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Grahamines A−E, Cyclobutane-Centered Tropane Alkaloids from the Aerial Parts of *Schizanthus grahamii*

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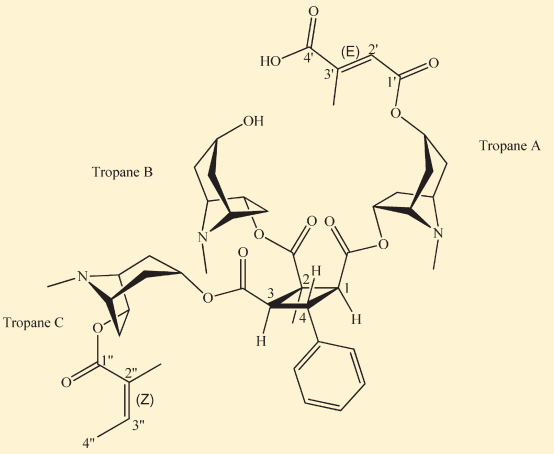
Supporting Information

ABSTRACT: *Schizanthus grahamii* is an endemic Chilean plant that is known to contain tropane alkaloids. Five new alkaloids, grahamines A−E (**1**−**5**), were isolated and characterized by extensive spectroscopic analysis. Their structures were determined to be 2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutanecarboxylic acid (**1**), 2-{[(3α-hydroxytropo-6β-yl)oxy]-carbonyl}-2-methyl-3-{[((6β-tigloyloxy)-3α-yl)oxy]carbonyl}-4-phenyl-cyclobutanecarboxylic acid (**2**), 1-methyl-2-{[(3α-hydroxytropo-6β-yl)-oxy]carbonyl}-4-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-3-phenylcy-clobutanecarboxylic acid (**3**), 1,2-bis{[(3α-hydroxytropo-6β-yl)oxy]-carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phe-nylcyclobutanecarboxylate (**4**), and 1-{[(3α-mesaconyloxytropo-6β-yl)-oxy]carbonyl}-2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutanecar-boxylate (**5**).



The *Schizanthus* genus belongs to the Solanaceae family and comprises 27 species distributed primarily in Chile except for *Schizanthus grahamii*, whose dispersal area extends to Argentina.[1] This species grows on the southwestern slopes of the Andes in fairly dry regions at altitudes up to 1300 m. It produces masses of vivid orchid-like flowers and thus is often cultivated for ornamental purposes. Numerous alkaloids have been isolated from this plant, mainly tropane ester derivatives with isomeric C_5 acids, namely, angelic, senecioic, tiglic, itaconic, and mesaconic acids, leading to numerous positional and configurational isomers such as 3α-senecioyloxytropane, 3α-hydroxy-6β-angeloyloxytropane, 3α-senecioyloxy-6β-hydroxytro-pane, and schizanthines C, D, E, and X.[2−5] A trimeric tropane alkaloid, grahamine, has also been isolated.[4] This complex molecule has a 2-methyl-4-phenylcyclobutane-1,2,3-tricarboxylate moiety as a central core. In the *Schizanthus* genus, grahamine is the only alkaloid containing this central cyclobutane ring substituted with three tropane units.

As part of our ongoing investigations on the Chilean species of this genus,[2,3,6] an investigation of the crude alkaloids extract of the aerial parts of *S. grahamii* Gill. was carried out. The paper reports the isolation and characterization of five new grahamines, named grahamines A−E (**1**−**5**), containing the same central structure as grahamine. Three isomeric alkaloid derivatives containing two tropane rings and two alkaloid derivatives containing three tropane rings are described.

RESULTS AND DISCUSSION

The crude alkaloid extract of the aerial parts of *S. grahamii* was first analyzed by UHPLC/ESITOFMS. Isomers of schizanthine X (572 Da), grahamine (871 Da), and other alkaloids with molecular weights over 500 Da were observed. Several purifications by MPLC were carried out and afforded two fractions rich in alkaloids with molecular weights over 500 Da. One of these (J11B) was fractionated by semipreparative chromatography to afford five tropane alkaloid derivatives. Compounds **1**−**3** were found to be isomers, each having a molecular formula of $C_{35}H_{47}N_2O_9$. For compound **1**, the presence of two tropane rings (A and B) disubstituted at C-3α and C-6β were deduced from the NMR spectra. The structures of the tropane rings are shown assuming the same chirality of similar compounds.[7] The characteristic chemical shifts of H-1, H-3, H-5, and H-6 of both tropane rings were observed in the ^{1}H NMR spectrum at δ_H 3.19−3.83 (br s), 4.00−4.74 (t, J = 3.9−5.2 Hz), 3.12−3.82 (br s), and 5.38−5.83 (dd, J = 7.7−7.8, 1.9−3.2 Hz), respectively.[8−11]

