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Assessment of the Antiprotozoal Activity of *Galphimia glauca* and the Isolation of New Nor-secofriedelanes and Nor-friedelanes

Maria del Rayo Camacho,*,† J. David Phillipson,† Simon L. Croft,‡ Dereck Marley,§ Geoffrey C. Kirby,‡ and David C. Warhurst‡

Centre for Pharmacognosy and Phytotherapy, and Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, U.K., and Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC 1E 7HT, U.K.

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Four new terpenoids, comprising three nor-secofriedelanes (**1**−**3**) and one nor-friedelane (**4**), were isolated from *Galphimia glauca*, together with the known flavonol quercetin and the sterols stigmasterol and sitosterol 3-*O*-*β*-D-glucoside. The structure elucidation of the new isolates was conducted by 1D and 2D NMR techniques. Compounds **1**−**4** were given the trivial names galphin A, galphin B, galphin C, and galphimidin, respectively. All isolates were tested for in vitro antiprotozoal and cytotoxic activities. Quercetin was the only substance isolated that showed any antiprotozoal activity, and this was weak; the IC_{50} values were 14 μM against *Plasmodium falciparum* K1, 13.2 μM against *Trypanosoma brucei brucei*, and 63.8 μM against *Leishmania donovani*. Quercetin was found to be inactive against KB cells (IC_{50} = 295.8 μM).

Galphimia glauca Cav. (Malphigeaceae), commonly known as "calderona amarilla", is widely distributed in Latin America. The plant is used in Mexican traditional medicine to alleviate heart pain, to calm the nerves,[1] and to treat diarrhea, dysentery, gastroenteritis, malaria,[2] and mental disorders.[3] An aqueous extract of *G. glauca* inhibited histamine release from human adenoidal mast cells[4] and displayed relaxant action on different types of smooth muscle.[5,6] A methanolic extract displayed sedative and anticonvulsant properties,[7] and a homoeopathic preparation of *G. glauca* has been used for the treatment of pollinosis.[8]

G. glauca has yielded various phenolic compounds as constituents, such as tetragalloylquinic acid, gallic acid, methyl gallate, ellagic acid, quercetin glucoside, quercetin galloylglucoside, and quercetin,[9,10] as well as nor-secotriterpenes, including galphimine B[11] and its 6-acetoxy derivative.[12] Quercetin has shown antiasthmatic and anticomplementary effects,[9,10] and galphimine B displayed depressant effects on the central nervous system.[11]

As part of the search for antiprotozoal drugs from plants, it was found that the *n*-BuOH and $CHCl_3$ fractions obtained from *G. glauca* had moderate activity against *Plasmodium falciparum* K1, *Trypanosoma brucei brucei*, *Leishmania donovani*, and KB (human epidermoid carcinoma of the nasopharynx) cells (Table 1). Chemical investigation of these fractions yielded four new terpenoids, comprising three nor-secofriedelanes, galphin A (**1**), galphin B (**2**), and galphin C (**3**), and one new nor-friedelane, galphimidin (**4**), along with the known flavonol quercetin[13] and the sterols stigmasterol[14] and sitosterol 3-*O*-*β*-D-glucoside.[15]

