

Metabolites from Roots of *Colubrina greggii* var. *yucatanensis* and Evaluation of their Antiprotozoan, Cytotoxic and Antiproliferative Activities

Dafne B. Domínguez-Carmona,^a Fabiola Escalante-Erosa,^a Karlina García-Sosa,^a Grace Ruiz-Pinell,^b David Gutierrez-Yapu,^b Manuel J. Chan-Bacab,^c Rosa E. Moo-Puc,^d Nigel C. Veitch,^e Alberto Giménez-Turba^b and Luis M. Peña-Rodríguez^{*,a}

^aUnidad de Biotecnología, Centro de Investigación Científica de Yucatán, Calle 43, N. 130, Col. Chuburná, Mérida, Yucatán, 97200 México

^bInstituto de Investigaciones Fármaco-Bioquímicas, Universidad Mayor de San Andrés, Av. Saavedra 2224, La Paz, Bolivia

^cDepartamento de Microbiología Ambiental y Biotecnología, Universidad Autónoma de Campeche, Agustín Melgar s/n, Campeche, Campeche, México

^dUnidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad, Centro Médico Ignacio García Téllez IMSS, Calle 41, N. 439, Col. Industrial, Mérida, Yucatán, 97150 México

^eJodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3AB UK

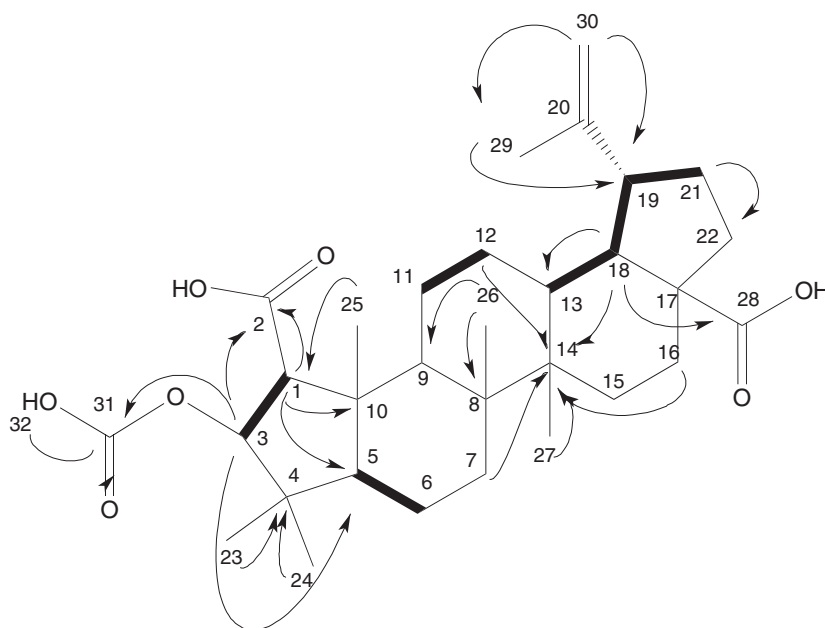


Figure S1. Principal ¹H-¹H COSY couplings (—) and HMBC (H→C) correlations in 3-*O*-acetyl-ceanothic acid (1).

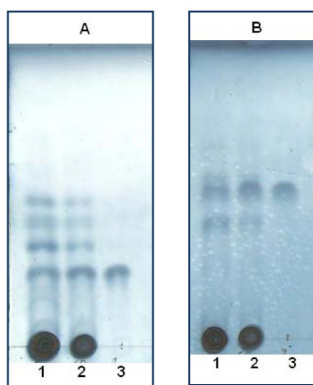


Figure S2. TLC analyses of 3-*O*-acetyl ceanothic acid (**1**) in the crude extract of root from *C. greggii*. 1) crude extract of root from *C. greggii*; 2) Co-chromatography of crude extract and 3-*O*-acetyl ceanothic acid (**1**); 3) 3-*O*-acetyl ceanothic acid (**1**); (A) hexane/Me₂CO 8:2; (B) ether/hexane 1:1.

