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Isolation and characterization of bioactive principles of the leaves and stems of *Physalis philadelphica*

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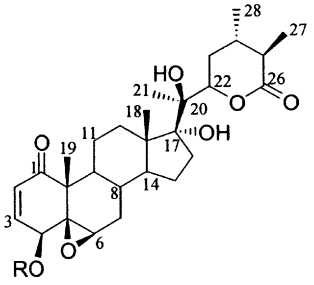
Abstract—An ethyl acetate-soluble extract of the leaves and stems of *Physalis philadelphica* has been investigated, leading to the isolation of three new withanolides, philadelphicalactones A (**1**) and B (**2**), and ixocarpalactone B (**3**), four known withanolides, ixocarpalactone A (**4**), withaphysacarpin (**5**), 18-hydroxywithanolide D (**6**), and withanone (**7**), one new ceramide, (2*S*,3*S*,4*R*,9*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-9-octadecene (**8**), two known ceramides, (2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-1,3,4-octadecanetriol (**9**), and (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1,3,4-octadecanetriol (**10**), as well as the known chlorophyllide *a* (**11**). The structures of the new compounds were elucidated based on spectroscopic and chemical methods. Single-crystal X-ray diffraction analysis was used to confirm the relative stereochemistry of compounds **1** and **4**. The absolute stereochemistry of compounds **1**–**4** and **8** was established by Mosher ester methodology and chemical transformation. All isolates were evaluated for their potential cancer chemopreventive properties utilizing in vitro assays to determine quinone reductase induction and inhibition of murine epidermal JB6 cell transformation.

1. Introduction

The whole plants of *Physalis philadelphica* Lam. (Solanaceae) have been used for the treatment of gastrointestinal disorders in Guatemala[1] and for treating leprosy, purifying the blood, and as a poison antidote in Mexico.[2] The fruits of *P. philadelphica*, known commonly as tomatillos, are used as an ingredient in foods such as enchiladas and salsas in some countries in Latin America, and they are also employed in some North American sauces and relishes as an acid source in place of tomatoes.[3] The former name for *P. philadelphica* is *Physalis ixocarpa* Brot.,[4] and the withanolides ixocarpalactone A (**4**), ixocarpalactone B 4-acetate (**3a**), ixocarpanolide, physalin B, and withaphysacarpin (**5**) have been isolated from the leaves and epigeal parts of this plant.[5–7] Three withanolides, 2,3-dihydro-3-methoxywithaphysacarpin, 24,25-dihydrowithanolide D, and withaphysacarpin (**5**), which showed significant induction of quinone reductase in hepa1c1c7 cells, were isolated from the fruits of this edible plant in our previous work.[8] As part of our continuing search for novel, plant-derived cancer chemopreventive agents,[9,10] we have now investigated an ethyl acetate-soluble extract of the leaves and stems of *P. philadelphica*, leading to the isolation of two new 17-hydroxywithanolides, philadelphicalactones A (**1**) and B (**2**), one new spiro-acetal withanolide, ixocarpalactone B (**3**), four known withanolides, **4**–**7**, one new (**8**) and two known (**9** and **10**) ceramides, as well as the known porphyrin derivative, chlorophyllide *a* (**11**). The structure elucidation of the new compounds was carried out by extensive spectral data interpretation as well as by chemical transformation. These isolates were evaluated for their potential cancer chemopreventive properties in a cell-based quinone reductase induction assay[11–13] and a



1 R = H
1r R = (*R*)-MTPA
1s R = (*S*)-MTPA

Keywords: *Physalis philadelphica*; Solanaceae; withanolides; ceramides; X-ray diffraction analysis; absolute configuration; quinone reductase induction; murine epidermal JB6 cell transformation.

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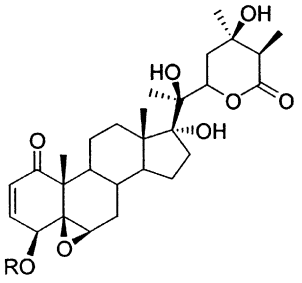
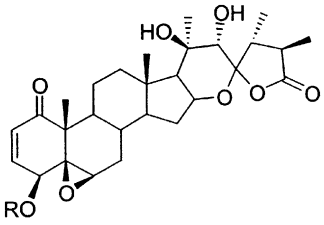
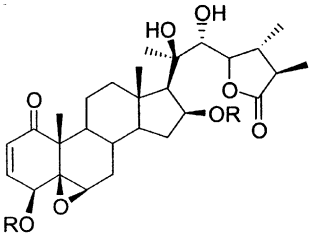
murine epidermal JB6 cell transformation assay.[14–16] The structural and absolute configuration determination of these compounds, as well as their biological evaluation, are the subject of this communication.



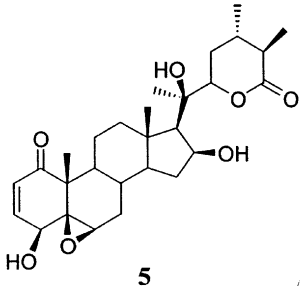
2 R = H
2r R = (*R*)-MTPA
2s R = (*S*)-MTPA



3 R = H
3a R = Ac
3r R = (*R*)-MTPA
3s R = (*S*)-MTPA



4 R = H
4r R = (*R*)-MTPA
4s R = (*S*)-MTPA



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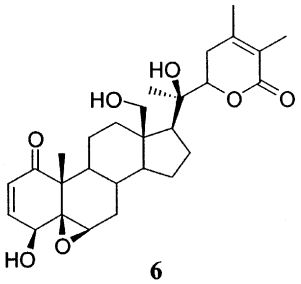

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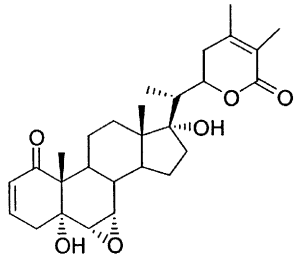

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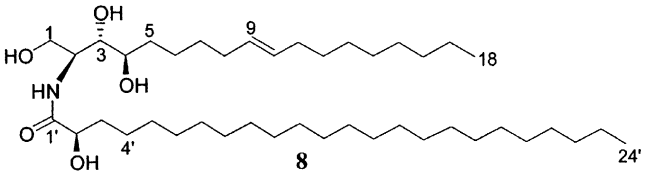

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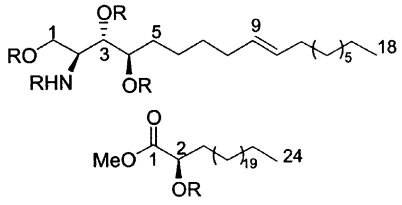
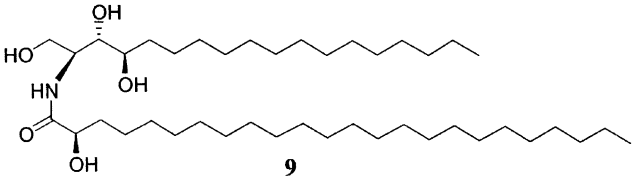


8a R = H
8c R = Ac

8b R = H
8d R = Ac



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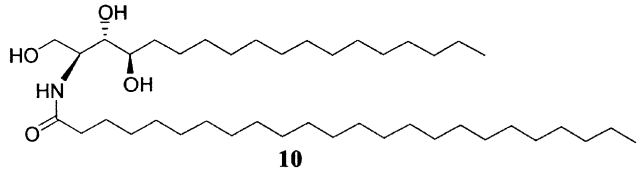

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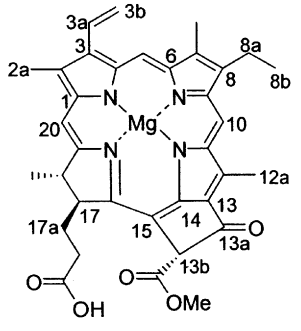

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Table 1. ^{1}H NMR data for compounds **1**, **1s**, and **1r**

Position	**1** (C_5D_5N)	**1** ($CDCl_3$)[a]	$\delta_{CDCl_3}-\delta_{C_5D_5N}$	**1s** (C_5D_5N)	**1r** (C_5D_5N)	$\delta_s-\delta_r$
2	6.42, d (9.8)	6.22, d (9.9)	−0.20	6.428, d (9.8)	6.515, d (9.8)	−0.087
3	7.21[b]	6.94, dd (9.9, 6.0)	−0.27	7.178[b]	7.235[b]	−0.057
4	4.01, d (6.2)	3.76, d (6.0)	−0.25	5.360, d (5.9)	5.395, d (6.0)	−0.035
6	3.21, br s	3.24, br s	+0.03			
7	2.26, m; 1.31, m	2.19–2.41, m; 1.53, m				
8	1.72–1.89, m	1.53, m				
9	1.08, ddd (10.8, 10.8, 4.5)	1.01, ddd (11.5, 11.5, 4.3)	−0.07	1.053, ddd (11.0, 11.0, 4.6)	1.033, ddd (11.0, 11.0, 4.6)	+0.020
11	2.11–2.22, m; 1.72–1.89, m	1.76–1.91, m; 1.49, m				
12	1.90–1.96, m; 2.11–2.22, m	2.19–2.41, m				
14	1.96–2.04, m	1.76–1.91, m				
15	1.72–1.89, m; 1.16–1.24[c]	1.76–1.91, m; 1.18, m				
16	3.12, br t (12.7); 2.11–2.22, m	2.19–2.41, m		3.062, br t (12.9)	3.051, br t (12.8)	+0.011
18	1.24, s	0.83, s	−0.41	1.188, s	1.161, s	+0.027
19	1.89, s	1.40, s	−0.49	1.487, s	1.401, s	+0.086
21	1.54, s	1.24, s	−0.30	1.527, s	1.523, s	+0.004
22	5.05, dd (11.8, 3.6)	4.42, dd (10.9, 3.7)	−0.63	5.057, dd (11.2, 3.5)	5.052, dd (11.3, 3.5)	+0.005
23	1.63–1.79, m; 2.16–2.26, m	2.19–2.41, m; 1.53, m				
24	1.58, m	1.76–1.91, m				
25	2.13–2.22, m	2.10, m				
27	1.18, d (6.5)	1.22, d (6.5)	+0.04	1.177, d (6.8)	1.173, d (6.9)	+0.004
28	0.95, d (6.6)	1.13, d (6.7)	+0.18	0.956, d (6.5)	0.953, d (6.5)	+0.003

Spectra taken at 300 MHz; chemical shift values presented in ppm; *J* values in parentheses given in Hz.
[a] Two drops of CD_3OD were added to improve solubility.
[b] Signals overlapped with solvent signals, and the chemical shifts of these signals were obtained from ^{1}H–^{1}H COSY spectra.
[c] Overlapped with CH_3-18 and CH_3-27 resonances.