Results and Discussion

The molecular formula of galphin A (**1**) was determined to be $C_{34}H_{49}O_{10}$ on the basis of HRFABMS, indicating 11 degrees of unsaturation. The IR spectrum gave bands for an alcohol (3500 cm^{-1}), ester (1720 cm^{-1}), and lactone (1750 cm^{-1}) groups. ^1H and ^{13}C NMR, DEPT, $^1H-^1H$ COSY, and HMQC experiments established the presence of three tertiary methyl groups, one secondary methyl group, two acetoxy groups, a carbomethoxy group, an epoxide function, an exocyclic methylene, a methylene and a methine bearing one primary and one secondary acetoxy group, respectively, a tertiary alcohol, and a lactone in a seven-membered ring. The above information was consistent with a nor-secofriedelane having a lactone in a heptacyclic ring.[12,16] The lactone function was confirmed through the carbon signals at δ_C 52.7 (C-1), 56.4 (C-2), and 168.6 (C-3), and its location in the seven-membered ring A was in accordance with the chemical shifts of carbons at δ_C 76.3 (C-4), 41.1 (C-5), 53.8 (C-10), and 13.0 (C-23) (Table 2). A long-range heteronuclear COLOC experiment allowed unambiguous identification of all resonances and the placement of the oxygenated functional groups in the nor-secofriedelane skeleton by identifying mainly ^2J and ^3J connectivities associated with the quaternary carbons and oxygenated functional groups (Table 3). The lactone signals at δ 168.6 (C-3) showed correlations with the methine protons H-1 (δ 3.55) and H-2 (δ 3.56) of the epoxide function at C-1/C-2. An HMQC correlation observed for a methine group (δ_H 5.16, brt, J = 8 Hz; δ_C 69.0) bearing a secondary acetoxy group and the chemical shifts of neighboring carbons at δ_C 31.8 (C-6) and 50.4 (C-8) suggested the location of the secondary acetoxy group at C-7. In turn, HMQC correlations observed for the methylene group (δ_H 3.63 and 5.07, each, d, J = 12 Hz; δ_C 68.2) and the chemical shifts of carbon C-5 (δ 41.1) indicated that the primary acetoxy group was located at C-24. A COLOC correlation observed for the quaternary carbon at δ 41.1 (C-5) with H-7 and H_2-24 confirmed these assignments, since C-5 showed further correlations with H-1, H-4, H_2-6, H-10, and Me-23. Another quaternary carbon at δ 37.9 (C-9) correlated with H-8, H-10, H_2-11, and Me-25. COLOC correlations were observed for C-13 with H_2-11, H_2-12, and Me-26. These were linked to the quaternary carbon at C-14 (δ 42.1), which correlated with Me-26 and H_2-15. Further, COLOC correlations observed

* To whom correspondence should be addressed. Mazatlan 40, Piloto A.L.M., C.P. 01290, Mexico, D.F., Mexico. Tel: 54233891.
† Centre for Pharmacognosy and Phytotherapy, University of London.
‡ London School of Hygiene and Tropical Medicine.
§ Department of Pharmaceutical and Biological Chemistry, University of London.

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Table 1. In Vitro Antiprotozoal and Cytotoxic Activities of Extracts of *Galphimia glauca* and Quercetin[a]

	$IC_{50} \pm$ SEM (μg/mL)			
sample	*Plasmodium falciparum* K1	*Trypanosoma brucei brucei*	*Leishmania donovani*	KB cells
MeOH extract	128.0 ± 1.9	125.0 ± 2.3	68.2 ± 0.2	163.8 ± 0.3
H_2O extract	>500	500.0 ± 7.0	103.7 ± 3.1	>500
hexane extract	>500	>500	>500	>500
$CHCl_3$ extract	86.7 ± 2.3	63.4 ± 1.3	58.1 ± 1.1	160.0 ± 2.6
n-BuOH extract	24.7 ± 1.2	36.7 ± 2.1	55.6 ± 1.4	68.4 ± 2.3
quercetin	6.5 ± 1.4	4.5 ± 0.9	29.5 ± 1.5	136.0 ± 1.9
	[14.2 ± 2.2 μM]	[13.2 ± 1.1 μM]	[63.8 ± 1.48 μM]	[295.8 ± 3.6 μM]
pentamidine	ND	$6 \times 10^{-4} \pm 2 \times 10^{-5}$	0.24 ± 0.05	0.11 ± 0.06
		[$3.4 \times 10^{-4} \pm 4 \times 10^{-5}$ μM]	[0.41 ± 0.18 μM]	[0.17 ± 0.12 μM]
chloroquine	0.22 ± 0.06	ND	ND	51.4 ± 1.7
	[0.59 ± 0.10 μM]			[160.5 ± 2.2 μM]
podophyllotoxin	ND	ND	ND	$6.21 \times 10^{-3} \pm 2 \times 10^{-4}$
				[0.015 ± 0.008 μM]