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Chart 1

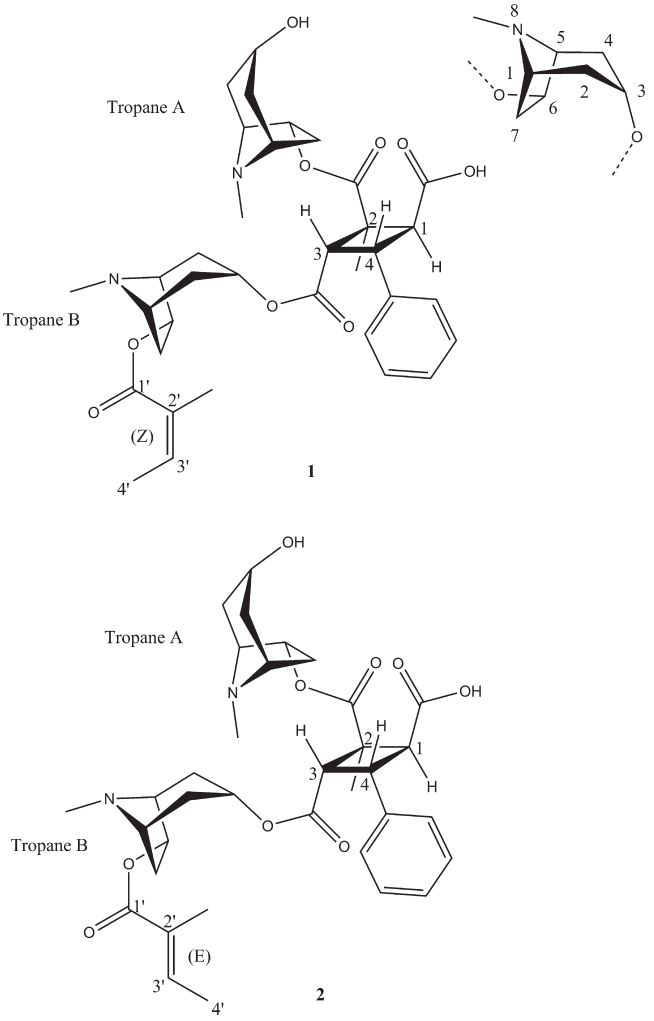
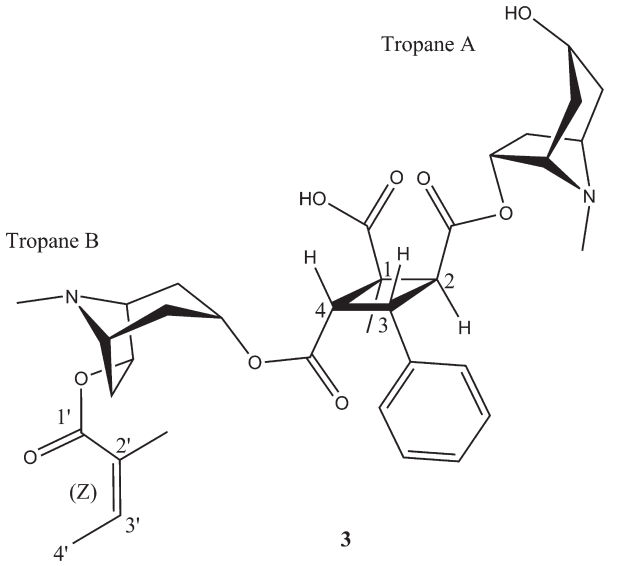


