–V), which were further purified to chromatographic homogeneity by application of preparative-scale recycling HPLC.7 In each of these analyzed eluates, one individual spot was observed by silica gel TLC. This chromatographic behavior was maintained even after 10 consecutive HPLC cycles (Figure S1, Supporting Information). However, despite the observed TLC homogeneity in all the NMR spectra of the eluted peaks, with the exception of peak III, a group of olefinic signals was recognized, suggesting the presence of a mixture. These signals were representative...
of an exomethylene vinylic moiety (H-2: δ 4.5 and 4.7; δC 109) and a trisubstituted endocyclic double bond (H-21: δ 5.1; δC 118). The results of the present investigation demonstrated, with the use of silver nitrate-impregnated silica gel plates, that each of these peaks was a mixture of isomers around the E-ring double bond. The eluates represented different pairs of double-bond isomers, of the C-20, C-21 endocyclic double bond (1–4) and of the C-20, C-29 exocyclic forms (5–8), which could be completely separated from each other by AgNO3-impregnated silica gel column chromatography (Figure S1, Supporting Information).

Figure 1. Minimum energy structure of galphimine A (3).

<table>
<thead>
<tr>
<th>MTPA ester</th>
<th>proton chemical shifts (ΔδH = δH - δT)</th>
<th>C-4 config</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>+0.062 3.809 -0.056 4.293 -0.110</td>
<td>R</td>
</tr>
<tr>
<td>13</td>
<td>+0.072 4.182 -0.048 4.274 -0.089</td>
<td>R</td>
</tr>
</tbody>
</table>

* Data measured in CDCl3 at 300 MHz.

Deduction of the basic skeleton as a nor-secofriedelane type for all of the isolated galphimines was achieved through comparative analysis of their NMR data (1H- and 13C HMBC, and NOESY) with those previously reported for related triterpenes6–11 and, in particular, 6-acetoxygalphimine B or galphimine E (2).4 The following common structural features for all the compounds were observed: the presence of three tertiary methyl groups (Me-23, Me-26, and Me-28); one secondary methyl group at C-4 (Me-23); a carboxethoxy group (C-30); one tertiary alcohol at C-18; and the seven-membered (Me-23); a carbomethoxy group (C-30); one tertiary alcohol at C-4 and one secondary methyl group at C-4 (Me-23) and one tertiary methyl group at C-7; and the seven-membered (Me-23); a carbomethoxy group (C-30); one tertiary alcohol at C-18; and the seven-membered cyclic lactone ring A. The last-mentioned functionality was confirmed through an HMBC-connectivity analysis where the carboxylic lactone resonance (δ 169) showed correlations with the vinylic protons H-1 (δ 6.4) and H-2 (δ 6.0). The latter two were also part of an ABX spin-system with H-10 (δ 2.5–2.7). The oxidation pattern of ring B was likewise deduced by comparison with 2.4 Whereas the methylene signal for C-6 (δ 37) in compounds 1, 5, and 9 was missing in the 13C NMR spectra of compounds 2–4 and 6–8, a hydroxylated (δ 65) or acetylated (δ 69) methine carbon appeared instead.

The molecular mechanics global minimum of compound 3 (E_MMX = 97.70 kcal/mol) displayed a conformation similar to that found in the X-ray structure of galphimine B (1)3 in that ring B in both substances adopts a classical boat conformation (Figure 1). The observed vicinal coupling constants (J 6,7 ≈ 5.5 Hz and J 7,8 ≈ 8 Hz) found in compounds 2–4 and 6–8 were similar to the calculated values generated from the molecular model of 3 (J 6,7 ≈ 4.6 Hz and J 7,8 ≈ 8 Hz). These values were obtained from the corresponding molecular mechanics dihedral angles H-6–C-6–C-7–H-7 = +40° and H-7–C-7–C-8–H-8 = +151° by using the Altona equation,12,13 thus defining an α-configuration for the functionalities (hydroxyl or acetyloxy groups) on these chiral centers. In addition, a NOESY cross-peak was clearly recorded between H-6 and Me-25, supporting the assignment for the functionalities (hydroxyl or acetyloxy groups) on these chiral centers. Without the NOESY cross-peak, the chemical shift difference (Δδ) between corresponding Me-23 methyl group protons was relatively deshielded compared to those signals in the (R)-MTPA derivatives (12r and 13r), respectively, the H-23 methyl group protons were relatively deshielded compared to those signals in the (R)-MTPA derivatives (12r and 13r). The chemical shift difference (Δδ) between corresponding Me-23 protons was positive (Table 1), allowing the confirmation of a C-4 (R) absolute configuration through the application of the configurational model proposed by Kakisawa and associates.15