2. Results and discussion

Compound **1** was isolated as colorless needles, mp 274–275°C and $[\alpha]_D^{20}=-6.2°$ (*c* 0.08, MeOH), and its molecular formula was determined as $C_{28}H_{40}O_7$ by HRFABMS (*m/z* 511.2630 $[M+Na]^+$). The ^{1}H NMR spectrum (in C_5D_5N) of compound **1** displayed characteristic signals for five methyl groups at δ_H 0.95 (3H, d, *J*=6.6 Hz, CH_3-28), 1.18 (3H, d, *J*=6.5 Hz, CH_3-27), 1.24 (3H, s, CH_3-18), 1.54 (3H, s, CH_3-21), and 1.89 (3H, s, CH_3-19), and two α,β-unsaturated olefinic protons at δ_H 6.42 (1H, d, *J*=9.8 Hz, H-2) and 7.21 (overlapped with solvent signal, H-3). In the ^{1}H–^{1}H COSY spectrum of **1**, H-3 correlated with H-2 and the oxygenated methine proton at δ_H 4.01 (1H, d, *J*=6.2 Hz, H-4), and indicated the presence of a 4β-hydroxy-2-en-1-one unit in the molecule.[6,7] The ^{13}C NMR and DEPT spectra of **1** disclosed 28 carbons, which were indicative of an α,β-unsaturated ketone (δ_C 202.6, s, C-1; δ_C 132.4, d, C-2; δ_C 144.9, d, C-3), a δ-lactone carbonyl (δ_C 175.9, s, C-26), two oxygenated methines (δ_C 70.4, d, C-4; δ_C 80.0, d, C-22), an epoxide functional group (δ_C 64.5, s, C-5; δ_C 60.1, d, C-6), two oxygenated quaternary carbons (δ_C 87.5, s, C-17; δ_C 78.1, s, C-20), in addition to five methyls, six methylenes, five other methines, and two other quaternary carbons. These NMR data were closely comparable to those of the known withanolide, withaphysacarpin (**5**),[8] and suggested that compound **1** is also a withanolide.[17,18]

Comparison of the ^{1}H and ^{13}C NMR spectral data of these two compounds indicated that they have the same substituent patterns and relative configurations in rings A–C and in their respective lactone ring. This was confirmed by observed correlations in their 2D NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC, and ROESY). The ^{13}C NMR and DEPT spectra of **1** and **5** suggested that these compounds vary at C-16 and C-17, with an oxygenated quaternary carbon at C-17 (δ_C 87.5, s) and a methylene at C-16 (δ_C 33.7, t) in **1**. In the HMBC spectrum of **1**, both the signals at δ_H 1.24 (CH_3-18) and 1.54 (CH_3-21) correlated with the oxygenated quaternary carbon (δ_C 87.5, C-17), which indicated that a hydroxyl group was attached to C-17. Pyridine is known to form weak hydrogen bonds and collision complexes with hydroxyl groups, and thereby produces shielding and deshielding cones and influences the chemical shift of the neighboring protons.[19] The shift differences observed are then useful to determine the position and orientation of hydroxyl groups.[19] In order to determine the orientation of OH-17, the ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY NMR spectra of compound **1** were run in both C_5D_5N and $CDCl_3$. Differences of the ^{1}H NMR chemical shifts ($\Delta=\delta_{CDCl_3}-\delta_{C_5D_5N}$) (Table 1 and Fig. 1) of the most diagnostic signals were found for H-22 ($\Delta=-0.63$), CH_3-18 ($\Delta=-0.41$), and CH_3-21 ($\Delta=-0.30$). These data suggested that OH-17 has an α-orientation in **1**. In this case, the bonds from C-17 to OH-17 and from C-22 to H-22 are nearly parallel, H-22 is 1,3-diaxial to OH-17, and the dihedral angle between OH-17 and CH_3-21 approximately 60° (Fig. 1). These relative configurations are consistent with the large shift of H-22 and the moderate shift of CH_3-21. On the other hand, CH_3-18 also showed a significant shift, due to OH-20 having a β-orientation and being very close to CH_3-18. A crystal was obtained from the solvent system $CHCl_3$–EtOAc–MeOH (5:20:1), and the X-ray diffraction analysis of compound **1** (Fig. 2) confirmed its structure and relative configuration. The absolute configuration of C-4 in **1** was determined using the Mosher ester procedure. Compound **1** was treated with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride in anhydrous pyridine at room temperature for 4 h, to afford the (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**, respectively). Negative values ($\Delta\delta_{S-R}$) were obtained for H-2 and H-3 (Table 1), indicating that the absolute

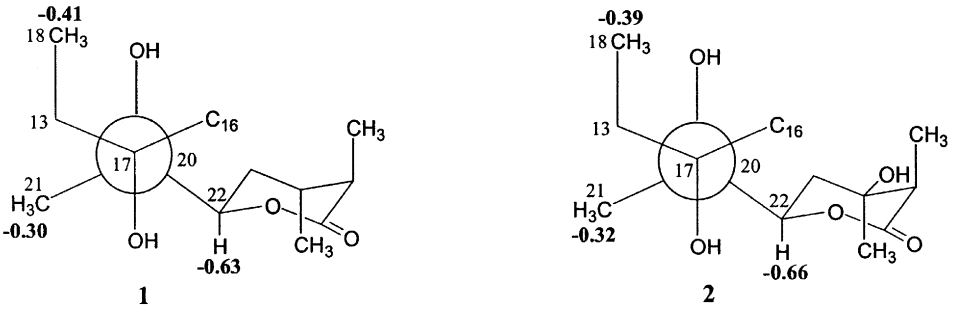

Figure 1. Newman projection formulas from C(17) to C(20) of compounds **1** and **2**.

configuration of C-4 is *S*. In this manner, philadelphicalactone A (**1**) was assigned as (17*R*,20*R*,22*R*,24*S*,25*R*)-4β,17α,20β-trihydroxy-5β,6β-epoxy-1-oxowitha-2-en-26, 22-olide.

Compound **2** was obtained as white amorphous powder, with mp 260–261°C and $[\alpha]_D^{20}=+74.3°$ (*c* 0.16, MeOH). The molecular formula of **2** was determined as $C_{28}H_{40}O_8$, one oxygen atom more than that obtained for **1**, based on its HRFABMS data (*m/z* 527.2596 $[M+Na]^+$). Both the ^1H and ^{13}C NMR spectral data of compound **2** were very close to those of **1**, but differences were evident between these substances in terms of the splitting pattern of CH_3-28 and the chemical shift of C-24. In the ^1H NMR spectrum of **2**, four methyl singlets and one methyl doublet signal were apparent. The ^{13}C NMR spectrum of **2** exhibited one more oxygenated quaternary carbon (δ_C 70.5, s, C-24) compared to **1**. In the HMBC spectrum of **2**, the signals at δ_H 5.13 (1H, dd, *J*=11.2, 3.8 Hz, H-22), 1.48 (3H, d, *J*=7.0 Hz, CH_3-27), and 1.50 (3H, s, CH_3-28) correlated to that at δ_C 70.5 (s, C-24). These 1D and 2D NMR data, in combination with the obtained HRFABMS result, suggested that a hydroxy group is attached to C-24 in compound **2**. A pyridine-induced shift ($\Delta=\delta_{CDCl3}-\delta_{C5D5N}$) experiment with compound **2** (Fig. 1 and Table 2) gave similar results to that performed on **1**, and indicated that OH-17 also has an α-orientation. The (*R*)- and (*S*)-MTPA ester derivatives (**2r** and **2s**) were also prepared, and established the absolute configuration of C-4 of compound **2** as *S*. Hence, philadelphicalactone B (**2**) was elucidated as (17*R*,20*R*,22*R*,24*R*,25*R*)-4β,17α,20β,24β-tetrahydroxy-5β,6β-epoxy-1-oxowitha-2-en-26,22-olide.

A molecular formula of $C_{28}H_{38}O_8$ was determined for **3** from its HRFABMS. The ^1H and ^{13}C NMR spectral data of **3** were closely comparable to those of the known withanolide, ixocarpalactone A (**4**),[6,7] which was also obtained in this investigation. However, when compared with **4**, the ^{13}C NMR spectrum of **3** displayed a downfield oxygenated quaternary carbon signal at δ_C 108.9 (s, C-23). In the HMBC spectrum of **3**, the proton signals of CH_3-28, H-25, H-22 and H-16 (Table 3) correlated with this oxygenated quaternary carbon signal. These correlations, in combination with the NMR data of H-16 (δ_H 4.68, 1H, m), C-16 (δ_C 75.6, d), and C-23 (δ_C 108.9, s), suggested that the downfield oxygenated quaternary carbon signal (δ_C 108.9) must be located at the C-23 position with an oxygen ether functional group present between C-16 and C-23. Thus, compound **3** contains an unusual spiro-acetal unit. A literature survey revealed this is the first isolation of compound **3**, although the 4-monoacetate of ixocarpalactone B (**3a**) was reported from *P. philadelphica* (formerly *P. ixocarpa*) in 1979.[6] In order to confirm the structure of **3**, **3a** was prepared from **3** by acetylation. The obtained NMR data of **3a** matched those of the reported values exactly.[6] However, the optical rotation values for **3** $\{[\alpha]_D^{20}=-19.6°$ (*c* 0.25, $CHCl_3$)$\}$ and **3a** $\{[\alpha]_D^{20}=+56.4°$ (*c* 0.23, $CHCl_3$)$\}$

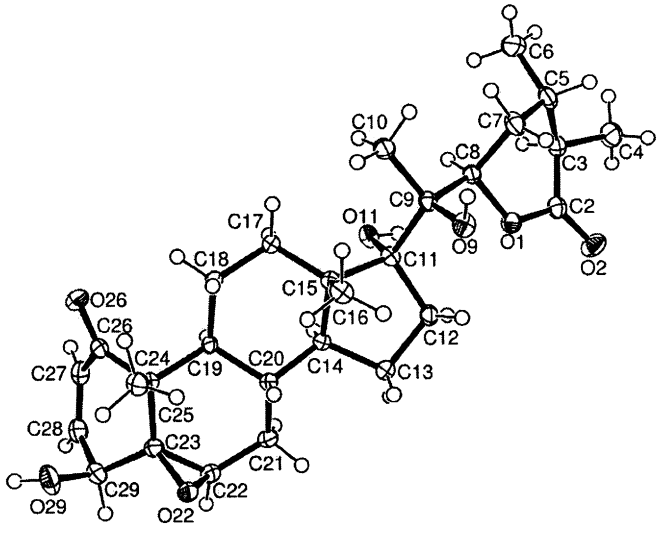

Figure 2. X-Ray structure of philadelphicalactone A (**1**). (Numbering does not follow that of Chemical Abstracts).