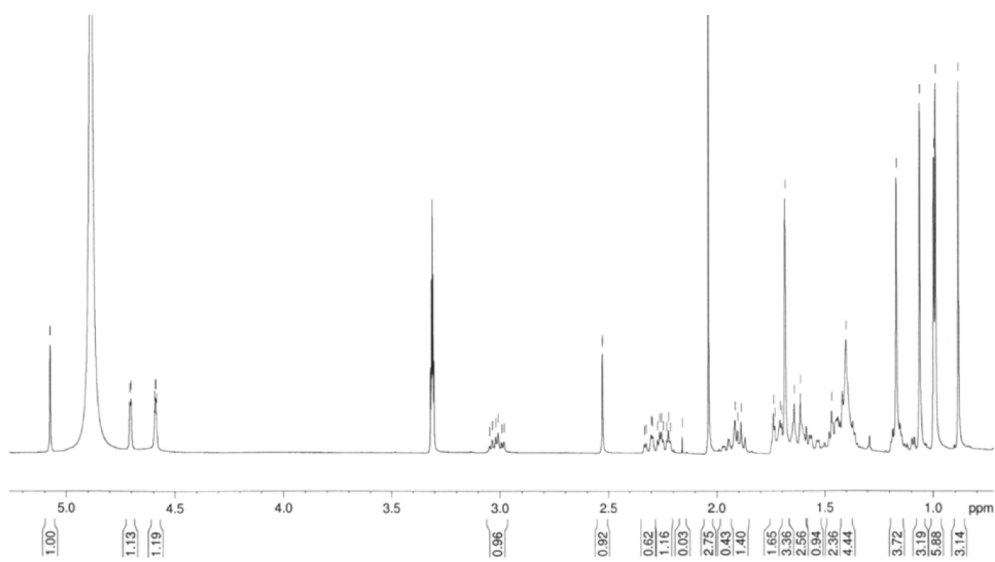


Figure S3. ¹H NMR spectrum of **1** (CD₃OD, 400 MHz).

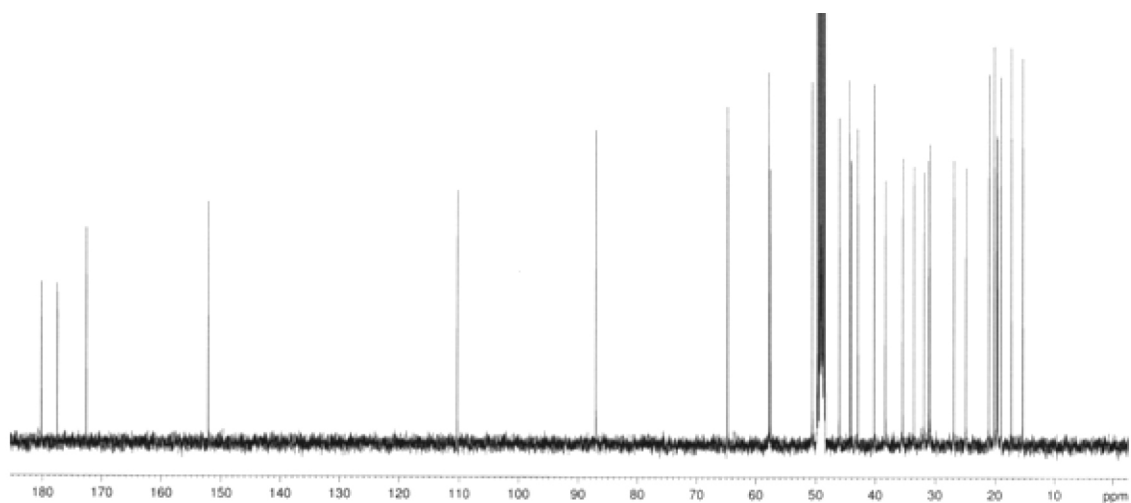
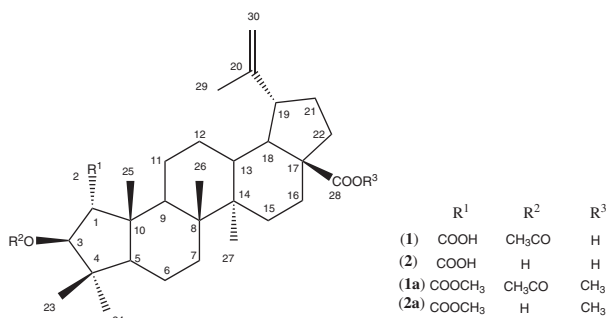
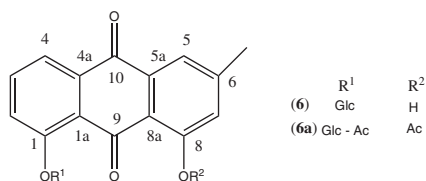