[a] Inhibitory concentration (IC_{50}) value of drug causing 50% inhibition of parasite or cell growth in the test was used to determine the activity of each sample. IC_{50} values and SEM were determined by linear regression analysis using the Minitab statistical program package. Three determinations were realized in different days ($n = 3$). $p > 95\%$. ND: not determined.

Table 2. ^1H and ^{13}C NMR Data for Compounds **1**−**4** (values in parentheses are *J* values in Hz)

	δ_H				δ_C			
position	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	3.55, m	3.55, m	3.56, m	1.55, 1.45, each, m	52.7	52.7	53.9	16.2
2	3.56, m	3.58, m	3.57, m	1.81, m	56.4	56.4	57.6	31.5
3				4.93, brs	168.6	168.5	169.7	74.7
4	5.32, q (6)	5.26, q (6)	5.20, q (6)	1.77, m	76.3	76.4	77.7	49.1
5					41.1	41.5	42.5	45.4
6α	1.73, dd (8, 12)	1.73, dd (8, 12)	1.71, dd (8, 12)	3.28, d (8)	31.8	31.8	32.9	86.3
6*β*	1.44, dd (8, 1)	1.43, dd (8, 1)	1.47, dd (8, 1)					
7α				3.98, brt (8)	69.0	68.7	70.0	72.5
7*β*	5.16, brt (8)	5.11, brt (7)	5.10, brt (7)					
8	2.15, brd (8)	1.96, brd (8)	2.01, brd (8)	1.66, brd (9)	50.4	51.3	52.4	50.0
9					37.9	37.8	38.9	37.5
10	1.66, brs	1.63, brs	1.62, brs	1.06, dd (10, 2.6)	53.8	54.2	55.8	59.3
11α	2.82, td (13, 4)	2.69, td (13, 4)	2.98, t (13)	1.55, m	27.1	26.9	35.2	30.4
11*β*	0.77, brd (12)	0.71, brd (12)	0.97, dd (12, 4)	3.22, dd (12,2)				
12α	1.18, m	1.47, m		1.43, m	33.9	26.8	76.3	29.6
12*β*	1.98, m	1.67, m	5.6, dd (12, 4)	2.18, m				
13					57.1	57.9	59.5	79.9
14					42.1	41.9	49.2	43.8
15	2.30, 1.98, each, m	1.62, 1.96, each, m	2.14, 1.95, each, m	1.55, m	40.4	40.4	41.4	39.8
16	1.07, m	1.98, m	1.98, m	1.40, m	23.4	23.9	25.6	25.3
17	2.25, m	2.15, m	1.71, m	1.95, m	38.2	43.1	43.9	41.5
18					77.0	76.7	80.7	79.5
19					42.6	41.9	42.8	45.0
20	2.08, 2.90, each, d (15)	2.11, 2.89, each, d (15)	2.16, 2.86, each, d (15)	2.37, 2.66, each, d (13)	144.2	138.6	139.0	144.1
21					29.1	34.9	37.0	33.2
22	1.07, 2.25, each, m	2.3, m	2.28, m	2.20, 2.54, each, m	36.1	70.9	70.0	52.7
	2.17, m	5.56, dd (12, 6)	6.04, dd (12, 6)	2.74, m				
	1.17 d (6)	1.17, d (6)	1.17, d (6)	1.35, d (7)				
23	3.63, 5.07, each, d (12)	3.61, 5.05, each, d (12)	3.61, 5.06, each, d (12)	4.50, 4.73, each, d (12)	13.0	13.0	14.3	16.7
24	1.31, s	1.36, s	1.52, s	1.18 s	68.2	68.2	69.4	65.1
25	1.34, s	1.30, s	1.35, s	1.02 s	22.7	20.9	23.1	23.0
26	3.49, s	3.62, s	3.65, s	3.65 s	20.5	23.1	25.1	19.5
27	1.09, s	1.00, s	1.25, s	1.27 s	51.3	51.3	52.7	51.4
28	4.46, 4.89, each, brs	4.6, 4.8, each, brs	4.61, 4.81, each, brs	4.79, 4.81, each, brs	25.7	18.3	16.0	22.8
29	1.99, s	1.98, s	1.98, s	2.04 s	109.7	113.2	114.6	110.4
OAc	2.07, s	2.05, s	1.98, s	2.11 s	174.6	173.7	174.3	174.8
		2.07, s	2.06, s		170.2	170.2	170.7	170.0
			2.03, s		170.9	170.2	171.4	170.6
						170.9	171.5	
							171.8	
					21.0	21.0	22.3	21.4
					21.6	21.2	22.6	21.2
						21.6	22.6	
							23.0	