Table 2. ^{1}H NMR data for compounds **2**, **2s**, and **2r**

Position	**2** (C_5D_5N)	**2** ($CDCl_3$)[a]	$\delta_{CDCl_3}-\delta_{C_5D_5N}$	**2s** (C_5D_5N)	**2r** (C_5D_5N)	$\delta_s-\delta_r$
2	6.42, d (9.8)	6.22, d (9.8)	−0.20	6.427, d (9.8)	6.516, d (9.8)	−0.089
3	7.21[b]	6.99, dd (9.8, 6.1)	−0.22	7.177[b]	7.235[b]	−0.258
4	4.01, d (6.0)	3.70, d (6.1)	−0.31	5.358, d (6.1)	5.391, d (6.1)	−0.033
6	3.22, br s	3.20, br s	−0.02			
7	2.11–2.23, m; 1.31, m	2.16–2.19, m; 1.52, m				
8	1.71–1.80, m	1.54, m				
9	1.07, ddd (10.8, 10.8, 4.5)	0.93, ddd (11.2, 11.2, 4.4)	–0.14	1.051, ddd (11.0, 11.0, 5.6)	1.030, ddd (11.0, 11.0, 5.6)	−0.021
11	2.11–2.23, m; 1.71–1.80, m	1.75–1.80, m; 1.49, m				
12	2.11–2.23, m; 1.89–1.96, m	2.16–2.19, m				
14	1.96–2.05, m	1.75–1.80, m				
15	1.71–1.80, m; 1.20, m	1.75–1.80, m; 1.13–1.28, m				
16	3.08, br t (12.6); 2.11–2.23, m	2.19–2.34, m				
18	1.24, s	0.85, s	–0.39	1.190, s	1.168, s	+0.022
19	1.88, s	1.41, s	−0.47	1.482, s	1.400, s	+0.082
21	1.56, s	1.24, s	−0.32	1.546, s	1.542, s	+0.004
22	5.13, dd (11.2, 3.8)	4.47, dd (10.7, 5.3)	−0.66	5.138, dd (11.4, 3.8)	5.134, dd (11.4, 3.9)	+0.004
23	2.69–2.77, m; 2.49, dd (14.1, 3.8)	2.29–2.34, m; 2.04, dd (14.5, 5.2)				
25	2.69–2.77, m	2.50, q (6.9)				
27	1.48, d (7.0)	1.25, d (6.9)	−0.23	1.466, d (7.0)	1.463, d (7.0)	+0.003
28	1.50, s	1.36, s	−0.14	1.492, s	1.488, s	+0.004

Spectra taken at 300 MHz for **2** (C_5D_5N) **2s**, and **2r** and 500 MHz for **2** ($CDCl_3$); chemical shift values presented in ppm; *J* values in parentheses given in Hz.

[a] Two drops of CD_3OD were added to improve solubility.

[b] Signals overlapped with solvent signals, and the chemical shifts of these signals were obtained from ^{1}H–^{1}H COSY spectra.

Table 3. ^{1}H NMR data for compounds **3**, **3s**, and **3r**

Position	**3** (C_5D_5N)	**3** ($CDCl_3$)	$\delta_{CDCl_3}-\delta_{C_5D_5N}$	**3s** (C_5D_5N)	**3r** (C_5D_5N)	$\delta_s-\delta_r$
2	6.42, d (9.8)	6.21, d (9.9)	−0.21	6.432, d (9.8)	6.517, d (9.8)	−0.085
3	7.20[a]	7.00, dd (9.9, 6.1)	−0.20	7.176[a]	7.231[a]	−0.055
4	4.05, d (6.1)	3.75, d (6.1)	−0.30	5.407, d (6.1)	5.444, d (6.1)	−0.037
6	3.31, br s	3.18, br s	−0.13	3.656, br s	3.564, br s	+0.092
7	2.18–2.24, m 1.23–1.40	2.12, m; 1.26, m				
8	1.74, m	1.63, m	−0.11			
9	1.10, m	0.92, m	−0.18			
11	1.94, m	1.72, m; 1.24, m				
12	2.64, br d (12.4); 1.23–1.40	2.21, m; 1.08–1.18, m				
14	0.89, m	0.85, m	–0.04	0.887, m	0.867, m	+0.020
15	2.18–2.24, m; 1.23–1.40	2.16, m; 1.34, m				
16	4.68, m	4.50, m	−0.18	4.682, m	4.679, m	+0.003
17	1.66, d (5.4)	1.46–1.51				
18	1.27, s	1.08, s	−0.19	1.230, s	1.219, s	+0.011
19	1.87, s	1.46, s	−0.41	1.649, s	1.568, s	+0.081
21	1.91, s	1.51, s	−0.40	1.927, s	1.921, s	+0.006
22	4.59, s	4.20, s	−0.39	4.606, s	4.601, s	+0.005
24	2.18–2.24, m	2.00, m				
25	3.07, m	2.68, m	−0.39			
27	1.24, d (7.1)	1.17, d (7.0)	−0.07	1.239, d (7.1)	1.235, d (7.1)	+0.004
28	1.34, d (6.9)	1.20, d (6.9)	−0.14	1.346, d (7.0)	1.342, d (7.0)	+0.004

Spectra taken at 300 MHz; chemical shift values presented in ppm; *J* values in parentheses given in Hz.
[a] Signals overlapped with solvent signals, and the chemical shifts of these signals were obtained from ^{1}H–^{1}H COSY spectra.

were of opposite sign. The absolute configuration of C-4 of **3** was established as *S* by means of Mosher ester methodology. Thus, ixocarpalactone B (**3**) was elucidated as (17*R*,20*R*,22*S*,23*S*,24*R*,25*R*)-4β,20β,22α-trihydroxy-5β,6β; 16β,23-diepoxy-1-oxowitha-2-en-26,23-olide.

In addition to withanolides **1**–**3**, four known withanolides, ixocarpalactone A (**4**),[6,7] withaphysacarpin (**5**),[8] 18-hydroxywithanolide D (**6**),[20] and withanone (**7**),[21,22] were isolated in the present investigation. The structures of these known compounds were characterized by spectroscopic data comparison with published values. Ixocarpalactone A (**4**) was the major withanolide (0.00363% w/w) of the leaves and stems of *P. philadelphica*. Its ^{1}H (Table 4) and ^{13}C NMR (Table 5) data were assigned based on the observed correlations from its 2D NMR spectra, and its structure was confirmed by X-ray diffraction analysis (Fig. 3). The absolute stereochemistry of C-4 of ixocarpalactone

Table 4. ^{1}H NMR data for compounds **3a** and **4**

Position	**3a** (C_5D_5N)	**3a** ($CDCl_3$)	**4** (C_5D_5N)	**4** ($CDCl_3$)[a]
2	6.44, d (9.8)	6.26, d (9.8)	6.46, d (9.8)	6.21, d (9.8)
3	7.21[b]	7.05, dd (9.8, 6.1)	7.22[b]	7.01, dd (9.8, 6.1)
4	5.07, d (5.8)	4.66, d (6.1)	4.05, d (6.2)	3.69, d (6.1)
6	3.43, br s	3.23, br s	3.29, br s	3.19, br s
7	2.13, m; 1.23–1.40[c]	2.14, m; 1.31, m	2.16, m; 1.34, m	2.12–2.17, m; 1.31, m
8	1.84–1.91, m	1.56–1.60, m	1.80, m	1.62, m
9	1.08, m	0.91, m	1.03–1.11, m	0.89, ddd (12.9, 12.9, 5.6)
11	1.87, m	1.50–1.60, m	2.00, m; 1.67, m	1.70, m; 1.54, m
12	2.66, br d (12.6) 1.23–1.40[c]	2.09, m; 1.12, m	2.21, m; 1.03–1.11, m	2.12–2.17, m; 1.14, m
14	0.87, m	0.87, m	0.85, m	0.79, m
15	2.20, m; 1.23–1.40[c]	2.19, m; 1.35, m	2.29–2.43, m; 1.59, m	2.22–2.29, m; 1.43, m
16	4.68, m	4.50, m	4.77, m	4.45, m
17	1.65, d (5.8)	1.49[d]	1.58, d (5.1)	1.36, d (4.5)
18	1.27, s	1.07, s	1.45, s	1.14, s
19	1.74, s	1.41, s	1.87, s	1.43, s
21	1.93, s	1.49, s	1.73, s	1.31, s
22	4.62, s	4.16, s	4.55, br s	4.16, s
23			4.85, d (8.3)	4.04, br s
24	2.24, m	2.03, m	2.29–2.43, m	2.22–2.29, m
25	3.10, m	2.63, m	3.02, m	2.68, m
27	1.25, d (7.1)	1.19, d (7.3)	1.20, d (6.7)	1.17, d (7.1)
28	1.35, d (6.7)	1.22, d (7.5)	1.20, d (6.7)	1.24, d (6.9)
OAc	1.93, s	2.06, s		

Spectra taken at 300 MHz; chemical shift values presented in ppm; *J* values in parentheses given in Hz.
[a] Two drops of CD_3OD were added to improve solubility.
[b] Signals overlapped with solvent signals, and the chemical shifts of these signals were obtained ^{1}H–^{1}H COSY spectra.
[c] Overlapped with CH_3-18, CH_3-27 and CH_3-28 signals.
[d] Overlapped with the signal of CH_3-21.