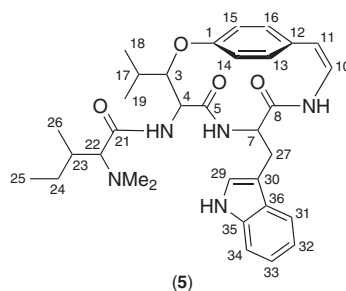
Figure S4. ¹³C NMR spectrum of **1** (CD₃OD, 100 MHz).

Table S1. ^{13}C and ^1H NMR data (δ in ppm) for 3-*O*-acetyl ceanothic acid (**1**), dimethyl ceanothate (**2a**), ceanothenic acid (**3**) (400 MHz and 100 MHz, respectively) and acetyl-dimethyl ceanothate (**1a**) (600 MHz and 150 MHz, respectively)

C/H	1			1a		2a		3	
	δ_{C} (mult)	δ_{H} (J Hz)	HMBC	δ_{C} (mult)	δ_{H} (J Hz)	δ_{C} (mult)	δ_{H} (J Hz)	δ_{C} (mult)	δ_{H} (J Hz)
1	64.7 (d)	2.52 d (0.8)	C-2, C-3, C-5, C-10	62.9 (d)	2.59 s	65.5 (d)	2.50 s	140.1 (d)	5.55 d (5.7)
2	177.4 (s)			174.2 (s)		175.9 (s)		-	-
3	87.0 (d)	5.07 d (0.8)	C-2, C-31	85.1 (d)	5.09 s	84.9 (d)	4.07 s	138.2 (d)	5.01 d (5.0)
4	44.0 (s)			42.9 (s)		43.4 (s)		44.0 (s)	
5	57.8 (d)	1.71 m	C-10	56.2 (d)	1.66 m	56.6 (d)	1.53 m	61.9 (d)	0.86 m
6	19.5 (t)	1.43 m		18.2 (t)	1.33 m	18.6 (t)	1.27 m	16.8 (t)	1.07 m
7	35.4 (t)	1.40 m	C-14	33.8 (t)	1.33 m	34.0 (t)	1.27 m	36.9 (t)	1.25 m
8	42.9 (s)	1.97 m		41.5 (s)	1.40 m	42.9 (s)		40.7 qC	1.33 m
9	46.1 (d)	1.71 m		44.6 (d)	1.46 m	44.7 (d)	1.36 m	47.4 (d)	1.48 dd (3.2, 12.3)
10	50.6 (s)			49.4 (s)		49.5 (s)		50.0 (s)	
11	24.8 (t)	1.43m	C-10	23.4 (t)	1.46 m	23.6 (t)	1.36 m	22.3 (t)	1.20 m
12	26.8 (t)	1.11m	C-14	25.2 (t)	0.95 m	25.5 (t)	0.82 m	25.2 (t)	1.30 m
13	40.1 (d)	1.62 m		38.4 (d)	1.60 m	38.7 (d)	1.83 m	39.0 (d)	1.74 m
14	44.3 (s)	2.27 m		42.7 (s)	2.18 td (11.0, 7.5)	41.7 (s)	2.08 m	39.0 (d)	2.02 m
15	31.8 (t)	1.43 m		30.5 (t)	1.36 m	30.9 (t)	1.27 m	59.3 (s)	1.00 m