Through acetylation, all members of each isomeric series, i.e., compounds 2–4 and 6–8, afforded the same peracetylated derivative 10 or 11, respectively. This chemical correlation, as well as that obtained by application of the Mosher esters methodology,14 provided conclusive evidence for the same C-4 absolute configuration in all galphimines. This reaction was performed in NMR tubes in deuterated pyridine, which allowed heating the solution to promote the esterification. In the NMR spectra of the (S)-MTPA ester of the galphimines B and E (MTPA derivatives 12s and 13s, respectively), the H-23 methyl group protons were relatively deshielded compared to those signals in the (R)-MTPA derivatives (12r and 13r). The chemical shift difference (Δδ) between corresponding Me-23 protons was positive (Table 1), allowing the confirmation of a C-4 (R) absolute configuration through the application of the configurational model proposed by Kakisawa and associates.15

The major HPLC peak II (tR = 26 min; Figure S1, Supporting Information) afforded an isomeric pair of compounds both with the molecular formula C30H44O7, which was subsequently resolved by argentation chromatography on a silica gel column into pure compounds 1 and 5. The second most prominent peak, eluate V (tR = 33.5 min), afforded a pair of compounds with the molecular formula C32H46O9, representing galphimine E (2) and its isomer 6. The molecular formula calculated for peak IV (tR = 30 min) was the same as that of peak V but was resolved...
into pure compounds 4 and 8. The isomerism of peaks IV and V was a consequence of the position interchange of the acetyl group residue between the hydroxylated C-6 and C-7 of the basic skeleton. Peak I \((t_{R} = 19 \text{ min})\) yielded 3 and 7, whose FABMS led to the molecular formula \(C_{38}H_{44}O_{9}\) and indicated that these pure compounds represented the deacetylated form of galphimine D as well as galphimine E. Galphimine C (peak II; \(t_{R} = 27.5 \text{ min}\)) was present in only one isomeric form (9) and possessed the same molecular formula as galphimine B \(C_{39}H_{42}O_{9}\). For compounds in both isomeric series, their structure elucidation was in total agreement with the NMR data included in the Experimental Section.

Galphimine C (9) represents the C-19, C-20 endocyclic double-bond isomer of compound 1. The NMR experiments allowed unambiguously for the identification of all resonances in ring E and the placement of the double bond between C-19 and C-20 (Figure S2, Supporting Information). The line width at half-height for the olefinic proton between C-19 and C-20 (Figure S2, Supporting Information) indicated a lack of significant cytotoxicity (ED50 > 10 \(\mu\)g/mL) toward the panel of four human tumor cell lines (KB, HCT-15, OVCAR, and SQC-1) indicated a lack of significant cytotoxicity (ED50 > 10 \(\mu\)g/mL) toward the panel of four human tumor cell lines (KB, HCT-15, OVCAR, and SQC-1).

**Plant Material.** Leaves of Galphimia glauca were collected at the "Municipio Doctor Mora", Guanajuato, Mexico, in July 2001. The voucher specimens were identified by Abigail Aguilar and deposited at Instituto Mexicano del Seguro Social Herbarium (IMSSM: 11061), Centro Médico Nacional Siglo XXI, Mexico City.