Chart 2



The difference in the chemical shifts between H-3 of the tropane A ring (δ_H 4.00) and H-3 of the tropane B ring (δ_H 4.74) can be explained by the presence of a free C-3 hydroxy group (δ_C 62.3) of the tropane A ring in contrast to the C-3 ester group of tropane B (δ_C 67.3).[10] Five aromatic protons at δ_H 7.16 (1H, m, *p*-Ph) and 7.26 (4H, m, *o*-Ph and *m*-Ph) suggested the presence of a phenyl group. An HMBC experiment revealed the presence of a 2-methyl-4-phenylcyclobutane-1,2,3-tricarboxylate moiety. Indeed, the connectivity of the three hydroxycarbonyl atoms connected to C-1, C-2, and C-3 of the cyclobutane ring at δ_C 178.5, 175.2, and 171.3, respectively, was revealed by the $^2J_{CH}$ and $^3J_{CH}$ coupling constants, respectively. In addition, the protons of the C-2 methyl group of the cyclobutane ring at δ_H 1.60 (3H, s) were coupled with three aliphatic saturated carbons C-1, C-2, and C-3 at δ_C 54.9 ($^3J_{CH}$), 47.9 ($^2J_{CH}$), and 49.3 ($^3J_{CH}$). H-1 and H-3 of the cyclobutane ring were coupled ($^2J_{CH}$) with C-4 as well as with an aromatic carbon at δ_C 141.0 (*i*-Ph, $^3J_{CH}$). Another long-range HMBC correlation between H-3 of the tropane B ring and the carbonyl group attached to C-3 confirmed the position of the tropane B ring in relation to the cyclobutane ring unambiguously. Attached to the C-6β tropane B ring was an angelic acid moiety, as shown by a proton at δ_H 6.14 (qq, J = 7.3, 1.5 Hz, H-3′) and two methyl groups at δ_H 1.90 (quint, H-2′) and 2.00 (dq, J = 7.3, 1.5 Hz, H-4′), characteristic of angelic acid.[12] The position of the tropane A ring with respect to the cyclobutane ring was not as straightforward. The tropane A ring possessed a C-3α hydroxy group and should be attached to the cyclobutane ring at C-6β. However, no long-range HMBC correlation between H-6 of the tropane A ring and a hydroxycarbonyl atom (attached either to C-1 or C-2) was observed. Therefore, it was not possible to position the tropane A ring with certitude, although the chemical shift of the carbonyl group at C-2 (δ_C 175.2) seemed to indicate an ester carbonyl C atom, whereas that of C-1 (δ_C 178.5) indicated a hydroxycarbonyl acid function.[13] To resolve the problem, methylation of the hydroxycarbonyl group was carried out with (trimethylsilyl)diazomethane (Figure 1). In the HMBC spectrum of the methylated compound, the correlation between H-6 of the tropane A ring and the ester C atom was still not observed. The protons of the C-2 methyl group of the cyclobutane ring at δ_H 1.60 (3H, s) coupled as previously for the nonmethylated compound **1** with the geminal C-2 carbonyl C at δ_C 174.4. The vicinal proton H-1 at δ_H 3.71 (1H, d, J = 11.1 Hz) correlated with a carbonyl C at δ_C 174.0, as did the protons of a methoxy group at δ_H 3.72 (3H, s) (Figure 1). From these observations we concluded that the tropane A ring was attached to the C-2 carbonyl of the cyclobutane ring. Finally, the relative orientations of H-1, H-3, and H-4, the C-2 methyl group, and the phenyl group were established by a NOESY experiment. The *o*-aromatic protons correlated with H-1 and those of the C-2 methyl group, suggesting that these moieties are cofacial. Despite the fact that the relative configuration of the cyclobutane moiety was unambiguously established, the absolute configuration is not known. Compound **1** was identified as 2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutanecarboxylic acid and named grahamine A.

For compound **2**, the NMR spectra were similar to those of **1**. The only difference was a resonance in the ^{1}H NMR spectrum at δ_H 6.86 (1H, q, J = 6.8 Hz, H-3′) instead of 6.14 (1H, qq, J = 7.3, 1.5 Hz, H-3′) in **1**. In a TOCSY experiment, H-3′ correlated with two methyl groups (Me-2′ and Me-4′) at δ_H 1.84 and 1.81, which is characteristic of tiglic acid.[12] Thus, for alkaloid **2**, a tiglic acid moiety is attached to the tropane B ring instead of the angelic acid

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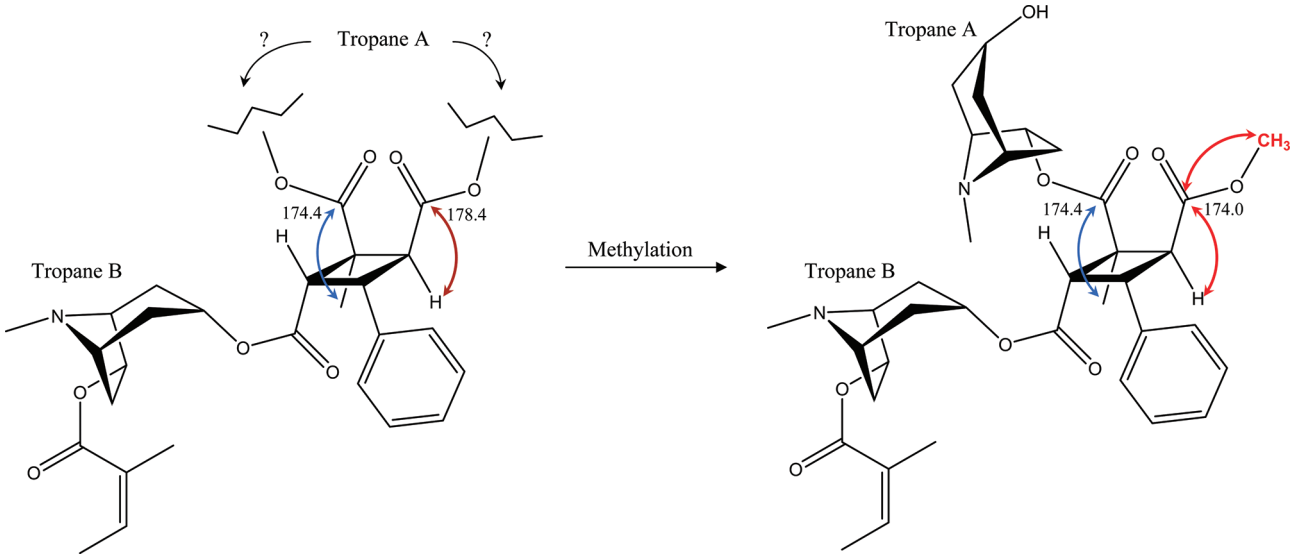