for the carbomethoxy group (δ_C 174.6) with H_2-12 (δ_H 1.18, 1.98, each, m) and H_2-19 (δ_H 2.08, 2.90, each, d, $J = 15$ Hz,), together with the similarity of the chemical shift values of C-13 (δ 57.1) and C-18 (δ 77.0) in **1**, and C-13 (δ 57.6) and C-18 (δ 76.8) in the galphimine B 6-acetoxy derivative previously isolated from *G. glauca*,[12] allowed the placement of the carbomethoxy group on C-13 and the tertiary alcohol group at C-18. The quaternary carbon at C-17 (δ 38.2) correlated with H_2-16, H_2-19, H_2-22, and Me-28. These were linked to C-18 (δ 77.0), which correlated in turn with Me-28 and H_2-19. The C-20 signal (δ 144.2) showed correlations with H_2-19 and H_2-21. Finally, the

Table 3. 2D NMR Spectral Data for Compounds **1** and **4**

			COLOC (C−H)					
	^{1}H−^{1}H COSY		**1**		**4**		NOESY	
position	**1**	**4**	^2J	^3J	^2J	^3J	**1**	**4**
1	H-2, H-10	H-2, H-10	H-2, H-10			H-3	H-2, H-10	
2	H-1	H-1, H-3	H-1	H-10			H-1, H-10	
3		H-2, H-4	H-2	H-1				H-4
4	Me-23	Me-23	Me-23	H_2-24	Me-23	H_2-24	H-8, H-10	H-6, H-8, H-10
5			H-4, H_2-6, H-10, H_2-24	Me-23, H-1, H-7	H_2-24	H-3		
6	H-7	H-7				H_2-24		H-4, H-10
7	H_2-6, H-8	H-8, H-6			H-8		H-6β, H-11β, Me-25	Me-25
8	H-7	H-7		H_2-6, Me-26	Me-25	H_2-11	H-10	H-10
9			H-8, H-10, H_2-11, Me-25					
10	H-1	H_2-1	H-1	H-2, H_2-6, Me-25		H_2-11, Me-25	H-1, H-2, H-4, H-8	H-4, H-6
11	H_2-12	H_2-12		Me-25				
12	H_2-11	H_2-11						
13			H_2-12	H_2-11, Me-26	H-11	Me-26		
14			H_2-15, Me-26		H_2-15, Me-26			
15	H_2-16	H_2-16		Me-26		Me-26		
16	H_2-15	H_2-15		Me-28		Me-28	Me-26, Me-28	
17			H_2-16, H_2-22, Me-28	H_2-19	H_2-16, H-22, Me-28			
18			H_2-19, Me-28		H_2-19	Me-28		
19		H_2-19	-	H_2-29		H_2-29	H_2-29	H_2-29
20			H_2-19, H_2-21					
21	H_2-22	H-22		H_2-29			H_2-29	H_2-21, H-22
22	H_2-21	H_2-21		Me-28				Me-28, H_2-21
23	H-4	H-4					H_2-24	H_2-24
24	H_2-24	H_2-24	H-10				Me-23, Me-25	M-23, Me-25
25							11β, H-7β, Me-26	Me-26, H-25, H-7β, H-11β, H-12β
26							H-16β, Me-28	Me-25, H-16β, Me-28
27			H_2-19, H_2-21	H_2-19	H-22		H-19α	
28							H-16β, Me-26	H-22
29							H-19α, H-21β	