**Extraction and Isolation.** The dried leaves (800 g) were powdered and defatted by maceration at room temperature with hexane. The residual material was extracted exhaustively with CHCl3 and MeOH to afford, after removal of the solvent, a dark green syrup (104 g) and a brownish oily residue (69 g). The crude mixture of galphimines was obtained after fractionation of the MeOH extract by open column chromatography over silica gel eluted with a gradient of MeOH in CHCl3. A total of 52 fractions (150 mL each) were collected and combined (fractions 11-16) to give a complex pool constituted by compounds 1-9 (2.5 g).

**Recycling HPLC Separation.** The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600E multi-solvent delivery system equipped with a Waters W996 diode array detector (232 nm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 32 software program (Waters). The analytical HPLC separations were done on a Symmetry C18 column (Waters; 5 \(\mu\)m, 4.6 \times 250 mm) with an isocratic elution of CH3CN-H2O (45:55), a flow rate of 0.65 mL/min, and a sample injection of 10 \(\mu\)L (1 mg/mL). The crude galphimine fraction was subjected to preparative HPLC on a reversed-phase C18 column (7 \(\mu\)m, 19 \times 300 mm). The elution was isocratic with CH3CN-H2O (45:55) using a flow rate of 10 mL/min. Eluates across the peaks with \(t_{R}\) of 18.3 min (peak I: 28 mg; 3 and 7), 26.2 min (peak II: 80 mg; 1 and 5), 27.7 min (peak III: 3 mg; 9), 29.9 min (peak IV: 20 mg; 4 and 8), and 33.4 min (peak V: 50 mg; 2 and 6) were collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode. The complete separation of HPLC peaks I-V was achieved to homogeneity (HPLC; Figure S1, Supporting Information) after five to ten consecutive cycles employing an isocratic elution with CH3CN-H2O (7:3).

**Argentation Chromatography.** TLC: precoted Si gel 60 F254 aluminum sheets (20 \times 20 cm) and HPTLC plates (10 \times 10 cm) were impregnated with silver nitrate by immersion (3 \(\times\)) in a solution of 2 g of AgNO3 in acetone-H2O (3:2, 50 mL) for 5 min. After each immersion, the plates were dried at 100 °C. Final activation was performed by heating overnight at 90 °C. Plates were appropriately stored to avoid deactivation by moisture and oxidation by exposure to air and light. Argentation TLC, using CHCl3-EtOAc (1:2), satisfactorily separated the galphimine mixture (TLC; Figure S1, Supporting Information). Spraying the plates with vanillin-sulfuric acid solution (0.1 g in 10 mL of H2SO4 98%) allowed the visualization of compounds.

**Column Chromatography.** Silica gel 60 (40-60 \(\mu\)m) was prepared by mixing the adsorbent (50 g) in a 2% AgNO3 solution of acetone-H2O (3:2, 120 mL). The impregnated adsorbent was dried following the above-described procedures for TLC plates. Complete resolution of all diasteromeric pairs (10 mg) was achieved by normal column chromatography using 5 g of the impregnated adsorbent and CHCl3-EtOAc (1:1) as elution solvent. These argentation techniques afforded pure compounds 3 (2 mg) and 7 (6 mg) from peak I; 1 (3 mg) and 5 (5 mg) from peak II; 4 (3 mg) and 8 (5 mg) from peak IV; and finally, 2 (3 mg) and 6 (5 mg) from peak V.