Table 5. ^{13}C NMR spectra data for compounds **1**–**4**, and **3a**

Position	**1**[a]	**1**[b]	**2**[a]	**2**[b]	**3**[a]	**3**[c]	**4**[a]	**4**[b]	**3a**[a]	**3a**[c]
1	202.6 s	201.8 s	202.6 s	202.6 s	202.4 s	202.3 s	202.5 s	202.9 s	202.8 s	201.1 s
2	132.4 d	132.6 d	132.5 d	132.6 d	132.3 d	132.4 d	132.4 d	132.6 d	135.9 d	134.0 d
3	144.9 d	141.7 d	144.9 d	142.9 d	145.0 d	143.0 d	145.1 d	143.3 d	142.0 d	139.8 d
4	70.4 d	70.0 d	70.4 d	70.0 d	70.3 d	69.9 d	70.4 d	69.9 d	74.4 d	72.2 d
5	64.5 s	63.9 s	64.5 s	64.0 s	64.6 s	64.1 s	64.5 s	64.1 s	63.3 s	61.1 s
6	60.1 d	62.4 d	60.1 d	61.3 d	59.9 d	61.0 d	60.1 d	60.9 d	62.0 d	60.1 d
7	31.9 t	31.0 t	32.0 t	31.2 t	31.7 t	31.2 t	31.7 t	31.3 t	32.9 t	31.0 t
8	30.0 d	29.5 d	30.1 d	29.6 d	29.6 d	29.1 d	29.5 d	29.1 d	30.9 d	28.9 d
9	44.5 d	43.4 d	44.5 d	43.6 d	44.8 d	44.4 d	44.7 d	44.3 d	46.2 d	44.3 d
10	48.6 s	47.6 s	48.6 s	47.9 s	48.6 s	48.0 s	48.6 s	48.1 s	50.4 s	48.3 s
11	21.6 t	21.6 t	21.6 t	21.2 t	21.2 t	21.0 t	21.5 t	21.2 t	22.8 t	20.6 t
12	32.7 t	32.0 t	32.8 t	32.0 t	40.2 t	39.6 t	40.3 t	40.0 t	41.8 t	39.5 t
13	49.0 s	47.0 s	49.0 s	47.3 s	43.2 s	42.8 s	43.7 s	43.3 s	44.9 s	42.8 s
14	51.2 d	50.3 d	51.2 d	50.5 d	54.4 d	54.4 d	54.7 d	54.5 d	56.0 d	54.4 d
15	24.2 t	22.9 t	24.2 t	23.1 t	34.2 t	33.7 t	38.1 t	37.1 t	35.8 t	33.6 t
16	33.7 t	32.0 t	33.8 t	32.3 t	75.6 d	75.2 d	72.7 d	72.7 d	77.2 d	75.1 d
17	87.5 s	90.3 s	87.6 s	89.6 s	63.4 d	62.5 d	58.1 d	57.5 d	65.0 d	62.4 d
18	15.5 q	16.1 q	15.6 q	15.9 q	15.1 q	14.9 q	15.0 q	14.5 q	16.8 q	14.9 q
19	17.1 q	17.2 q	17.1 q	16.6 q	17.1 q	16.9 q	17.2 q	16.7 q	17.8 q	15.6 q
20	78.1 s	75.7 s	78.1 s	76.1 s	74.2 s	74.5 s	79.6 s	79.4 s	75.9 s	75.1 s
21	20.7 q	22.4 q	20.6 q	21.7 q	27.1 q	26.8 q	22.8 q	21.9 q	28.9 q	27.0 q
22	80.0 d	80.9 d	79.7 d	80.1 d	72.0 d	71.7 d	73.9 d	73.0 d	73.7 d	71.8 d
23	31.7 t	30.1 t	41.2 t	38.5 t	108.9 s	108.0 s	79.2 d	79.0 d	110.6 s	107.6 s
24	31.6 d	30.8 d	70.5 s	69.9 s	50.1 d	49.4 d	42.4 d	42.0 d	51.9 d	49.3 d
25	40.4 d	40.4 d	45.3 d	45.1 d	43.2 d	42.5 d	40.7 d	40.3 d	44.9 d	42.4 d
26	175.9 s	174.3 s	175.0 s	174.6 s	177.8 s	177.9 s	180.5 s	181.7 s	179.4 s	177.6 s
27	14.3 q	14.1 q	9.4 q	8.6 q	14.4 q	14.0 q	14.5 q	14.1 q	16.2 q	13.9 q
28	20.9 q	21.0 q	29.2 q	28.3 q	12.2 q	12.1 q	13.2 q	12.9 q	14.0 q	12.2 q
OAc									171.6 s	170.1 s
									22.2 q	20.8 q

TMS was used as internal standard; the data of compound **2** in $CDCl_3$ were obtained at 125 MHz, while other spectra were taken at 75 MHz; all data were assigned based on their ^1H–^1H COSY, HMQC and HMBC spectra.

[a] C_5D_5N was used as solvent.

[b] $CDCl_3$ was used as solvent, and a few drops of CD_3OD were added to improve the solubility.

[c] $CDCl_3$ was used as solvent.

A (**4**) was also determined for the first time as *S*, by means of Mosher ester methodology.

Compound **8** was isolated as white amorphous powder, mp 140–142°C, $[\alpha]_D^{20}=+7.8°$ (*c* 0.30, pyridine). A molecular formula of $C_{42}H_{83}NO_5$ was determined for compound **8** from its HRFABMS data (*m/z* 704.6181 $[M+Na]^+$). The ^1H NMR spectrum of **8** displayed a downfield doublet at δ_H 8.56 (1H, d, *J*=8.7 Hz, NH), six olefinic, oxygenated or other heteroatomized protons between δ_H 4.26–5.52, as well as the signals of six methyl protons at δ_H 0.87 (6H, br t, *J*=7.0 Hz, H-18 and H-24′) and a very strong aliphatic methylene band at δ_H 1.26–1.32. The ^{13}C NMR and DEPT spectral data of **8** were supportive of the above analysis, showing a carbonyl group at δ_C 175.2 (C-1′, s), one double bond at δ_C 131.0 (C-9 or C-10, d) and 130.8 (C-9 or C-10, d), five oxygenated or other heteroatomized carbons at δ_C 77.0 (C-3, d), 73.0 (C-4, d), 72.6 (C-2′, d), 62.1 (C-1, t), and 53.1 (C-2, d), aliphatic methylenes between δ_C 23.1–35.8, and two methyls at δ_C 14.4 (C-18 and C-24′, q). The downfield doublet at δ_H 8.56 (NH) was deuterium-exchangeable, and there was no correlation from this signal to any carbon in the HMQC spectrum of **8**. On the other hand, a correlation from δ_H 8.56 (NH) to δ_H 5.08 (1H, m, H-2), and the correlations from δ_H 8.56 (NH) to δ_C 175.2 (C-1′, s), 72.6 (C-2′, d), 62.1 (C-1, t), 53.1 (C-2, d), and 77.0 (C-3, d) were observed in the ^1H–^1H COSY and HMBC spectra of **8**, respectively. These 1D NMR data and 2D NMR correlations established the presence of an amide function in the molecule of **8**, and suggested this compound is a ceramide.[23,24] The chemical shifts of the allylic methylene carbons in **8** were assigned at δ_C 33.5 (C-8 or C-11) and 33.1 (C-8 or C-11) based on the clearly observed HMBC correlations from the olefinic signals at δ_H 5.52 (2H, m, H-9 and H-10) to these two carbon signals. Since the chemical shifts of allylic methylene carbons are different when alkene double bonds are *cis*-oriented ($\delta_C<27$ ppm) compared with when *trans*-oriented ($\delta_C>30$ ppm),[25,26] the double bond in **8** was assigned in the *E* configuration.

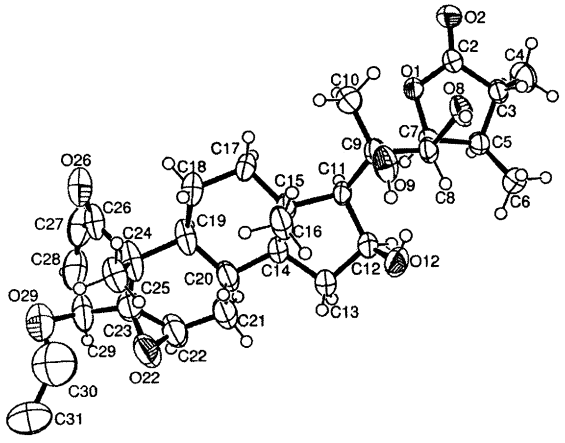

Figure 3. X-Ray structure of ixocarpalactone A (**4**). (Numbering does not follow that of Chemical Abstracts).