exocyclic methylene at C-29 (δ 109.7) correlated with H_2-19 and H_2-21.

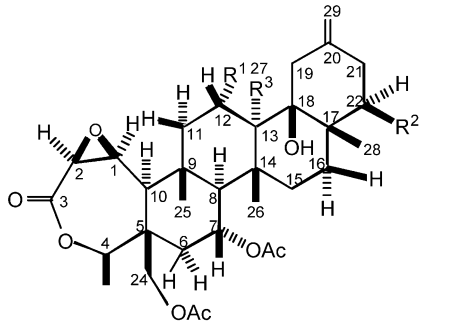
Once the planar structure of galphin A (**1**) was established, the configuration and conformation of the molecule was determined by a NOESY experiment (Table 3). The small coupling constants for H-1/H-2 and H-1/H-10 in the ^{1}H NMR spectrum and correlations observed for H-2/H-10 and H-1/H-10 in the NOESY spectrum permitted the β-configuration of the epoxide group at C-1/C-2 to be determined. The α-axial configuration of the acetoxy group at C-7 was determined by the small coupling constants observed for H-6β/H-7β (J = 1 Hz), H-6α/H-7β (J = 12 Hz), and H-7β/H-8α (J = 8 Hz) in the ^{1}H NMR spectrum and NOESY correlations for H-7/H-11/Me-25. NOESY correlations observed for H_2-24/Me-25, Me-25/H-11β/Me-26, Me-26/H-16β, and Me-28/H-16β were consistent with a twist-boat-*trans*-chair-*trans*-chair-*cis*-half-chair arrangement for rings B/C/D/E. This was in agreement with an α- and β-axial configuration of the carbomethoxy group on C-13 and the tertiary alcohol group on C-18, respectively. On this basis, compound **1** was identified as (4*R*)-7α,24-diacetoxy-13α-carbomethoxy-1β,2β-epoxy-18β-hydroxy-30-nor-3,4-secofriedela-20(29)-en-3,4-olide and named galphin A.

Galphin B (**2**) had the molecular formula $C_{36}H_{51}O_{12}$ on the basis of its HRFABMS data. Its NMR data (Table 2) were almost identical to those of **1**, except that **2** lacked the C-22 methylene signal seen for **1**, and instead a methine (δ 5.56, dd, J = 12, 6 Hz, H-22) bearing a secondary acetoxy group (δ_C 170.2; δ_C 21.2; δ_H 2.05) was observed. In addition, the chemical shifts for carbons C-17 (δ_C 43.1), C-21 (δ_C 34.9), and C-22 (δ_C 70.9); COLOC correlations for C-17 with H_2-16, H_2-19, H-22, Me-28; and NOESY correlations observed for CO_2Me-27α/H-22α/H-21α/H-11α were consistent with a β-equatorial acetoxy group at C-22. On this basis **2** (galphin B) was assigned as (4*R*)-7α,22β,24-triacetoxy-13α-carbomethoxy-1β,2β-epoxy-18β-hydroxy-30-nor-3,4-secofriedela-20(29)-en-3,4-olide.