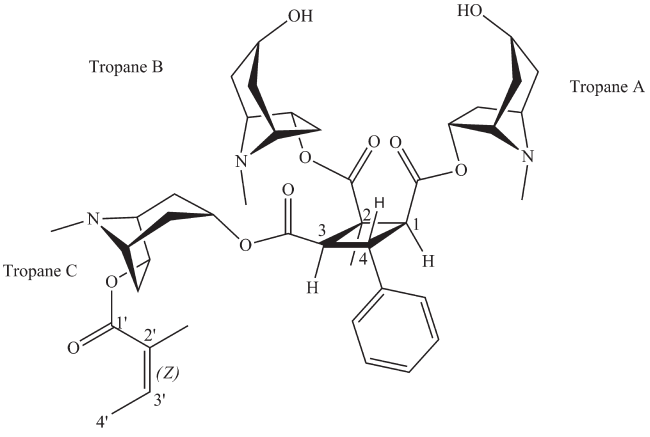
Figure 1. Methylation of compound **1**.

in **1**. Compound **2** was established as 2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-tigloyloxy)-3α-yl)oxy]-carbonyl}-4-phenylcyclobutanecarboxylic acid and named grahamine B.

For compound **3**, an HMBC experiment showed that the protons of the C-1 methyl group at δ_H 1.56 (3H, s) correlated with a carbonyl carbon at δ_C 179.7, whereas for **1** and **2**, the chemical shifts of the carbonyl carbons were δ_C 175.2 and 174.6, respectively. The two tropane groups A and B were thus attached to C-2 and C-4, and the hydroxycarbonyl group was attached to C-1. A TOCSY experiment showed that, as for **1**, H-3′ (δ_H 6.14, q, J = 7.1 Hz) formed a spin system with CH_3-2′ (δ_H 1.89, s) and CH_3-4′ (δ_H 2.00, d, J = 7.1), which is characteristic of angelic acid. Thus, compound **3** was elucidated as 1-methyl-2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-4-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-3-phenylcyclobutanecarboxylic acid and named grahamine C.

Compound **4** was found to have the molecular formula $C_{43}H_{59}N_3O_{10}$. The ^{1}H NMR spectrum was similar to those of compounds **1**−**3**, but additional signals were observed. Regarding the tropane rings, three C-6 protons resonated at δ_H 5.26, 5.76, and 5.81 as well as three C-3 protons at δ_H 3.95, 3.99, and 5.03. This implied that compound **4** possessed three tropane rings. HMBC correlations between H-6 of the tropane A ring (δ_H 5.76), H-6 of the tropane B ring (δ_H 5.81), and H-3 of the tropane C ring (δ_H 5.03) and the C-1, C-2, and C-3 ester carbonyl carbons attached to the cyclobutane ring (δ_C 172.1, 174.6, and 172.0), respectively, confirmed the position of the tropane moieties. Their orientation with respect to the cyclobutane ring was shown by a NOESY experiment. Correlations were observed between the H-1 and H-3 of the cyclobutane ring with the protons of the C-2 methyl group in addition to the *o*-protons of the phenyl group. This suggested that the three tropane rings were cofacial, unlike in the previous three compounds. TOCSY correlations between H-3′ (δ_H 6.16, 1H, qq, J = 7.3, 1.5 Hz) and H-2′ (δ_H 1.88, 3H) and H-4′ (δ_H 2.00, 3H) indicated the presence of an angelic acid moiety. This angelic acid moiety was attached to the tropane C ring via appropriate HMBC correlations. Thus, the structure of compound **4** was elucidated as 1,2-bis{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutanecarboxylate and named grahamine D.

Chart 3



Compound **5** was found to have the molecular formula $C_{48}H_{64}N_3O_{13}$. The ^{1}H NMR spectrum was similar to that of compound **4**, where three tropane rings were also observed. Three C-6 protons resonated at δ_H 5.26, 5.53, and 5.76 and three C-3 protons at δ_H 3.93, 4.98, and 5.02. Like compound **4**, all three tropane rings were observed to be cofacial by a NOESY experiment. An additional signal at δ_H 6.40 and a methyl group at δ_H 2.27 were detected in the ^{1}H NMR spectrum, revealing the structure of mesaconic acid, which was attached to the tropane A ring by HMBC correlations. Compound **5** was identified as 1-{[(3α-mesaconyloxytropo-6β-yl)oxy]carbonyl}-2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutanecarboxylate and named grahamine E.