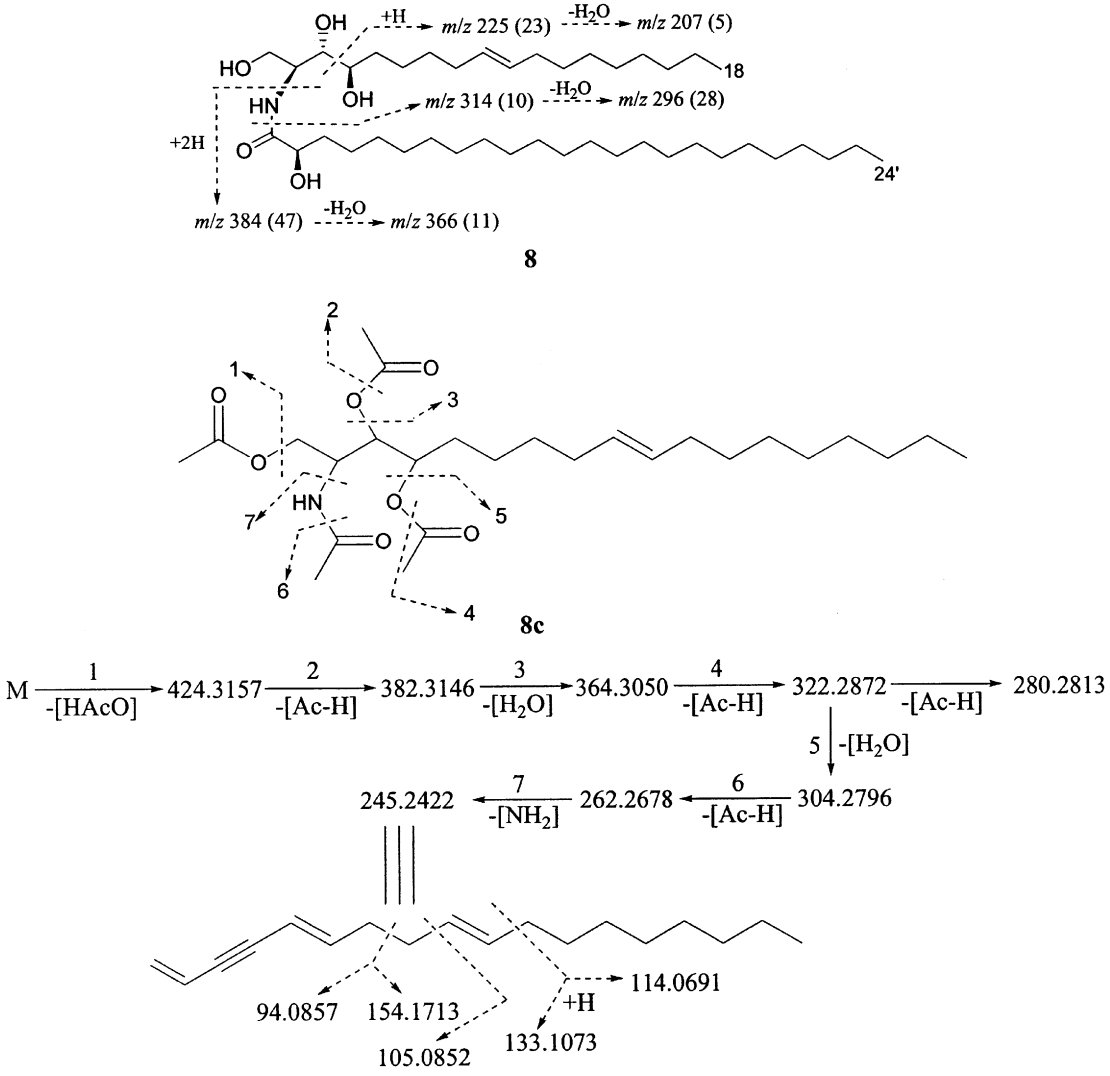

Figure 4. EIMS fragmentation pattern of **8** and MS–MS fragmentation (*m/z* values) of **8c**.

In order to determine the lengths of the sphingosine and fatty acid chains, the position of the double bond, and the absolute configuration of **8**, the acid methanolysis method of Gaver and Sweeley[27] was used. A sphingosine (**8a**) and a fatty acid methyl ester (**8b**) were obtained by methanolysis of compound **8**, with **8a** and **8b** then acetylated in the usual manner, to afford **8c** and **8d**, respectively. The ^{1}H and ^{13}C NMR data of **8a** and **8c** (Section 3) indicated that the double bond was located in the sphingosine unit in **8**. The HRFABMS of **8c** and **8d** gave protonated molecular ion peaks at *m/z* 484.3246 and 441.3935, corresponding to the molecular formulas of $C_{26}H_{45}NO_7$ and $C_{27}H_{52}O_4$, respectively. These molecular formulas verified that the lengths of the sphingosine and fatty acid units of compound **8** are 18 and 24 carbons, respectively, and were supported from the results obtained by analysis of the major fragment ion peaks of EIMS of **8** (Fig. 4). In order to determine the position of the double bond, MS–MS determinations of **8c** were conducted under different collision energies (20, 25 and 30 eV), and the results indicated the double bond to be between C-9 and C-10 (Fig. 4). By comparison with the literature values of synthetic sphingamines,[28,29] the obtained optical rotation value of **8c** $\{[\alpha]_D^{20}=+22.5°$ (*c* 0.25, $CHCl_3)\}$ indicated an absolute configuration of 2*S*, 3*S*, and 4*R* for compound **8**. In the same manner, the optical rotation value of **8d** $\{[\alpha]_D^{20}=+12.4°$ (*c* 0.30, $CHCl_3)\}$ suggested the absolute configuration of C-2 of the fatty acid to be *R*.[29] Thus, the structure of compound **8** was assigned as (2*S*,3*S*,4*R*,9*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-9-octadecene.

The ^{1}H and ^{13}C NMR data of compounds **9** and **10** were very similar to those of compound **8**, which disclosed that they were also ceramides. The structures of these two compounds were determined as (2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-1,3,4-octadecanetriol (**9**) and (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1,3,4-octadecanetriol (**10**) based on their spectral and physical data (1D and 2D NMR, HRFABMS, EIMS, and $[\alpha]_D$), and confirmed by comparison with literature values.[30–33] The structure of compound **9** was confirmed by X-ray diffraction analysis in 1972.[34] Ceramides have been isolated previously from sponges,[35–37] sea stars,[38–40] gorgonians,[41] green algae,[42] and dinoflagellates.[43] To the best of our knowledge, this is the first isolation of ceramides from a plant in the genus *Physalis* or the family Solanaceae.

Table 6. Quinone reductase (QR)-inducing activity and inhibition of transformation of murine epidermal JB6 cells by compounds **1–11**

Compound	QR			JB6		
	CD (μM)[a]	IC_{50} (μM)[b]	CI[c]	[IC_{50} (μM)][d]	[ED_{50} (μM)][e]	CI[c]
1	0.04	0.47	11.8	0.14	0.61	4.3
2	0.28	0.63	2.3	2.62	0.58	0.2
3	0.21	1.42	6.8	0.19	0.60	3.2
3a	0.09	0.39	4.2	0.13	0.37	2.8
4	0.32	7.54	24	0.26	1.79	6.9
5	0.03	0.08	3.1	0.04	0.21	5.0
6	0.06	0.33	5.3	0.64	0.62	1.0
7	>21.2	>42.4	ND	ND	ND	ND
8	1.76	3.96	2.3	0.57	>5.87	10.3
9	2.45	>7.32	>3.0	>5.86	1.61	<0.27
11	>16.3	>32.6	ND	0.49	8.96	18.3
4′-Bromoflavone[f]	0.013	>166	>17000			
13-*cis*-Retinoic acid[f]				0.02	>10	>500

[a] Concentration required to double QR activity (μM).
[b] Concentration required to inhibit cell growth by 50% (μM).
[c] Chemopreventive index (CI)=IC_{50}/CD (QR) or ED_{50}/IC_{50} (JB6).
[d] Concentration required to inhibit colony formation by 50% (μM).
[e] Cytotoxic concentration required to inhibit tumor growth by 50% in a 3-day incubation using trypan blue staining (μM).
[f] 4′-Bromoflavone[53] and 13-*cis*-retinoic acid[54] were used as positive control substances.

Chlorophyllide *a* (**11**) was isolated from a relatively less polar fraction, and identified based on interpretation of its spectroscopic data. Part of the ^{1}H NMR data of chlorophyllide *a*[44] and the ^{13}C NMR data of methyl chlorophyllide *a*[45] have been reported, and the structure of ethyl chlorophyllide *a* was established by X-ray single crystal diffraction analysis.[46] The full ^{1}H and ^{13}C NMR data of chlorophyllide *a*, which were assigned by the observed correlations of its 2D NMR spectra, are given in the present paper (Section 3).

The potential of all isolates (except for **10**, since its solubility was very limited in DMSO) to induce quinone reductase,[11–13] and to inhibit the transformation of murine epidermal JB6 cells,[14–16] are summarized in Table 6. All withanolides (**1–6**, **3a**) except compound **7** exhibited significant quinone reductase-inducing activity, with CD values in the range of 0.03–0.32 μM (Table 6). These same seven withanolides also exhibited significant inhibition of murine epidermal JB6 cell transformation. The ceramide **8** also was found to be active in both assays, while compound **9** only exhibited significant quinone reductase-inducing activity. The known porphyrin derivative, chlorophyllide *a* (**11**), which was obtained in the present investigation, was found to be inactive (CD>10 μM) in the quinone reductase assay, but it was significantly active as an inhibitor of JB6 cell transformation. Chlorophyllin, a related compound, has been subjected to clinical trial as a cancer chemopreventive agent in the People's Republic of China.[47]

Among the withanolides, the greatest activity was demonstrated by compounds **1** and **4**. It appears that the presence of 4β-hydroxy-2-en-1-one and 5β,6β-epoxy units of withanolides are necessary for activity in the quinone reductase induction and JB6 cell transformation assays. Ixocarpalactone A (**4**) was not only the major withanolide (0.00363% w/w) of the leaves and stems of *P. philadelphica*, but also exhibited significant quinone reductase-inducing activity and inhibition of murine epidermal JB6 cell transformation, with chemopreventive indices of 24 and 6.9, respectively. Accordingly, this compound has been selected for further in vivo biological testing in our program of research on naturally occurring cancer chemopreventive agents.

3. Experimental

3.1. General procedures

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectral data were recorded at room temperature on Bruker Avance DPX-300 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. FABMS and HRFABMS were obtained on a VG 7070E-HF sector-field mass spectrometer, EIMS, CIMS, and HRCIMS on a Finnigan/MAT 90/95 sector-field mass spectrometer, and HRQTOFMS and MS–MS were obtained on a Micromass QTOF II mass spectrometer. X-Ray crystallographic analysis data collection for compounds **1** and **4** were carried out on an Enraf-Nonius Kappa CCD area detector with a rotating anode Mo X-ray tube. The SIR-92 direct methods package[48] was used to locate the non-hydrogen atoms, and the WinGX package[49] was used for completing the structure determination, with PLUTON[50] and ORTEP[51] used for the figures. Column chromatography was carried out with Si gel G (Merck, 230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thick Merck Si gel 60 F_{254} aluminum plates, while preparative thin-layer chromatography was performed on precoated 1000 μm thick Merck Si gel 60 F_{254} glass plates.