**Galphimine B (1):** white amorphous powder; mp 194–195 °C; \([\alpha]_{D}^{25} = -11^\circ\) (c 0.16, CHCl3); \(^{1}H\) NMR (CDCl3, 500 MHz) \(\delta 6.41 (1H, dd, J = 12.0, 9.0 \text{ Hz}, H-1), 6.05 (1H, dd, J = 12.0, 9.0 \text{ Hz}, H-2), 5.15 (1H, m, H-1'), 4.22 (1H, m, H-7'), 4.22 (1H, d, J = 12.0, H-2b), 3.52 (1H, m, H-4), 3.52 (1H, m, H-2a), 3.51 (3H, s, OCH3), 2.92 (1H, dd, J = 13.1, 3.3 Hz, H-15c), 2.67 (1H, dd, J = 15.9, 6.6 Hz, H-6u), 2.65 (1H, m, H-19b), 2.54
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Galphimine E (6-acetylgalphimine B, 2): white amorphous powder; mp 177–179 °C; [α]D20 = +24° (c 0.23, CHCl3); 1H NMR (CDCl3, 500 MHz) δ 6.40 (1H, dd, J = 12.2, 8.7 Hz, H-1), 6.07 (1H, dd, J = 12.2, 6.8 Hz, H-2), 5.27 (1H, br, H-4), 2.89 (1H, d, J = 7.8 Hz, H-8a), 2.15 (3H, s, OAc), 2.03 (2H, m, H-12), 1.97 (1H, m, H-16a), 1.82 (1H, d, J = 15.0 Hz, H-16a); 13C NMR (CDCl3, 125.7 MHz) δ 175.1 (C-30), 169.7 (C-10a), 163.4 (C-3), 144.7 (CH-1), 133.2 (C-2), 128.3 (C-3), 118.5 (CH-2), 115.8 (C-12), 76.6 (C-11), 69.4 (C-21), 65.3 (C-12), 64.1 (CH-1), 53.2 (C-15), 52.8 (CH 2, C-12), 52.5 (C-14), 51.6 (OCH3), 42.1 (C-14), 40.5 (CH-1), 40.1 (CH-22), 38.9 (C-9), 38.5 (CH-22), 38.1 (C-17), 32.3 (CH-16), 29.1 (CH-21), 26.5 (CH-23, 28), 23.4 (CH-12), 22.9 (CH-25), 21.7 (CH-26), 21.4 (CH-23), 20.9 (CH, OAc), 18.0 (CH3-C-23); positive FABMS m/z 597 [M + Na]+, 557 [M + H – H2O]+; HR FABMS m/z 537.3148 ([M + H]+, calcd for C25H39O7S 537.3146 ppm error).

Galphimine F (5): white amorphous powder; mp 148–150 °C; [α]D20 = –38° (c 0.4, CHCl3); 1H NMR (CDCl3, 300 MHz) δ 6.43 (1H, dd, J = 12.4, 6.6 Hz, H-1), 6.04 (1H, d, J = 12.4, 7.8 Hz, H-2), 4.68 (1H, q, J = 2.4 Hz, H-29b), 4.46 (1H, q, J = 2.4 Hz, H-29a), 4.23 (1H, d, J = 12.0 Hz, H-5b), 4.10 (1H, d, J = 6.3 Hz, H-4), 3.52 (1H, q, J = 6.3 Hz, H-4), 2.91 (1H, d, J = 15.0 Hz, H-19b), 2.54 (1H, dd, J = 8.6 Hz, H-15a, 8.6 Hz, H-15b), 2.30 (1H, d, J = 8.8 Hz, H-8a), 2.10 (1H, m, H-16a), 2.05 (1H, d, J = 15.0 Hz, H-19a), 1.98 (1H, m, H-21a), 1.46 (1H, m, H-6b), 1.44 (1H, m, H-11b), 1.35 (3H, s, CH3-C-26), 1.28 (1H, m, H-16a), 1.24 (1H, m, H-11a), 1.20 (1H, ddd, J = 12.0, 4.5, 2.4 Hz, H-15a), 1.10 (3H, s, CH3-C-28), 1.05 (1H, m, H-22a), 0.95 (3H, s, OAc), 0.99 (3H, s, OCH3), 48.3 (C-5), 42.5 (CH3-C-19), 41.5 (C-14), 40.3 (C-1), 39.3 (C-9), 38.7 (CH-22), 37.1 (C-17), 31.9 (CH2-C-16), 29.2 (CH2-C-15), 26.5 (CH3-C-28), 23.4 (C, CH-12), 22.9 (CH3-C-25), 21.7 (CH3-C-26), 21.4 (CH3-C-25), 20.9 (CH3, OAc), 18.0 (CH3-C-23); positive FABMS m/z 575 [M + H]+, 537 [M + H + H2O]+; HR FABMS m/z 575.3203 ([M + H]+, calcd for C24H34O6S 575.3205 ppm error).