To date, grahamine is the only alkaloid identified in the *Schizanthus* genus to contain a 2-methyl-4-phenylcyclobutane-1,2,3-tricarboxylate moiety as a central structure.[4] We have isolated another five compounds with the same central

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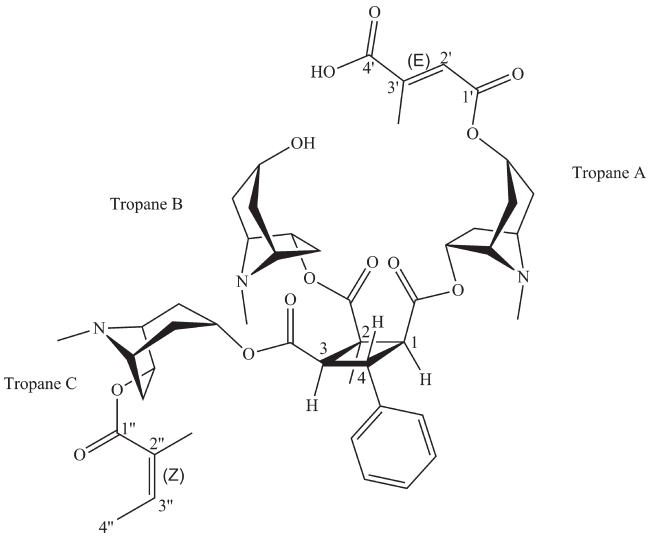
Chart 4



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skeleton. The hypothesis that the formation of these four-membered rings takes place through [2+2] photocycloaddition is supported by the absence of cyclobutane-containing tropane alkaloids in the root extract and has been reported to occur in other plants containing cinnamic acid and related moieties.[14−17] Complete rationalization of the biosynthesis is not yet possible, but some points are worth mentioning. First, the common cyclobutane structure speaks in favor of a regioselective cycloaddition. The regioselectivity of the photochemical [2+2] cycloaddition of cinnamic ester[18] is usually low unless templating can impose a given geometry.[19−21] Outside the dimerization process, little is known about the reactions and selectivity of cinnamic ester with other double-bond-containing substrates. We can only speculate that the common structure of **1**−**5** can be explained by a combination of electronic and steric effects of the vinylic methyl group. Simple molecular orbital calculations indicated no significant differences in the coefficients of the frontier orbitals of the mesaconic moiety, making it impossible to argue in terms of molecular orbitals. Only a detailed computational study might confirm whether this common structure is driven by steric or electronic effects. The [2+2] cyclizations are known to occur in two steps.[22] First, the photoexcitation of the cinnamic chromophore results in a reactive $^3(\pi,\pi^*)$ state, forming a bond with one carbon of the double bond of the mesaconic group. This results in a 1,4-biradical triplet, and the second bond can be formed only after the singlet state is reached. It is not known which of the two bonds is formed first, but the fact is that all known compounds have pairs of esters at C-1 and C-2 after initial formation of the C-1/C-2 bond (Figure 2). Concerning the conformation of the mesaconate moiety, the diversity of the stereochemistry observed for C-3 may be explained by a rotation of the C-2/C-3 bond during the triplet-state period. The rotation may be favorable for the sterically hindered ester to reach an equatorial position. Why the rotation would only occur with the trisubstituted compounds **4** and **5** but not with the disubstituted compounds **1**−**3** is not clear but may be a means to reduce the steric hindrance or is related to the presence of a hydroxycarbonyl group at C-1 in **1**−**3**.

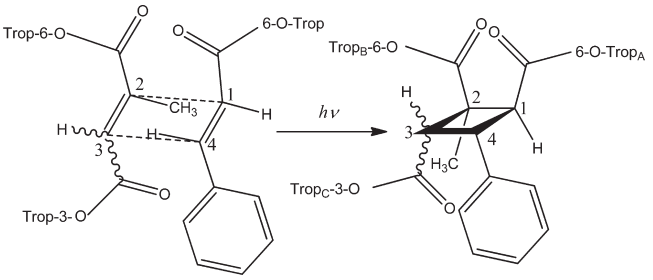

Figure 2. Probable relative orientations of the components of the [2+2] photocyclization.

If the cyclophotoaddition is concerted, the partners of formation of the cyclobutane would be, on one hand, (*E*)-cinnamic acid, and, on the other hand, mesaconic derivatives or the corresponding citraconic equivalent. According to the [2+2] cyclophotoaddition hypothesis, **1**, **4**, and **5** would be obtained by the photoreaction of schizanthine D, which is known to occur in *S. grahamii*. The isomer **2** could have schizanthine Y as precursor even if it has been observed only in other species of the *Schizanthus* genus. Concerning compound **3**, the precursor could be the hydrolyzed form of 3α-(1-methylmesaconyl)-6β-angeloyloxytropane previously described in this genus.[23] Cinnamic ester derivatives have not yet been identified in *S. grahamii*, but 6β-cinnamoyloxytropan-3α-methylmesaconate is present in *S. litoralis*.[24]