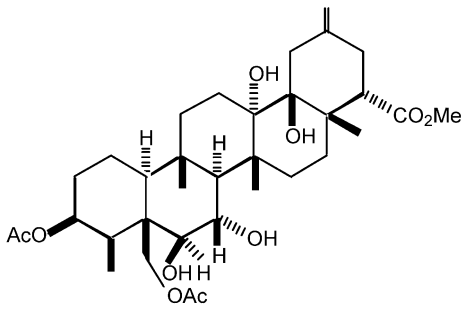
Galphin C (**3**) displayed the molecular formula $C_{38}H_{53}O_{14}$ on the basis of its HRFABMS. Its NMR data (Table 2) were almost identical to those of **2**, except that **3** lacked the C-12 methylene resonance evident for **2**, and instead a methine (δ 5.6, dd, J = 12, 4 Hz, H-12) bearing a secondary acetoxy group (δ_H 1.98; δ_C 22.97; δ_C 171.5) was observed. Further, the chemical shifts for C-12 (δ_C 76.3) and C-11 (δ_C 35.2); COLOC correlations for C-13 (δ 59.5) with H_2-11, H-12, and Me-26; and NOESY correlations of H-11β/H-12β/Me-25/Me-26 were consistent with an α-equatorial configuration for the acetoxy group at C-12. On this basis, **3** was identified as (4*R*)-7α,22β,12α,24-tetraacetoxy-13α-carbomethoxy-1β,2β-epoxy-18β-hydroxy-30-nor-3,4-secofriedela-20(29)-en-3,4-olide and named galphin C.

Galphimidin (**4**) gave the molecular formula $C_{34}H_{53}O_{10}$ on the basis of its HRFABMS, indicating nine degrees of unsaturation. Its IR spectrum showed as main features the presence of alcohol (3400 cm^{-1}) and ester (1720 cm^{-1}) groups. 1D and 2D NMR data (Tables 2 and 3) showed

signals for three tertiary methyl groups, one secondary



1: R^1 = H R^2 = H R^3 = CO_2Me
2: R^1 = H R^2 = OAc R^3 = CO_2Me
3: R^1 = OAc R^2 = OAc R^3 = CO_2Me



4

methyl group, two acetoxy groups, a carbomethoxy group, two secondary alcohol groups, two tertiary alcohol groups, an exocyclic methylene, a methylene, and a methine bearing a primary and a secondary acetoxy group, respectively. This information together with the absence of the lactone group observed in **1**−**3** pointed to the basic skeleton of a nor-friedelane terpenoid.[17−19] The structure elucidation of compound **4** was carried out in a manner similar to that described for compounds **1**−**3**. Chemical shifts of carbons at δ_C 31.5 (C-2), 74.7 (C-3), and 49.1 (C-4); the broad singlet observed for H-3 (δ 4.93) in the ^{1}H NMR spectrum; and COLOC correlations for C-1 (δ 16.2) with H-3 (δ 4.9), and C-5 (δ 45.4) with H-3, were consistent with a β-axial configuration of the acetoxy group at C-3. Placement of a primary acetoxy group at C-24 was determined in view of COLOC correlations observed for C-5 with the methylene protons (δ 4.50 and 4.73, each, d, J = 12 Hz) located at C-24 (δ 65.1). Chemical shifts at δ_C 45.4 (C-5), 86.3 (C-6), 72.5 (C-7), 50.0 (C-8); COLOC correlations of C-6 with H_2-24, and C-7 with H-8; the coupling constants observed for H-6α/H-7β (J = 8 Hz) and H-7β/H-8α (J = 8 Hz); and the NOESY correlations for H-4/H-6/H-8/H-10 and H-7β/Me-25 helped determine the orientation of the secondary alcohol groups at C-6 and C-7, which were assigned with a β-equatorial and an α-axial configuration, respectively. The tertiary alcohol groups were placed at C-13 and C-18 based on COLOC interactions observed for C-13 (δ 79.9) with H_2-11, Me-26 and C-18 (δ 79.5) with H_2-12, H_2-19, Me-28, respectively (Table 3). NOESY correlations observed for H-24/Me-25, Me-25/Me-26, and Me-26/H-16β/Me-28 were consistent with a chair-*trans*-chair-*trans*-chair-*trans*-chair-*cis*-half-chair arrangement for the A/B/C/D/E rings and allowed the determination of the α- and β-axial configuration of the tertiary alcohol groups at C-13 and C-18. COLOC interactions observed for the carbomethoxy group at δ_C 174.8 with H-22 (δ_H 2.74, m) and the chemical shifts of carbons at δ_C 33.2 (C-21) and 52.7 (C-22) helped determine the location of the carbomethoxy group at C-22. NOESY correlations observed for H-22β and Me-28 established the α-axial configuration of the carbomethoxy group at C-22. On this basis **4** was assigned as 3β,24-diacetoxy-6β,7α,13α,18β-tetrahydroxy-30-nor-friedela-20(29)-en-22α-carboxylate and given the trivial name galphimidin.