3.2. Evaluation of quinone reductase-inducing activity of isolates

As described previously.[11,13]

3.3. Evaluation of transformation of murine epidermal JB6 cells

As described previously.[15,16]

3.4. Plant material

The seed of *P. philadelphica* was purchased from Johnny's Selected Seed, Albion, ME, USA, and a voucher specimen (number 144) has been deposited at the University of Illinois Pharmacognosy Field Station. The leaves and stems (6 kg) of *P. philadelphica* were harvested in the year 2000 before fruiting, at the University of Illinois Pharmacognosy Field Station, Downers Grove, IL, USA. Harvesting was performed about every two weeks starting in the end of July and ending in the middle of September. A voucher specimen (PB3070) of the leaves and stems were deposited at the University of Illinois Pharmacognosy Field Station.

3.5. Extraction and isolation

The dried and milled leaves and stems (6 kg) were extracted by maceration with MeOH–H_2O (9:1) three times (3×15 L) at room temperature, for three days each. After filtration and evaporation of the solvent under reduced presure, the combined crude methanolic extract was suspended in H_2O (1200 mL) to afford an aqueous MeOH solution (~85%), then partitioned in turn with petroleum ether (3×800 mL) and EtOAc (3×1000 mL), to afford dried petroleum ether- (72.5 g), EtOAc- (137.6 g), and H_2O-soluble (192.5 g) residues.

The EtOAc-soluble extract was chromatographed over a Si gel column (9.5×85 cm, 2 kg 70–230 mesh Si gel), and eluted with pure $CHCl_3$ initially, then with gradient mixtures of $CHCl_3$–MeOH (from 50:1 to 4:1), to afford ten fractions (F01–F10).

Fraction F04, eluted with $CHCl_3$–MeOH (50:1), was subjected to Si gel column (3.2×60 cm) chromatography, using the isocratic solvent system $CHCl_3$–MeOH (from 100:1 to 30:1), to give the semi-pure compound **11** (eluted with $CHCl_3$–MeOH, 40:1). This was finally purified by preparative TLC (Merck 60 Å Si gel, 20×20 cm, 1000 μm), developed with petroleum ether–acetone (3:1; R_f=0.70), to afford compound **11** (35 mg).

Fraction F05, eluted with $CHCl_3$–MeOH (30:1), was chromatographed over a Si gel column (3.8×70 cm) and eluted with petroleum ether–acetone mixtures of increasing polarity (from 3:1 to 1:1), to give eight subfractions (F0501–F0508). Compound **10** (28 mg) was obtained as an amorphous white solid from the $CHCl_3$–MeOH (~10:1) mother liquor of F0501 (eluted with petroleum ether–acetone, 3:1). F0503, eluted with petroleum ether–acetone (3:1), was chromatographed over a Si gel column (2.8×55 cm), and eluted with gradient mixtures of petroleum ether–EtOAc–MeOH (from 10:10:1 to 10:10:3), to afford pure compounds **1** (43 mg; eluted with petroleum ether–EtOAc–MeOH, 10:10:1), **5** (84 mg; eluted with petroleum ether–EtOAc–MeOH, 10:10:1), and **7** (8 mg; eluted with petroleum ether–EtOAc–MeOH, 10:10:2), as well as a mixture (eluted with petroleum ether–EtOAc–MeOH, 10:10:3). The mixture was then purified further over a Sephadex LH-20 column (2.8×55 cm) and eluted with MeOH, to give pure compound **3** (37 mg). Four amorphous mixtures were obtained from subfractions F0504 (eluted with petroleum ether–acetone, 3:1), F0505 (eluted with petroleum ether–acetone, 3:1), F0506 (eluted with petroleum ether–acetone, 3:2), and F0507 (eluted with petroleum ether–acetone, 3:2). These amorphous powders were combined and further purified over a Si gel column (2.8×55 cm), and eluted with gradient mixtures of $CHCl_3$–MeOH (from 30:1 to 10:1), to give pure compounds **1** (12 mg; eluted with $CHCl_3$–MeOH, 25:1), **5** (112 mg; eluted with $CHCl_3$–MeOH, 20:1), and **6** (5 mg; eluted with $CHCl_3$–MeOH, 15:1).

Fractions F06 (eluted with $CHCl_3$–MeOH, 20:1), F07 (eluted with $CHCl_3$–MeOH, 16:1) and F08 (eluted with $CHCl_3$–MeOH, 12:1) were combined and chromatographed over a Si gel column (3.5×65 cm), eluted with a petroleum ether–acetone gradient (from 3:1 to 1:1, then pure acetone), to give seven subfractions (F0601–F0607). Compounds **9** (8 mg) and **8** (72 mg) were afforded as white amorphous solids from the subfractions F0601 (eluted with petroleum ether–acetone, 3:1) and F0604 (eluted with petroleum ether–acetone, 2:1), respectively. F0602 (eluted with petroleum ether–acetone, 3:1) was further purified over a Sephadex LH-20 column (2.8×55 cm), eluted with MeOH, and afforded pure compound **2** (36 mg). F0605 (eluted with petroleum ether–acetone, 3:2) and F0606 (eluted with petroleum ether–acetone, 1:1) were combined and subjected to passage over a Si gel column (3.2×60 cm), developed with $CHCl_3$–MeOH (from 30:1 to 6:1) as solvent system, to afford, in turn, compounds **2** (13 mg; eluted with $CHCl_3$–MeOH, 15:1) and **4** (218 mg; eluted with $CHCl_3$–MeOH, 10:1).

3.5.1. Philadelphicalactone A (1). Colorless needles; mp 274–275°C; $[\alpha]_D^{20}=-6.2°$ (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.92), 336 (2.34) nm; IR ν_{max} (film) 3450, 1713, 1667, 1456, 1373, 1177, 1082, 920 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 5, respectively; EIMS *m/z* 488 $[M]^+$ (2), 470 $[M-H_2O]^+$ (6), 452 (3), 343 (20), 283 (22), 281 (18), 238 (16), 225 (16), 215 (14), 172 (97), 161 (14), 154 (96), 136 (36), 121 (74), 111 (65), 98 (91), 91 (38), 71 (95), 43 (100); HRFABMS *m/z* 511.2630 $[M+Na]^+$ (calcd for $C_{28}H_{40}O_7Na$, 511.2672).

3.5.2. X-Ray crystallography of philadelphicalactone A (1). A colorless crystal was obtained from $CHCl_3$–EtOAc–MeOH (5:20:1). Crystal size: 0.20×0.20×0.20 mm^3. Cell parameters: a=7.4527 (2) Å, α=90°; b=9.9782 (3) Å, β=100.2660 (10)°; c=16.4134 (16) Å, γ=90°; V=1201.03 (12) $Å^3$, space group $P2_1$, Z=2, D_{calc}=1.351 g/cm^3, λ=0.71073 Å, μ(Mo Kα)=0.096 mm^{-1}, F(000)=528, T=150 (1) K. Data collection yielded 9444 reflections resulting in 4044 unique, averaged reflections with $I>2\sigma_I$. Full-matrix least-squares refinement led to a final R, R (all),

and GOF values of 0.0408, 0.0562, and 1.002. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 171421. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.5.3. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of 1. Two portions (each 1.2 mg) of compound **1** were treated with (*S*)-(+)α- and (*R*)-(−)α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (5 μL) in anhydrous pyridine (0.5 mL) at room temperature for 4 h, and afforded the (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**) of **1**, respectively. ^{1}H NMR data of **1s** and **1r**, see Table 1.

3.5.4. Philadelphicalactone B (2). White amorphous powder; mp 260–261°C; $[\alpha]_D^{20}$=+74.3° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.89), 330 (2.23) nm; IR ν_{max} (film) 3250, 1704, 1667, 1457, 1376, 1176, 921 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 5, respectively; EIMS *m/z* 504 $[M]^+$ (2), 486 $[M-H_2O]^+$ (4), 343 (23), 299 (33), 283 (26), 281 (36), 255 (16), 225 (20), 197 (16), 188 (22), 187 (23), 175 (25), 169 (28), 159 (25), 133 (27), 124 (83), 117 (99), 105 (37), 99 (44), 72 (80), 43 (100); HRFABMS *m/z* 527.2596 $[M+Na]^+$ (calcd for $C_{28}H_{40}O_8Na$, 527.2621).

3.5.5. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of 2. The (*R*)- and (*S*)-MTPA ester derivatives (**2r** and **2s**) of **2** were obtained by the same method as described for compound **1**. ^{1}H NMR spectral data of **2s** and **2r**, see Table 2.

3.5.6. Ixocarpalactone B (3). White amorphous powder; mp 145–148°C; $[\alpha]_D^{20}$=−19.6° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215 (3.90), 326 (2.21) nm; IR ν_{max} (film) 3570, 1762, 1676, 1457, 1376, 1222, 1116, 1022, 951, 853, 757 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 5, respectively; EIMS *m/z* 502 $[M]^+$ (1), 485 $[M-OH]^+$ (2), 484 $[M-H_2O]^+$ (1), 361 (100), 356 (43), 341 (43), 283 (17), 189 (14), 175 (14), 159 (21), 147 (14), 142 (26), 124 (56), 105 (20), 95 (15), 91 (22), 74 (54); *m/z* HRFABMS 525.2456 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

3.5.7. Acetylation of 3. Compound **3** (10.5 mg) was acetylated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. The product was purified by Si gel column chromatography (2.0×20 cm), eluting with $CHCl_3$–MeOH (30:1), affording the 4-monoacetate of **3** (**3a**, 9 mg). Amorphous powder, mp 168–171°C (lit.[6] 170–172°C); $[\alpha]_D^{20}$=+56.4° (*c* 0.23, $CHCl_3$) {lit.[6] $[\alpha]_D^{25}$=+47.2° (solvent and concentration were not reported)}; UV (MeOH) λ_{max} (log ε) 214.5 (3.78), 331.5 (2.23) nm; IR ν_{max} (film) 3506, 2977, 1750, 1746, 1682, 1376, 1226, 1117, 1021, 952, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 4 and 5, respectively; EIMS *m/z* 544 $[M]^+$ (45), 526 $[M-H_2O]^+$ (12), 502 $[M-Ac]^+$ (8), 484 $[M-H_2O-Ac]^+$ (37), 361 (100), 283 (13), 184 (23), 105 (27), 95 (10), 43 (38).

