Tirucallane, Apotirucallane, and Octanorapotirucallane Triterpenes of *Simarouba amara*

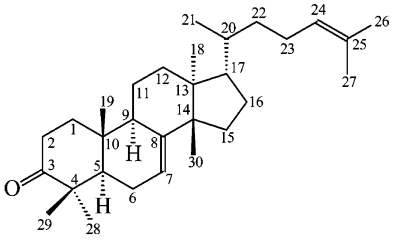
Sumieya N. J. Grosvenor,[†] Keisha Mascoll,[†] Stewart McLean,[‡] William F. Reynolds,[‡] and Winston F. Tinto*[,†]

Laboratory of Bioorganic Chemistry, Department of Biological & Chemical Sciences, The University of the West Indies, Cave Hill Campus, Bridgetown, P. O. Box 64, Barbados, BB11000, and Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 3H6, Canada

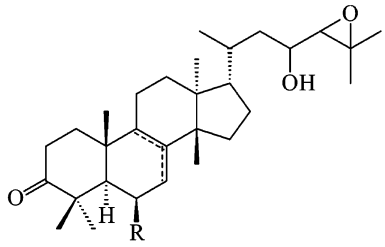
Received May 12, 2006

A chemical investigation of the bark of *Simarouba amara*, collected in Barbados, resulted in the isolation of six new triterpenes (**3**−**8**), in addition to two known compounds, 3-oxatirucalla-7, 24-dien-23-ol (**1**) and niloticin (**2**). Compound **3** is a tirucallane triterpene, while compounds **4**−**7** are apotirucallane derivatives containing an ϵ-lactone in ring A. Compounds **6** and **7** were obtained as a mixture that could not be separated, while compound **8** is an octanorapotirucallane derivative that lacks the C_8 side chain. The structures of all compounds were determined by interpretation of physical data.

Simarouba amara Aubl. (Simaroubaceae) is a medium-sized tropical tree found in Central and South America and the Caribbean Islands. The plant has an extensive history of medicinal use, especially as a source of antimalarial drugs.[1] In the Caribbean, the bark of the plant is used as a tonic and to reduce fevers.[2] Previous investigations of *S. amara* resulted in the isolation of quassinoids, alkaloids, and triterpenes.[3−8] The quassinoids are by far the most abundant metabolites of this plant and have been shown to be responsible for the antimalarial as well as antitumor activity.[1,3] As part of our investigation of the chemical constituents of the bark of *S. amara*, collected in Barbados, we now describe the isolation and structural elucidation of six new secondary metabolites along with the known triterpenes 3-oxotirucalla-7,24-dien-23-ol and niloticin.

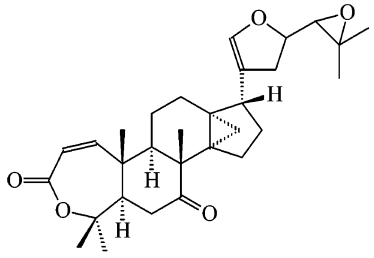


(1)

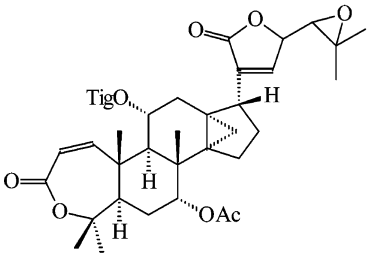


(2) Δ^7, R= H

(3) Δ^8, R= OH



(4)



(5)

Results and Discussion

Compound **3** was obtained as white needles and had the molecular formula $C_{30}H_{48}O_4$, as determined by HREIMS. The IR spectrum has absorptions characteristic of hydroxy (3383 cm^{-1}) and saturated ketone (1700 cm^{-1}) functionalities. The ^1H NMR spectrum was similar to that of compound **2**, the major differences being the absence of an olefinic proton at C-7 and the presence of an additional oxymethine proton, which resonated at δ 4.40 (1H, m, H-6). T-ROESY cross-peaks between H-5 and H-6 indicated that the 6-OH group was β-oriented. A tetrasubstituted double bond had ^{13}C NMR signals at δ 131.1 (C-8) and 148.9 (C-9). An examination of the H−H COSY, HSQC, and HMBC data confirmed the assignments for this triterpene. Compound **3** is therefore characterized as 24,25-epoxy-3-oxotirucalla-8-en-23-ol.