16	33.5 (t)	1.90 m	C-14	29.7 (t)	1.87 m	32.3 (t)	1.80 m	27.4 (t)	1.74 m
17	57.5 (s)	1.43 m		56.4 (s)	1.13 m	56.7 (s)	1.78 m	33.7 (t)	0.95 m
18	50.5 (d)	2.27 m	C-13, C-14, C-17, C-19, C-28	49.2 (d)	1.36 m	49.6 (d)	2.11 m	55.6 (s)	1.99 m
19	48.6 (d)	1.62 m	C-29	46.8 (d)	1.54 t (11.4)	47.0 (d)	1.45 t (11.4)	50.9 (d)	1.36 m
20	152.0 (s)	3.01 dt (10.8, 4.5)		150.3 (s)	2.97 dt (10.5, 4.7)	150.5 (s)	2.88 dt (10.9, 4.6)	46.6 (d)	2.72 dt (7.5, 4.0)
21	31.1 (t)		C-22	32.1 (t)		30.7 (t)		149.7 (s)	
22	38.3 (t)	1.62 m		36.9 (t)	2.23 m	37.0 (t)	1.27 m	29.8 (t)	1.00 m
23	30.8 (q)	1.90 m	C-3, C-4, C-5, C-24	30.2 (q)	1.36 m	29.8 (q)	1.27 m	180 m	1.57 m
24	20.1 (q)	1.16 s	C-3, C-4, C-5, C-23	19.5 (q)	1.87 m	29.9 (q)	1.78 m	36.5 (t)	1.00 m
25	18.9 (q)	0.88 s	C-1, C-5, C-9, C-10	18.0 (q)	1.19 s	14.8 (q)	1.16 s	28.6 (q)	0.59 s
26	17.2 (q)	1.06 s	C-7, C-9	16.4 (q)	0.85 s	18.5 (q)	1.03 s	20.5 (q)	0.53 s
27	15.3 (q)	0.98 s	C-8, C-13, C-14, C-15	14.6 (q)	0.92 s	16.6 (q)	0.83 s	17.2 (q)	0.68 s
28	180.1 (s)	0.99 s		176.6 (s)	0.91 s	176.8 (s)		178.5 (s)	
29	19.7 (q)	1.68 s	C-19	19.4 (q)	1.67 s	19.3 (q)	1.57 s	17.9 (q)	1.32 s
30	110.3 (t)	4.58 dd (2.2, 1.4)	C-19, C-29	109.6 (t)	4.60 sa	109.7 (t)	4.50 sa	109.1 (t)	4.22 d (2.2)
		4.70 d (2.0)			4.72 sa		4.63 sa		4.35 d (2.1)
C=O	172.5 (s)			170.5 (s)		176.8 (s)		177.8 (s)	
Me	21.0 (q)	2.03 s	C-31	21.0 (q)	2.04 s	51.4 (q)	3.50 s		
MeO				51.5 (q)	3.68 s	51.4 (q)	3.50 s		
MeO				51.3 (q)	3.66 s				