Finally, **4** and **5** would be formed either by the direct reaction of (*E*)-cinnamic acid with the citraconic derivatives, also known to occur in this genus,[23] or after isomerization of the double bond of mesaconic derivatives if it proceeds in two steps.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Perkin-Elmer Lambda-20 UV−vis spectrophotometer (Wellesley, MA, USA) in MeOH. IR spectra were measured on a Perkin-Elmer Spectrum 100 in $CHCl_3$. 1D and 2D NMR spectra were recorded in methanol-d_4 on a Varian Unity Inova 500 MHz NMR instrument (Palo Alto, CA, USA). For compounds **2**, **3**, and **5** (quantities less than 1 mg), spectra were recorded on the same instrument by direct injection of 5 μL samples in methanol-d_4 in a microflow probe (active volume: 1.5 μL) from Protasis/MRM (Savoy, IL, USA). An HMBC spectrum of **5** was recorded on a Bruker Avance 500 MHz spectrometer equipped with a cryoprobe to confirm its structure. Chemical shifts (δ) are in ppm relative to Me_4Si as internal standard, and coupling constants (J) are in Hz. HR-MS was obtained on a Micromass LCT Premier time-of-flight mass spectrometer (Waters, Milford, MA, USA) using electrospray in the positive mode. Capillary voltage was set at 2.8 kV, cone voltage at 40 V, source temperature at 120 °C, desolvation temperature at 250 °C, cone gas flow at 20 L/h, and desolvation gas flow at 600 L/h. UHPLC was performed on an Acquity UPLC System (Waters) with an Acquity BEH C18 column (1.7 μm; 50 × 2.1 mm i.d.; Waters). All separations by semipreparative HPLC were carried out using a Varian 9012 pump coupled with a triple quadrupole mass spectrometer (TSQ700, Finnigan MAT, San Jose, CA, USA) equipped with a Finnigan ESI interface. ESI conditions were as follows: capillary temperature 200 °C, source voltage 4.5 kV, sheath gas nitrogen 50 psi, acquisition in positive ion mode, full scan m/z 100−1000 Da, scan time 0.5 s. An adjustable flow splitter Quicksplit (El Sobrante, CA, USA) was used to split the flow to the MS detector and to manually collect samples.

Plant Material. *Schizanthus grahamii* Gill. was collected in Rengo (Central Chile) in January 2004 and authenticated by Prof. Fernanda Perez (Departamento de Botánica, Facultad de Ciencias, Universidad

Table 1. NMR Data (500 MHz, methanol-d_4, δ in ppm, J in Hz) of Compounds 1−3 Obtained Using Capillary NMR

	1		2		3	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	54.9	3.72 d (11.1)	54.0	3.67 d (10.9)	50.2	
1-CO	178.5		177.9		179.7	
1-CH_3					20.4	1.56 s
2	47.9		47.3		50.2	3.81 d (11.4)
2-CO	175.2		174.6		173.8	
2-CH_3	20.1	1.60 s	19.3	1.58 s		
3	49.3	3.91 d (9.7)	48.6	3.86 d (9.5)	40.1	4.34 t (10.3)
3-CO	171.3		170.5			
4	42.0	4.31 t (10.4)	41.1	4.29 t (10.6)	51.3	3.77 d (9.2)
4-CO					171.4	
i-Ph	141.0		140.5		140.0	
o-Ph	128.2	7.26 m	127.5	7.22 m	127.1	7.19 d (7.7)
m-Ph	128.8	7.26 m	127.5	7.22 m	127.8	7.25 t (7.2)
p-Ph	127.1	7.16 m	126.2	7.13 t (6.0)	126.2	7.16 t (7.7)
			Tropane A			
1	63.0	3.83 br s	61.5	3.45 br s	61.6	3.49 br s
2		2.20 dt (14.7, 3.9), 1.92 d (14.7)	37.2	2.08 m, 1.75 d (13.7)	37.3	2.10 m, 1.79 d (14.9)
3	62.3	4.00 t (3.9)	62.6	3.95 t (4.1)	62.6	3.96 t (3.8)
4		2.25 dt (15.8, 3.9), 2.12 d (15.8)	32.6	2.09 m, 1.98 m	36.2	2.11 m, 2.02 m
5	68.2	3.82 br s	67.0	3.43 br s	66.7	3.61 br s
6	77.9	5.83 dd (7.8, 1.9)	79.5	5.75 dd (6.9, 2.0)	77.9	5.77 dd (7.4, 2.9)
7	35.0	3.02 dd (14.6, 7.8) 2.55 m	34.6	2.78 dd (12.3, 6.9), 2.36 m	34.1	2.80 dd (13.6, 7.4), 2.35 m
8 (N-CH_3)		2.91 s	40.3	2.63 s	40.4	2.60 s
			Tropane B			
1	60.9	3.19 br s	59.9	3.17 br s	60.0	3.14 br s
2	34.9	1.87 dt (15.1, 5.2), 1.05 d (15.1)	33.8	1.87 m, 1.07 d (15.1)	34.2	1.86 m, 1.06 d (15.7)
3	67.3	4.74 t (5.2)	66.5	4.72 t (4.1)	66.1	4.78 t (4.9)
4	33.6	2.02 dt(15.1, 4.4), 1.66 d (15.1)	32.6	2.01 m, 1.62 m	32.4	1.95 m, 1.37 d (15.1)
5	66.9	3.12 br s	65.9	3.07 br s	65.7	3.06 br s
6	79.4	5.38 dd (7.7, 3.2)	78.7	5.35 dd (7.5, 2.8)	78.7	5.36 dd (7.4, 2.7)
7	36.0	2.43 dd (13.4, 7.7), 2.12 d (13.4)	35.3	2.41 m, 2.11 m	35.5	2.43 m, 2.10 m
8 (N-CH_3)	40.1	2.44 s	39.1	2.42 s	39.2	2.42 s
1′	168.7		168.0		167.7	
2′	128.7		128.6		127.9	
2′-CH_3	20.4	1.90 quint (1.5)	10.9	1.84 s	19.5	1.89 s
3′	138.9	6.14 qq (7.3, 1.5)	137.6	6.86 q (6.8)	137.6	6.14 q (7.1)
4′	15.7	2.00 dq (7.3, 1.5)	13.2	1.81 d (7.1)	14.7	2.00 d (7.1)