The methanolic extract, fractions, and isolated compounds from *G. glauca* were tested for antiprotozoal activity against *P. falciparum* K1, *T. b. brucei*, and *L. donovani*. The cytotoxic activity of isolated compounds against KB cells was also determined, since KB cells have an intermediate sensitivity to a large number of cytotoxic agents. Results in Table 1 show that the $CHCl_3$ and the *n*-BuOH extracts showed the highest antiprotozoal activity. Chemical investigation of both fractions yielded galphin A (**1**), galphin B (**2**), galphin C (**3**), galphimidin (**4**), quercetin, stigmasterol, and sitosterol 3-*O*-β-D-glucoside. Compounds **1**−**4**, stigmasterol, and sitosterol 3-*O*-β-D-glucoside were inactive (IC_{50} >100 μM) against all organisms tested (data not shown). Quercetin was the only compound that showed weak antiplasmodial, antitrypanosomal, and antileishmanial activities compared with the standard drugs (Table 1), and it was not toxic to KB cells. Thus, it is likely to be more selective against protozoal parasites than to mammalian cells. To our knowledge, this is the first report of the antitrypanosomal and antileishmanial activity of quercetin, although the antigiardial,[20] antiamoebic activity,[20] and antiplasmodial activity[21] of quercetin have already been reported and are in agreement with our results. It is likely that the antiplasmodial activity of quercetin found in this study may contribute to the use of *G. glauca* in folk medicine. It is important to point out that this study was not carried out by bioassay-guided fractionation, thus it is possible that other minor components present in the extract may contribute to the antiplasmodial effects of *G. glauca*. In addition, other biological effects, e.g., antiinflammatory, antipyretic, and immunomodulation, may contribute to the antiplasmodial effects of *G. glauca*. However, this species will require additional investigation for its biological activities in the future.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotations were measured on a Bellingham & Stanley model P 506 polarimeter using a sodium lamp operating at 589 nm in $CHCl_3$ solution. IR spectra were recorded on KBr using a Perkin-Elmer 841 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AMX-400 instrument, operating at 400 and 100 MHz, respectively, using TMS as internal reference. All 2D NMR experiments were performed on the same spectrometer. FAB mass spectra were recorded on a VG analytical ZAB SE spectrometer with xenon as the atom source at 8 eV. TLC analysis was performed on Si gel SiF_{254} (Merck) and visualized with the spray reagents cerium sulfate (0.1% $CeSO_4$/2 N H_2SO_4) or vanillin (1% vanillin/5% H_2SO_4).

Plant Material. Aerial parts of *Galphimia glauca* were collected in Ayutla, Guerrero, Mexico, in September 1994. A voucher specimen (MC-94-5) was deposited at the Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, London.