Table S2. ^{13}C and ^1H NMR data for compounds **6** and **6a**

C/H	6			6a				
	δ_{C} (mult)	δ_{H} (J Hz)	δ_{C} (mult)	δ_{H} (J Hz)	Me	δ_{C}	C=O	δ_{C}
1	147.5 (s)		149.6 (s)		6''	21.0 (q)	6'''	170.4 (s)
1a	114.7 (s)		123.9 (s)					
2	124.0 (d)	7.17 dd (1.6, 0.7)	130.6 (d)	7.20 dd (1.8, 0.6)				
3	118.2 (s)		145.5 (s)					
4	119.3 (d)	7.47 dd (1.6, 0.7)	125.5 (d)	7.97 dd (1.8, 0.6)				
4a	132.1 (s)		134.2 (s)					
5	120.5 (d)	7.85 m	122.1 (d)	8.00 dd (7.5, 1.2)				
5a	136.1 (s)		134.8 (s)					
6	135.8 (d)	7.85 m	134.3 (d)	7.65 dd (7.6, 7.6)				
7	122.4 (d)	7.70 dd (2.8, 1.3)	123.3 (d)	7.45 dd (8.4, 1.2)				
8	158.1 (s)		156.4 (s)					
8a	121.2 (s)		123.8 (s)					
9	187.4 (s)		180.5 (s)					
10	182.0 (s)		182.7 (s)					
1'	100.5 (d)	5.08 d (5.1)	99.8 (d)	5.13 d (7.8)				
2'	77.2 (d)	3.46 m	70.4 (d)	5.50 dd (9.3, 7.8)	2''	20.5 (q)	2'''	169.6 (s)
3'	73.2 (d)	3.46 m	72.6 (d)	5.31 dd (9.6, 9.6)	3''	20.6 (q)	3'''	170.2 (s)
4'	69.5 (d)	3.21 m	68.2 (d)	5.22 dd (9.5, 9.5)	4''	20.6 (q)	4'''	169.3 (s)
5'	76.5 (d)	3.31 m	72.2 (d)	3.90 ddd (12.3, 9.5, 2.6)	5''	20.8 (q)	5'''	170.1 (s)
6'	60.6 (t)	3.46 m 3.70 dd (10.0, 5.5)	61.8 (t)	4.20 ddd (12.3, 9.5, 2.6) 4.28 ddd (12.3, 9.5, 2.6)				
11	21.4 (q)	2.41 s	21.6 (q)	2.49 s				

Table S3. ^{13}C and ^1H NMR data for compound **5** in CD_3OD at $30\text{ }^\circ\text{C}$ (400 MHz)

5			
C/H	δ_{C} (mult.)	δ_{H} (J Hz)	HMBC
1	157.7 (s)		
3	82.0 (d)	4.86 dd (8.4, 1.7)	C-1, C-4, C-5, C-17, C-18, C-19
4	56.8 (d)	4.48 d (8.4)	C-3, C-5, C-17, C-21
5	172.4 (s)		
7	55.5 (d)	4.26 dd (9.1, 5.0)	C-8, C-27, C-30
8	172.1 (s)		
10	127.0 (d)	5.97 br s	
11	131.0 (d)	6.67 br s	
12	132.4 (s)		
13/16 ^a	131.4 (d)	6.90 m	C-1
	130.4 (d)	6.96 m	C-1
14/15 ^a	122.2 (d)	6.96 m	C-12
	119.2 (d)	6.97 m	C-12
17	30.0 (d)	2.19 m	C-18, C-19
18/19 ^a	20.8 (q)	1.19 d (6.9)	C-3, C-17, C-18/19
	15.4 (q)	1.01 d (6.8)	C-3, C-17, C-18/19
21	173.2 (s)		
22	74.4 (d)	2.64 d (8.4)	C-21, C-23, C-24, C-26, NMe ₂
23	35.6 (d)	1.81 m	C-21, C-22, C-24, C-26
24	27.1 (t)	1.59 m 1.09 m	C-22, C-23, C-25, C-26 C-22, C-23, C-25, C-26
25	11.4 (q)	0.87 t (7.4)	C-23, C-24
26	15.6 (q)	0.74 d (6.7)	C-22, C-23, C-24
NMe ₂	42.5 (q)	2.24 s	C-21, C-22
27	29.6 (t)	2.99 br dd (14.0) 2.74 br dd (14.0)	
29	124.7 (d)	6.99 s	C-7, C-27, C-30, C-35, C-36
30	110.4 (s)		
31	119.3 (d)	7.44 br d (7.9)	C-30, C-33, C-35, C-36
32	119.8 (d)	6.96 ddd (8.0, 7.0, 1.1)	C-31, C-34, C-36
33	122.5 (d)	7.06 ddd (8.2, 7.0, 1.2)	C-31, C-34, C-35
34	112.3 (d)	7.29 dt (8.1, 0.8)	C-32, C-36
35	138.1 (s)		
36	128.6 (s)		

^aAssignments interchangeable.