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Galphimine H (7): white amorphous powder; mp 167–178 °C; [α]D20 = –21° (c 0.7, CHCl3); 1H NMR (CDCl3, 300 MHz) δ 6.41 (1H, dd, J = 12.2, 8.8 Hz, H-1), 6.07 (1H, d, J = 12.2, 7.9 Hz, H-2), 5.51 (1H, d, J = 5.9 Hz, H-6a), 4.67 (1H, q, J = 2.4 Hz, H-29a), 4.46 (1H, q, J = 2.4 Hz, H-29a), 4.11 (1H, d, J = 7.8, 5.9 Hz, H-7b), 4.11 (1H, d, J = 12.2 Hz, H-24b), 3.95 (1H, q, J = 6.4 Hz, H-4), 2.90 (1H, d, J = 9.0 Hz, H-19b), 2.59 (1H, d, J = 9.0 Hz, H-10a), 2.48 (1H, d, J = 7.9 Hz, H-8a), 2.03 (2H, m, H-12), 2.00 (1H, m, H-16a), 1.81 (1H, d, J = 15.3 Hz, H-19a), 1.54 (3H, s, CH3-C-29), 1.51 (1H, m, H-22a), 1.46 (1H, m, H-11b), 1.37 (1H, m, H-11a), 1.33 (3H, s, CH3-C-26), 1.32 (1H, m, H-15a), 1.20 (1H, m, H-16a), 1.09 (3H, s, CH3-C-28), 0.98 (3H, d, J = 6.3 Hz, CH3-C-23), 0.92 (3H, s, CH3-C-25); 13C NMR (CDCl3, 125.7 MHz) δ 175.6 (C-30), 169.7 (C-3), 144.8 (CH-1), 132.0 (C-128), 132.9 (C-2), 118.5 (CH-2), 115.8 (C-12), 76.6 (C-11), 69.4 (C-21), 65.2 (C-12), 64.6 (CH-1), 53.8 (C-15), 52.9 (CH 2, C-12), 52.5 (C-14), 51.6 (OCH3), 42.1 (C-14), 40.5 (CH-1), 40.1 (CH-22), 38.8 (C-9), 38.5 (CH-22), 38.1 (C-17), 32.3 (CH-16), 29.1 (CH2-C-15), 26.6 (CH3-C-28), 23.3 (CH2-C-12), 22.8 (CH3-C-29), 21.4 (CH3-C-26), 21.2 (CH3-C-25), 17.6 (CH3-C-23), 12.0 (CH3-C-24); positive FABMS m/z 533 [M + H]+; HR FABMS m/z 533.3137 ([M + H]+, calcd for C25H39O7S + 0.4 ppm error).
Acetylation of Galphimines 2–4 and 6–7. Each individual compound (1–2 mg) was dissolved in Ac₂O–pyridine (1:4) (2.5 mL) and stood at room temperature for 24 h. The derivatives were precipitated by addition of cold water, and their purification was performed by semipreparative HPLC on a reversed-phase C₁₈ column (300 × 7.8 mm, 6 μm), using CH₃CN–H₂O (7:3, flow rate = 3 mL/min), to give derivatives 10 (1.5 mg) and 11 (2 mg).

Peracetylated derivative 10: white amorphous powder; [α]D25 +14° (c 0.2, CHCl₃); H NMR (CDCl₃, 300 MHz) ð 6.30 (1H, dd, J = 12.6, 8.4 Hz, H-1), 6.06 (1H, dd, J = 12.6 Hz, H-2), 5.66 (1H, d, J = 4.8 Hz, H-6), 5.53 (1H, m, H-7), 5.46 (1H, H, J, 4), 5.15 (1H, brs, H-21), 4.40 (1H, d, J = 12.4 Hz, H-24b), 4.08 (1H, d, J = 12.4 Hz, H-24a), 3.52 (3H, s, OCH₃), 2.50 (1H, d, J = 12.4 Hz, H-12), 2.40 (1H, ð, J = 12.4 Hz, H-24a), 2.10 (3H, s, OAc), 0.38 (3H, s, OAc), 2.00 (3H, s, OAc), 1.56 (3H, s, CH₃-29), 1.36 (3H, s, CH₃-26), 1.23 (3H, d, J = 6.3 Hz, CH₃-23), 1.07 (6H, s, CH₃-25, 28).