Extraction and Isolation. The dried and powdered plant material (2.5 kg) was extracted sequentially with MeOH and H_2O at room temperature. The concentrated MeOH extract (225 g) was diluted with H_2O and partitioned between hexane, $CHCl_3$, and *n*-BuOH. Each fraction was dried under vacuum to give dried hexane (34 g), $CHCl_3$ (120 g), *n*-BuOH (54 g),

and H_2O (68 g) extracts. Their antiprotozoal activity was determined against *Plasmodium falciparum* K1, *Trypanosoma brucei brucei*, and *Leishmania donovani*. The cytotoxic activity was determined against KB cells. Both the *n*-BuOH and $CHCl_3$ extracts showed the highest antiprotozoal and cytotoxic activities (Table 1). The *n*-BuOH fraction (10 g) was chromatographed over Sephadex LH-20 (Pharmacia) and eluted with a gradient of $CHCl_3$/MeOH (from 50:50 to 0:100) to yield 81 mg of quercetin as the major component. The active $CHCl_3$ extract was column chromatographed over Si gel and eluted with a gradient of hexane/acetone (from 100:0 to 10:90) solvent system. Each fraction was monitored by TLC and then sprayed with either vanillin or cerium sulfate in H_2SO_4. Fractions with the same TLC behavior were combined and further column chromatographed over Si gel eluted with a gradient solvent system of hexane/EtOAc (from 100:0 to 20:80). Seven compounds were obtained, galphin A (**1**, 46 mg), galphin B (**2**, 30 mg), galphin C (**3**, 43.67 mg), galphimidin (**4**, 401.8 mg), quercetin (27 mg), stigmasterol (400 mg), and sitosterol 3-*O*-β-D-glucoside (37 mg).

Galphin A (1): white needles ($CHCl_3$); mp 246 °C (MeOH); $[\alpha]_D$ 0° (*c* 0.058, $CHCl_3$); IR (KBr) ν_{max} 3500, 1750, 1720, 1250, 1050 cm^{-1}; ^1H NMR, ^{13}C NMR, DEPT, $^1H-^1H$ COSY, HMQC, COLOC, and NOESY data, see Tables 2 and 3; HRFABMS *m/z* $[M + H]^+$ 617.3299 (calcd for $C_{34}H_{49}O_{10,}$ 617.331998).

Galphin B (2): white needles ($CHCl_3$); mp 285 °C (MeOH); $[\alpha]_D$ −18° (*c* 0.061, $CHCl_3$); IR (KBr) ν_{max} 3400, 1750, 1720, 1250, 1050 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS *m/z* $[M + H]^+$ 675.3398 (calcd for $C_{36}H_{51}O_{12}$, 675.337475).

Galphin C (3): white needles ($CHCl_3$); mp 271 °C (MeOH); $[\alpha]_D$ −23° (*c* 0.055, $CHCl_3$); IR (KBr) ν_{max} 3400, 1750, 1720, 1250, 1050 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS *m/z* $[M + H]^+$ 733.3414 (calcd for $C_{38}H_{53}O_{14}$, 733.342953).

Galphimidin (4): white needles ($CHCl_3$); mp 234 °C (MeOH); $[\alpha]_D$ +46° (*c* 0.090, $CHCl_3$); IR (KBr) ν_{max} 3400, 1720, 1250, 1050 cm^{-1}; ^1H NMR, ^{13}C NMR, DEPT, $^1H-^1H$ COSY, HMQC, COLOC, and NOESY data, see Tables 2 and 3; HRFABMS *m/z* $[M + H]^+$ 621.2345 (calcd for $C_{34}H_{53}O_{10}$, 621.3632).

Antiprotozoal Assays. The antiplasmodial activity against *P. falciparum* K1 strain was measured as previously described.[22] Chloroquine was used as a positive control substance. Antitrypanosomal activity against *T. b. brucei* (strain S427) blood stream trypomastigote forms and antileishmanial activity against *L. donovani* (MHOM/ET/67/L82 strain) promastigote forms were measured as previously reported.[23] Pentamidine was used as a positive control substance.

Cytotoxicity Assay. Cytotoxic activity against KB cells was performed as previously described.[24] Podophyllotoxin was used as a standard drug.

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