de Chile); a voucher specimen (no. 2000-4) has been deposited in the Faculty of Chemistry at the same University.

Extraction and Isolation. The dried and powdered aerial parts (2.6 kg) were extracted with EtOH at room temperature, and the filtered solution was evaporated to dryness. The residue was suspended in 0.1 M HCl, filtered, and washed with DCM (3 × 2 L). The aqueous solution was basified to pH 12 with NH_4OH and further extracted with DCM (3 × 2 L), yielding a gummy alkaline residue (5.8 g).

A portion of this residue (4.3 g) was fractionated by MPLC on a LiChroprep C_{18} (40−63 μm, 450 × 45 mm i.d., Merck) column with a Büchi 681 pump equipped with a Knauer UV detector, using a H_2O (+0.1% NH_3)/MeOH (+0.1% NH_3) gradient of 90:10 to 0:100. Peaks were detected at 220 nm, giving 14 fractions (A−N). Fraction J (1.7 g) was further separated by MPLC under the same conditions as previously mentioned and gave 12 fractions (J1 to J12). Fraction J11 (406 mg) was purified on a Sephadex LH-20 open column (310 mm × 25 mm i.d.) with 100% MeOH to give seven fractions (J11A to J11G). Fraction J11B (40 mg) was purified by semipreparative HPLC (X-Terra, 150 mm × 19 mm i.d., Waters) using H_2O (+0.1% NH_3)/MeCN (+0.1% NH_3) with a gradient of 95:5 to 20:80 in 70 min (flow rate: 5 mL/min) to afford 14 fractions (J11B1 to J11B14). Finally, fraction J11B9 (8 mg) was purified by semipreparative HPLC (X-Terra, 150 mm × 19 mm i.d., Waters) with 81% H_2O (+0.1% NH_3)/19% MeCN (+0.1% NH_3) in isocratic mode (flow rate: 5 mL/min), affording 2.2 mg of **1**, 0.5 mg of **2**, and 0.4 mg of **3**. Fraction J11B12 (30 mg) was purified by semipreparative HPLC (X-Bridge, 250 mm × 10 mm i.d., Waters) with 63% H_2O (+0.1% NH_3)/37% MeCN (+0.1% NH_3) in isocratic mode (flow rate: 1.9 mL/min) to give 3.0 mg of **4**. Fraction J11B10 (5.6 mg) was purified by semipreparative HPLC (X-Terra, 150 mm × 19 mm i.d., Waters) with 77% H_2O (+0.1% NH_3)/23% MeCN (+0.1% NH_3) in isocratic mode (flow rate: 5 mL/min) to give 0.5 mg of **5**.

Table 2. NMR Data (500 MHz, methanol-d_4, δ in ppm, J in Hz) of Compound 4 Obtained Using Capillary NMR