Peracetylated derivative 11: white amorphous powder; [α]D25 +9° (c 0.2, CHCl₃); H NMR (CDCl₃, 300 MHz) ð 6.30 (1H, dd, J = 12.6, 8.4 Hz, H-1), 6.06 (1H, dd, J = 12.6 Hz, H-2), 5.66 (1H, d, J = 4.8 Hz, H-6), 5.53 (1H, m, H-7), 5.46 (1H, H, J, 4), 4.69 (1H, q, J = 2.3 Hz, H-29b), 4.44 (1H, q, J = 2.3 Hz, H-29b), 4.40 (1H, d, J = 12.4 Hz, H-24b), 4.08 (1H, d, J = 12.4 Hz, H-24a), 3.52 (3H, s, OCH₃), 2.50 (1H, d, J = 7.8 Hz, H-8), 2.42 (1H, d, J = 8.4 Hz, H-10), 2.10 (3H, s, OAc), 0.28 (3H, s, OAc), 2.00 (3H, s, OAc), 1.34 (3H, s, CH₃-26), 1.23 (3H, d, J = 6.3 Hz, CH₃-23), 1.08 (6H, s, CH₃-25, 28).

Determination of the Absolute Configuration. Each individual solution (2.0 mg) of galphimines B (1) and E (2) was treated with 4-(dimethylaminopyridine (3 mg, previously heated at 70 °C for 3 h) and dry pyridine-d₅ (0.75 mL) in NMR tubes. (S)-(+)-α-Methoxy-α-trifluoromethylphenacyl (MTPA) chloride was added (20 μL). The reactions were allowed to stand at 70–75 °C under an atmosphere of N₂. NMR spectra were then recorded at 300 MHz by acquiring the reaction mixtures. Further purification was performed as follows. The mixtures were transferred from the NMR tubes into vials. Saturated aqueous NaHCO₃ and Et₂O were added to the mixtures and stirred vigorously for 5 min. Water (5 mL × 2) was added and extracted with CHCl₃. The organic phases were washed with 0.5 N HCl, dried with anhydrous Na₂SO₄, and concentrated. Each crude residue was purified by column chromatography over silica gel using CHCl₃–EtOAc (1:4) as eluent to give the (R)-MTPA ester (1.5–2.0 mg). NMR spectra in CDCl₃ were recorded after purification. Treatment of the same galphimines with (R)-(−)-MTPA chloride as described above yielded the (S)-MTPA esters.
H-4), 4.55 (1H, d, J = 12.3 Hz, H-24b), 4.44 (1H, d, J = 12.3 Hz, H-24a), 1.77 (3H, d, J = 6.0 Hz, CH₃-23).

Molecular Modeling Calculations. The molecular mechanics minimum energy structure of galphimine A (3) was generated using the MMX force field as implemented in the PCMODEL molecular modeling program V 6.00 (Serena Software, Box 3076, Bloomington, IN 47402-3076). The X-ray Cartesian coordinates of galphimine B (3)¹ were used as the starting point for the molecular modeling calculations. A systematic conformational search for all the rings of 3, according to DREIDING models, was achieved considering dihedral angle rotations of ca. 20° for those bonds that allowed such a movement. The E₉ MX values were monitored throughout the calculation process.

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Supporting Information Available: Chromatographic profiles (HPLC and TLC) of the galphimine mixture investigated and NMR spectra for galphimine ring E double-bond isomers. This information is available free of charge via the Internet at http://www.pubs.acs.org.

References and Notes


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