3.5.8. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of 3. The (*R*)- and (*S*)-MTPA ester derivatives (**3r** and **3s**) of **3** were prepared by the same method as described for compound **1**. ^{1}H NMR data of **3s** and **3r**, see Table 3.

3.5.9. Ixocarpalactone A (4). Colorless needles; mp 292–293°C (lit.[6] 294–295°C; lit.[7] 291–292°C); $[\alpha]_D^7$=+90.0° (*c* 0.20, MeOH) {lit.[6] $[\alpha]_D^{25}$=+84° (CH_3CN); lit.[7] $[\alpha]_D^7$=+68.9° (*c* 1.93, CH_3CN)}; UV (MeOH) λ_{max} (log ε) 214.5 (3.89), 326.5 (2.29) nm; ^{1}H and ^{13}C NMR data, see Tables 4 and 5, respectively.

3.5.10. X-Ray crystallography of ixocarpalactone A (4). A colorless crystal was obtained from $CHCl_3$–EtOAc–MeOH (~5:20:1). Cell parameters: *a*=7.8685 (10) Å, α=90.00°; *b*=10.7377 (2) Å, β=90.00°; *c*=31.2402 (6) Å, γ=90.00°; *V*=2639.47 (8) Å^3, space group $P2_12_12_1$, *Z*=4, D_{calc}=1.34 g/cm^3, λ=0.71073 Å, μ(Mo Kα)=0.096 mm^{-1}, *F*(000)=1151.9, *T*=150 (1) K. A total of 15,081 reflections were collected, yielding a set of 3794 unique, averaged reflections with $I>2\sigma_I$. Full-matrix least-squares refinement led to a final *R*, *R* (all), and GOF values of 0.0974, 0.1390, and 1.043. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 171420. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.5.11. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of 4. Two portions (each ~2.0 mg) of compound **4** were treated with (*S*)-(+) α- and (*R*)-(−) α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (8 μL) and a catalytic amount (~0.1 mg) of 4-dimethylaminopyridine in anhydrous pyridine (0.5 mL) at room temperature overnight, and afforded the (*R*)- and (*S*)-MTPA ester derivatives (**4r** and **4s**) of **4**, respectively. ^{1}H NMR data of **4r** (500 MHz, C_5D_5N) δ 7.252 (1H, dd, *J*=9.8, 6.1 Hz, H-3), 6.545 (1H, d, *J*=9.8 Hz, H-2), 5.438 (1H, d, *J*=6.1 Hz, H-4), 2.909 (1H, m, H-25), 1.494 (3H, s, H-19), 1.327 (3H, s, H-21), 1.247 (3H, d, *J*=6.8 Hz, H-28), 1.148 (3H, d, *J*=7.1 Hz, H-27); ^{1}H NMR data of **4s** (500 MHz, C_5D_5N) δ 7.205 (overlapped with solvent signal), 6.450 (1H, d, *J*=9.8 Hz, H-2), 5.381 (1H, d, *J*=6.1 Hz, H-4), 2.983 (1H, m, H-25), 1.663 (3H, s, H-19), 1.377 (3H, s, H-21), 1.297 (3H, d, *J*=6.9 Hz, H-28), 1.143 (3H, d, *J*=7.1 Hz, H-27).

3.5.12. Withaphysacarpin (5). White amorphous powder; mp 276–280°C (lit.[8] 270–273°C); $[\alpha]_D^{20}$=+23.5° (*c* 0.08, MeOH) {lit.[8] $[\alpha]_D^{22}$=+20.0° (*c* 0.05, $CHCl_3$)}; UV (MeOH) λ_{max} (log ε) 214 (3.82), 333 (2.24) nm; IR ν_{max} (film) 3383, 1716, 1665, 1364, 1034 cm^{-1}; spectral data (^{1}H, ^{13}C NMR and MS) consistent with literature values.[8]

3.5.13. 18-Hydroxywithanolide D (6). White amorphous powder; mp 171–173°C (lit.[20] 175–177°C); $[\alpha]_D^{20}$=+52.0° (*c* 0.15, MeOH) {lit.[20] $[\alpha]_D^{25}$=+59.8° (*c* 0.26, CH_3OH)}; UV (MeOH) λ_{max} (log ε) 222 (3.78) nm; spectral data (^{1}H, ^{13}C NMR and MS) consistent with literature values.[20]

3.5.14. Withanone (7). White amorphous powder; mp 264–267°C (lit.[21] 275–276°C); $[\alpha]_D^{20}$=+64.5° (*c* 0.08, MeOH) {lit.[21] $[\alpha]_D$=+81° (*c* 0.50, the solvent used was not reported)}; UV (MeOH) λ_{max} (log ε) 222 (3.78) nm; ^{1}H NMR and EIMS data consistent with literature values,[21] and its ^{13}C NMR data were also identical with the published values.[21,22]

3.5.15. (2*S*,3*S*,4*R*,9*E*)-1,3,4-Trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-9-octadecene (8). White amorphous powder; mp 140–142°C; $[\alpha]_D^{20}$=+7.8° (*c* 0.30, pyridine); UV (MeOH) λ_{max} (log ε) 204 (3.67), 220 (3.19) nm; IR ν_{max} (film) 3390, 2919, 2852, 1620, 1543, 1466 cm^{-1}; ^{1}H NMR data (C_5D_5N, 300 MHz) δ 8.56 (1H, d, *J*=8.7 Hz, NH), 5.52 (2H, m, H-9 and H-10), 5.08 (1H, m, H-2), 4.60 (1H, dd, *J*=7.5, 3.6 Hz, H-2′), 4.47 (1H, dd, *J*=10.8, 4.8 Hz, H-1a), 4.41 (1H, dd, *J*=10.8, 4.6 Hz, H-1b), 4.32 (1H, dd, *J*=6.2, 5.1 Hz, H-3), 4.26 (1H, m, H-4), 2.18–2.15 (4H, m, H-5a, H-8a, H-9a and H-3′a), 1.94–2.05 (5H, m, H-5b, H-8b, H-9b, H-3′b and H-4′a), 1.71–1.77 (3H, m, H-6a, H-6b and H-4′b), 1.26–1.32 (methylene band), 0.87 (6H, br t, *J*=7.0 Hz, H-18 and H-24′); ^{13}C NMR data (C_5D_5N, 75 MHz) δ 175.2 (C-1′, s), 131.0 (C-9 or C-10, d), 130.8 (C-9 or C-10, d), 77.0 (C-3, d), 73.0 (C-4, d), 72.6 (C-2′, d), 62.1 (C-1, t), 53.1 (C-2, d), 35.8 (C-3′, t), 34.0 (C-5, t), 33.5 and 33.1 (C-8 and C-11, t), 32.3 (C-16′ and C-22′, t), 30.4–29.6 (methylenes), 26.9 (C-4′, t), 26.0 (C-6, t), 23.1 (C-17 and C-23′, t), 14.4 (C-18 and C-24′, q); CIMS *m/z* 682 $[M+H]^+$ (10), 664 $[M-H_2O+H]^+$ (100), 636 (57), 384 (22), 356 (30), 328 (25), 326 (37), 318 (27), 316 (33), 300 (29), 272 (42), 242 (32), 75 (71); EIMS *m/z* 681 $[M]^+$ (2), 663 $[M-H_2O]^+$ (25), 645 $[M-2H_2O]^+$ (23), 467 (12), 436 (17), 422 (17), 412 (37), 408 (29), 396 (30), 384 (47), 366 (11), 339 (51), 337 (37), 314 (10), 296 (28), 280 (46), 262 (42), 225 (23), 121 (31), 109 (50), 95 (85), 83 (97), 55 (100); HRFABMS *m/z* 704.6181 $[M+Na]^+$ (calcd for $C_{42}H_{83}NO_5Na$, 704.6170).

3.5.16. Methanolysis of compound 8. Compound **8** (30 mg) was added to a mixture of HCl (15 mL, 1N) and MeOH (50 mL), with this solution refluxed for 16 h under magnetic stirring. Then, 50 mL H_2O were added to the refluxed mixture, which was extracted with *n*-hexane (3×30 mL). The fatty acid methyl ester (**8b**, 12 mg) was afforded after the purification of the *n*-hexane extract over a Si gel column (1.2×25 cm) with *n*-hexane–acetone (10:1) as solvent. The MeOH/H_2O phase was evaporated under reduced pressure. The residue obtained was purified over a Si gel column (1.2×25 cm), and eluted with $CHCl_3$–MeOH (15:1), and yielded a sphingosine (**8a**, 10 mg).

3.5.17. Sphingosine 8a. $[\alpha]_D^{20}$=+6.8° (*c* 0.30, MeOH) {lit.[52] $[\alpha]_D^{23}$=+7.6° (*c* 1.0, pyridine)}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.42 (2H, m, H-9 and H-10), 3.92–3.94 (1H, m), 3.61–3.88 (3H, m), 3.46 (1H, m), 3.28 (1H, m), 1.98–2.03 (4H, m, H-8 and H-11), 1.28 (methylene band), 0.89 (3H, t, *J*=7.0 Hz, H-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 131.7 and 131.1 (C-9 and C-10, d), 73.3 and 73.1 (C-3 and C-4, d), 58.7 (C-1, t), 56.3 (C-2, d), 34.9 (C-5, t), 33.6 and 33.0 (C-8 and C-11, t), 30.7–30.2 (methylenes), 26.3 (C-6, t), 23.6 (C-17, t), 14.4 (C-18, q).

3.5.18. Fatty acid methyl ester 8b. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.21 (1H, dd, *J*=7.4, 4.1 Hz, H-2), 3.81 (3H, s, OMe), 1.78–1.82 (1H, m, H-3a), 1.62–1.66 (1H, m, H-3b), 1.30 (m, partly overlapped with δ_H 1.27, H-22), 1.27 (methylene band), 1.24 (m, partly overlapped with δ_H 1.27, H-23), 0.90 (3H, t, *J*=6.8 Hz, H-24); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.3 (C-1, s), 70.9 (C-2, d), 52.9 (OMe, q), 34.8 (C-3, t), 32.3 (C-22, t), 25.1–30.1 (methylenes, t), 23.1 (C-23, t), 14.5 (C-24, q).