Table S4. Cytotoxic activity [CC_{50} ($\mu\text{g mL}^{-1}$)] in HeLa, KB, HEp-2 and VERO cells of organic crude extract from *C. greggii*, low and medium polarity fractions, compounds **1-6**, and semisynthetic derivatives **1a**, **2a** and **6a**

Extract/ fraction/ compound	Cytotoxic activity						
	HeLa		KB		HEp-2		VERO
	CC_{50}	SI	CC_{50}	SI	CC_{50}	SI	CC_{50}
CG-1	249.8	-	533.3	-	8.9	-	NT
CG-2A	20.6	-	19.6	-	6.9	-	NT
CG-2B	136.9	-	140.4	-	13.1	-	NT
1	36.2 ± 5.1	2.8	46.9 ± 9.2	2.1	389.0 ± 10.9	0.2	103.1 ± 1.8
2	NA	-	56.0 ± 2.1	2.3	68.7 ± 3.4	1.9	131.2 ± 3.2
1a	NT		NT		NT		NT
2a	NT		NT		NT		NT
3	67.7 ± 10.2	1.4	35.2 ± 3.2	2.8	54.5 ± 3.6	1.8	98.6 ± 1.2
4	15.5 ± 4.7	9.3	43.3 ± 4.2	3.3	174.6 ± 2.1	0.8	145.0 ± 2.9
5	43.9 ± 3.9	4.5	66.1 ± 7.2	3.0	179.5 ± 2.1	1.1	199.1 ± 2.7
6	69.3 ± 4.2	7.5	86.8 ± 11.3	6.0	102.7 ± 4.1	5.0	521.0 ± 6.3
6a	120.5 ± 9.1	1.0	46.0 ± 2.1	2.6	65.7 ± 3.4	1.8	123.8 ± 4.2
Docetaxel	0.20 ± 0.01	5.5	0.23 ± 0.03	4.7	0.08 ± 0.01	13.7	1.1 ± 0.05

SI: selectivity index were calculated as the ratio CC_{50} of VERO cells/ CC_{50} of each cell line; CG-1: crude extract of *C. greggii*; CG-2A: low polarity fraction; CG-2B: medium polarity fraction

Table S5. Inhibition of the growth [IG_{50} ($\mu\text{g mL}^{-1}$)] in HeLa, KB, HEp-2 and VERO cells of compounds **1-6** from *C. greggii*, and derivative **6a**

Compound	Antiproliferative activity			
	HeLa	KB	HEp-2	VERO
1	192.3 ± 2.4	45.0 ± 4.5	70.2 ± 4.5	146.8 ± 6.7
2	141.9 ± 1.6	55.4 ± 5.6	53.6 ± 5.6	189.7 ± 5.2
3	48.8 ± 3.1	33.8 ± 3.1	89.8 ± 4.9	89.2 ± 2.1
4	107.7 ± 1.2	46.0 ± 4.5	152.4 ± 3.2	221.5 ± 5.6
5	125.4 ± 2.3	56.9 ± 3.4	140.0 ± 2.5	201.4 ± 3.8
6	73.9 ± 2.9	66.6 ± 7.8	98.4 ± 2.8	351.0 ± 8.9
6a	55.9 ± 2.5	19.22 ± 2.3	44.7 ± 5.6	143.5 ± 6.2
Docetaxel	0.03 ± 0.01	0.05 ± 0.04	0.06 ± 0.02	0.11 ± 0.02