position	δ_C	δ_H
1	53.6	3.16 d (10.2)
1-CO	172.1	
2	49.8	
2-CO	174.6	
2-CH_3	24.8	1.71 s
3	52.1	3.43 d (10.7)
3-CO	172.0	
4	44.0	4.30 t (10.3)
i-Ph	142.2	
o-Ph	128.4	7.46 d (7.4)
m-Ph	129.6	7.29 t (7.6)
p-Ph	128.3	7.21 tt (7.4, 1.1)
	Tropane A	
1	63.3	3.61 br d (6.3)
2	38.0	2.12 m, 1.80 d (15.6)
3	63.2	3.95 t (4.4)
4	37.0	2.16 m, 1.97 m
5	68.7	3.36 br s
6	79.0	5.76 dd (7.9, 3.0)
7	35.9	2.88 t (8.3), 2.21 m
8 (N-CH_3)	40.9	2.52 s
	Tropane B	
1	63.3	3.69 br d (6.8)
2	38.1	2.15 m, 1.85 d (13.9)
3	63.3	3.99 t (4.4)
4	37.1	2.20 m, 2.04 m
5	68.5	3.55 br s
6	79.7	5.81 dd (8.0, 3.2)
7	35.3	2.86 t (8.5), 2.32 m
8 (N-CH_3)	41.3	2.77 s
	Tropane C	
1	61.3	3.35 br s
2	35.1	2.16 m, 1.59 d (15.1)
3	68.2	5.03 t (5.1)
4	33.9	2.18 m, 1.90 m
5	67.3	3.25 br s
6	79.3	5.26 dd (7.8, 3.3)
7	35.8	2.21 m, 2.01 m
8 (N-CH_3)	40.4	2.50 s
1′	168.9	
2′	128.9	
2′-CH_3	20.8	1.88 quint (1.5)
3′	139.5	6.16 qq (7.3, 1.5)
4′	16.1	2.00 dq (7.3, 1.5)

Grahamine A (1): colorless oil; UV (MeOH) λ_{max} 273 nm; IR ($CHCl_3$) 2930, 1730, 1590, 1472, 1385, 1203 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HR-ESIMS m/z 639.7417 ($C_{35}H_{47}N_2O_9$, $[M + H]^+$, requires 639.7477).

Grahamine B (2): colorless oil; ^1H and ^{13}C NMR see Table 1; HR-ESIMS m/z 639.7454 ($C_{35}H_{47}N_2O_9$, $[M + H]^+$, requires 639.7477).

Table 3. NMR Data (500 MHz, methanol-d_4, δ in ppm, J in Hz) of Compound 5 Obtained Using Capillary NMR

position	δ_C	δ_H
1	53.7	3.07 d (10.3)
1-CO	172.2	
2	50.0	
2-CO	174.3	
2-CH_3	25.2	1.70 s
3	52.1	3.33
3-CO	172.2	
4	43.6	4.30 t (10.4)
i-Ph	142.6	
o-Ph	128.5	7.40 d (7.4)
m-Ph	129.7	7.27 t (7.4)
p-Ph	128.4	7.18 t (7.4)
	Tropane A	
1	67.9	3.05 br s
2	36.6	2.09 m, 1.73 m
3	67.6	4.98 t (5.2)
4	35.1	2.09 m, 1.90 m
5	62.1	3.32
6	80.8	5.53 dd (7.9, 3.1)
7	36.3	2.56 dd (14.4, 7.9), 2.20 m
8 (N-CH_3)	41.7	2.36 s
1′	167.7	
2′	121.2	6.4 q (1.5)
3′	156.2	
3′-CH_3	16.4	2.27 d (1.5)
4′	175.8	
	Tropane B	
1	68.1	3.19 br s
2	38.8	2.02 m, 1.72 d (14.1)
3	64.3	3.93 t (4.1)
4	37.9	2.03 m, 1.88 m
5	62.5	3.36
6	81.6	5.76 dd (7.6, 3.1)
7	36.1	2.66 dd (13.7, 7.6), 2.21 m
8 (N-CH_3)	42.0	2.54 s
	Tropane C	
1	67.4	3.13 br s
2	35.3	2.12 m, 1.57 d (15.0)
3	68.5	5.02 t (5.2)
4	34.2	2.12 m, 1.87 m
5	61.3	3.26 br s
6	80.2	5.26 dd (7.6, 3.2)
7	36.2	2.13 m, 2.00 m
8 (N-CH_3)	40.7	2.43 s
1″	169.1	
2″	129.1	
2″-CH3	20.9	1.86 quint (1.5)
3″	139.5	6.13 qq (7.2, 1.5)
4″	16.2	1.99 dq (1.5)

Grahamine C (3): colorless oil; ^1H and ^{13}C NMR see Table 1; HR-ESIMS m/z 639.7418 ($C_{35}H_{47}N_2O_9$, $[M + H]^+$, requires 639.7477).

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Grahamine D (4): white solid; UV (MeOH) λ_{max} 267 nm; IR ($CHCl_3$) 2911, 1717, 1583, 1450, 1370, 1222, 1011 cm^{-1}; ^1H and ^{13}C NMR see Table 2; HR-ESIMS m/z 778.6478 ($C_{43}H_{60}N_3O_{10}$, $[M + H]^+$, requires 778.9268).

Grahamine E (5): white solid; ^1H and ^{13}C NMR see Table 3; HR-ESIMS m/z 890.8945 ($C_{48}H_{64}N_3O_{13}$ $[M + H]^+$, requires 891.0342).

■ ASSOCIATED CONTENT

Supporting Information. ^1H NMR, COSY, HMBC, HSQC, and NOESY spectra of compounds **1**−**5**. This information is available free of charge via the Internet at http://pubs.acs.org.

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