3.5.19. Acetylation of 8a and 8b. Both **8a** (10 mg) and **8b** (12 mg) were acetylated using acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. The acetylation products of **8a** and **8b** were purified over small Si gel columns (1.0×15 cm) with $CHCl_3$–MeOH (40:1) and *n*-hexane–acetone (20:1) as solvent systems, to afford **8c** (8.5 mg) and **8d** (10 mg), respectively.

3.5.20. Tetraacetate of sphingosine 8c. $[\alpha]_D^{20}$=+22.5° (*c* 0.25, $CHCl_3$) {lit.[52] $[\alpha]_D^{20}$=+21.9° (*c* 1.1, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.97 (1H, d, *J*=9.2 Hz, NH), 5.31–5.44 (2H, m, H-9 and H-10), 5.09 (1H, dd, *J*=8.2, 3.1 Hz, H-3), 4.94 (1H, dt, *J*=8.2, 3.5 Hz, H-4), 4.46 (1H, m, H-2), 4.29 (1H, dd, *J*=11.6, 4.9 Hz, H-1a), 4.00 (1H, dd, *J*=11.6, 3.1 Hz, H-1b), 1.96–2.13 (16H, H-8, H-11, and 4×OAc), 1.63 (2H, m, H-5), 1.26 (methylene band), 0.88 (3H, t, *J*=7.0 Hz, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1, 170.9, 170.1, 169.7 (4×OAc, s), 131.2 and 129.3 (C-9 and C-10, d), 72.9 (C-4, d), 72.1 (C-3, d), 62.8 (C-1, t), 47.7 (C-2, d), 32.6, 32.2, and 31.2 (C-8, C-11, and C-5, t), 25.5–29.6 (methylenes, t), 23.3 (OAc, q), 22.7 (C-17, q), 21.04, 20.75, 20.74 (3×OAc, q), 14.1 (C-18, q) (^{1}H and ^{13}C NMR data of this compound were assigned based on the observed correlations of its ^{1}H–^{1}H COSY, HMQC and HMBC spectra); EIMS *m/z* 483 $[M]^+$ (14), 441 $[M-Ac-H]^+$ (5), 423 (20), 364 (16), 310 (11), 244 (8), 184 (53), 144 (52), 138 (4), 114 (11), 102 (51), 84 (100), 60 (32), 43 (52); HRFABMS *m/z* 484.3246 $[M+H]^+$ (calcd for $C_{26}H_{46}O_7N$, 484.3274); MS–MS (20 eV) *m/z* 424.3157 (100), 382.3146 (10), 364.3050 (10), 322.2872 (6), 304.2796 (15), 280.2810 (4), 262.2678 (27), 245.2422 (11); MS–MS (25 eV) *m/z* 424.3241 (25), 382.3146 (10), 364.2973 (10), 322.2872 (13), 304.2796 (21), 280.2813 (12), 262.2678 (100), 245.2358 (18), 133.1073 (8); MS–MS (30 eV) *m/z* 424.3241 (4), 382.3146 (4), 364.2973 (6), 322.2872 (10), 304.2796 (10), 280.2813 (14), 262.2678 (100), 245.2422 (10), 154.1713 (2), 133.1073 (10), 114.0691 (3), 105.0852 (3), 94.0857 (3).

3.5.21. Monoacetate of fatty acid methyl ester 8d. $[\alpha]_D^{20}$=+12.4° (*c* 0.30, $CHCl_3$) {lit.[29] $[\alpha]_D^{24}$=+14.1° (*c* 1.75, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.99 (1H, dd, *J*=6.4, 6.3 Hz, H-2), 3.74 (3H, s, OMe), 2.14 (3H, s, OAc), 1.78–1.85 (2H, m, H-3), 1.26 (methylene band), 0.88 (3H, t, *J*=6.8 Hz, H-24); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9 (C-1, s), 170.6 (C=O, OAc), 72.4 (C-2, d), 52.2 (OMe, q), 31.2 (C-3, t), 31.1 (C-22, t), 25.1–29.7 (methylenes, t), 22.7 (C-23, t), 20.7 (OAc, q), 14.1 (C-24, q); EIMS *m/z* 440 $[M]^+$ (2), 426 $[M-Me+H]^+$ (15), 412 (10), 398 (44), 370 (12), 366 (10), 156 (8), 142 (9), 110 (11), 97 (19), 83 (17), 57 (21), 43 (100); HRFABMS *m/z* 441.3935 $[M+H]^+$ (calcd for $C_{27}H_{53}O_4$, 441.3944).

3.5.22. (2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-Hydroxytetracosanoylamino]-1,3,4-octadecanetriol (9). White amorphous

powder, mp 123–125°C; $[\alpha]_D^{20}=+12.6°$ (*c* 0.45, pyridine) {lit.[30] $[\alpha]_D=+11.1°$ (*c* 0.26, pyridine)}; IR ν_{max} (film) 3436, 1623, 1542 cm^{-1}; ^1H and ^{13}C NMR data, consistent with the literature values;[30] EIMS *m/z* 683 $[M]^+$ (3), 665 $[M-H_2O]^+$ (13), 647 $[M-2H_2O]^+$ (22), 439 (25), 409 (31), 394 (27), 384 (69), 370 (23), 357 (63), 339 (83), 308 (20), 278 (22), 123 (23), 97 (70), 83 (86), 69 (74), 57 (100); HRFABMS *m/z* 706.6309 $[M+Na]^+$ (calcd for $C_{42}H_{85}NO_5Na$, 706.6326).

3.5.23. (2*S*,3*S*,4*R*)-2-Tetracosanoylamino-1,3,4-octadecanetriol (10). White amorphous powder, mp 114–116°C; $[\alpha]_D^{20}=+19.5°$ (*c* 0.20, pyridine); IR ν_{max} (film) 3402, 1622, 1540 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) δ 8.45 (1H, d, *J*=8.7 Hz, NH), 5.10 (1H, m, H-2), 4.50 (2H, br d, *J*=3.0 Hz, H-1), 4.40 (1H, m, H-3), 4.29 (1H, m, H-4), 2.47 (2H, t, *J*=7.5 Hz, H-2′), 2.44 (1H, m, H-5a), 1.70–1.98 (5H, m, H-5b, H-6, and H-3′), 1.32 (methylene band), 0.88 (6H, t, *J*=3.8 Hz, H-18 and H-24′); ^{13}C NMR (C_5D_5N, 75 MHz) δ 173.3 (C-1′, s), 76.8 (C-3, d), 73.1 (C-4, d), 62.2 (C-1, t), 53.8 (C-2, d), 36.9 (C-2′, t), 34.0 (C-5, t), 32.2 (C-16 and C-22′, t), 29.6–30.3 (methylenes, t), 26.7 and 26.4 (C-6 and C-3′, t), 23.0 (C-17 and C-23′, t), 14.3 (C-18 and C-24′, q); EIMS *m/z* 667 $[M]^+$ (0.8), 649 $[M-H_2O]^+$ (4), 631 $[M-2H_2O]^+$ (20), 602 (17), 440 (26), 410 (25), 406 (23), 392 (54), 378 (35), 368 (85), 364 (34), 340 (54), 336 (100), 323 (38), 264 (29), 141 (19), 97 (44), 83 (52), 60 (98), 57 (84); HRFABMS *m/z* 690.6356 $[M+Na]^+$ (calcd for $C_{42}H_{85}NO_4Na$, 690.6376).

3.5.24. Chlorophyllide *a* (11). Black amorphous powder; mp 198–199°C; $[\alpha]_D^{20}=-60.0°$ (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 326.5 (3.04), 413.5 (4.46), 507.5 (2.74), 538.0 (2.72), 611.5 (2.71), 668.8 (3.86) nm; IR ν_{max} (film) 3243, 1993, 1736, 1695, 1498, 1223, 1036, 988, 896, 752 cm^{-1}; ^1H NMR data ($CDCl_3$, 300 MHz) δ 9.39 (1H, s, H-10), 9.23 (1H, s, H-5), 8.52 (1H, s, H-20), 7.87 (1H, dd, *J*=17.9, 11.6 Hz, H-3a), 6.22 (1H, s, H-13a), 6.13 (2H, m, H-3b), 4.42 (1H, br d, *J*=6.2 Hz, H-18), 4.19 (1H, br d, *J*=8.1 Hz, H-17), 3.85 (3H, s, COOMe), 3.62 (3H, s, H-12a), 3.56 (2H, q, *J*=7.5 Hz, H-8a), 3.33 (3H, s, H-2a), 3.11 (3H, s, H-7a), 2.58 and 2.25 (each 2H, m, H-17a and H-17b), 1.80 (3H, d, *J*=7.1 Hz, H-18a), 1.62 (3H, t, *J*=7.5 Hz, H-8b); ^{13}C NMR data ($CDCl_3$, 75 MHz) δ 189.6 (C-13a, s), 178.2 (C-17c, s), 172.0 (C-19, s), 169.6 (C-13c, s), 161.1 (C-16, s), 155.5 (C-6, s), 150.8 (C-9, s), 149.6 (C-14, s), 145.0 (C-8, s), 142.0 (C-1, s), 137.8 (C-11, s), 136.4, 136.1, 136.0 (C-3, C-4 and C-7, s), 131.8 (C-2, s), 128.90 (C-12 and C-13, s), 128.89 (C-3a, d), 122.6 (C-3b, t), 105.1 (C-15, s), 104.2 (C-10, d), 97.4 (C-5, d), 93.0 (C-20, d), 64.6 (C-13b, d), 52.9 (COOMe, q), 51.0 (C-17, d), 50.1 (C-18, d), 30.9 (C-17a, t), 29.6 (C-17b, t), 23.1 (C-18a, q), 19.3 (C-8a, t), 17.3 (C-8b, q), 12.0 (C-2a and C-12a, q), 11.0 (C-7a, q); HRQTOFMS *m/z* 613.2411 $[M-H]^+$ and 591.2615 $[M-Mg-H]^+$ (calcd for $C_{35}H_{33}N_4O_5Mg$, 613.2293, and for $C_{35}H_{33}N_4O_5$, 591.2599).

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