Simaroubin A (**4**) was isolated as a pale yellow gum and had the molecular formula $C_{30}H_{40}O_5$, on the basis of HREIMS. The IR spectrum had absorbances due to ester (1755 cm^{-1}) and saturated ketone (1704 cm^{-1}) functionalities. The ^1H NMR spectrum had resonances due to six methyl groups at δ 1.47 (H_3-19), 1.44 (H_3-28), 1.46 (H_3-29), 1.43 (H_3-30), 1.35 (H_3-27), and 1.32 (H_3-26). The α,β-unsaturated ϵ-lactone in ring A had olefinic protons at 6.43 (d, J = 12.0 Hz, H-1) and 5.92 (d, J = 12.0 Hz, H-2), while the double bond in the dihydrofuran ring had a resonance at δ 6.13 (m, H-21). The C-18 methylene protons of the cyclopropane ring resonated as doublets at δ 0.74 and 0.36 (6.7 Hz), while two correlating (COSY) oxymethine protons occurred at δ 4.32 (dt, J = 10.5, 8.0 Hz, H-23) and 2.94 (d, J = 8.0 Hz, H-24). The latter resonance was associated with a trisubstituted epoxide moiety, with ^{13}C NMR signals at δ 65.7 (C-24) and 57.4 (C-25). In addition, the ^{13}C NMR spectrum had a low-field signal due to the saturated ketone at δ 212.4, and this was placed at C-7 since it showed

* To whom correspondence should be addressed. Tel: (246) 417-4357. Fax: (246) 417-4325. E-mail: wtinto@uwichill.edu.bb.
[†] University of the West Indies.
[‡] University of Toronto.

10.1021/np060214f CCC: $33.50
Published on Web 08/19/2006

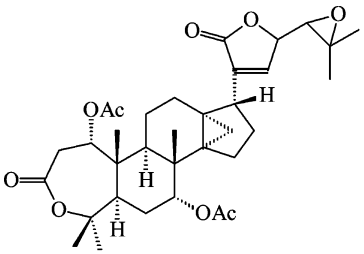
Table 1. ^{1}H NMR Data of Compounds **3**−**8** (*J* in Hz)

H	3	4	5	6	7	8
1	1.96 (m), 1.68 (m)	6.43 (d, 12.0)	6.30 (d, 12.5)	4.93 (d, 7.2)	4.81 (t, 4.1)	6.42 (d, 12.1)
2	⟨2.53⟩[a] (m)	5.92 (d, 12.0)	5.71 (d, 12.5)	3.16 (d, 15.6) 3.04 (dd, 15.6, 7.2)	⟨3.14⟩[a] (m)	5.92 (d, 12.1)
3						
4						
5	2.18 (m)	2.38 (dd, 14.5, 3.5)	2.52 (dd, 12.0, 4.0)	2.52 (dd, 12.0, 4.0)	2.51 (dd, 12.0, 4.0)	2.37 (dd, 14.5, 5.0)
6	⟨4.40⟩[a] (m)	2.86 (dd, 14.5, 13.5) 2.15 (dd, 13.5, 3.5)	⟨1.86⟩[a] (m)	1.88 (m), 1.80 (m)	1.96 (m), 1.90 (m)	2.84 (t, 14.5) 2.14 (dd, 14.5, 5.0)
7	2.22 (m), 1.47 (m)		4.98 (t, 3.2)	5.01 (dd, 3.8, 1.9)	5.19 (dd, 4.0, 2.1)	
8						
9		1.37 (m)	2.05 (d, 8.5)	1.86 (m)	2.52 (m)	1.37 (m)
10						
11	⟨2.10⟩[a] (m)	1.67 (m), 1.39 (m)	5.29 (ddd, 8.5, 7.0, 4.1)	1.36 (m), 1.22 (m)	1.54 (m), 1.42 (m)	1.67 (m), 1.36 (m)
12	⟨1.75⟩[a] (m)	1.94 (m), 1.64 (m)	2.38 (dd, 15.0, 7.0) 1.83 (dd, 15.0, 4.1)	1.86 (m), 1.56 (m)	2.02 (m), 1.68 (m)	1.30 (m), 1.27 (m)
13						
14						
15	2.03 (m), 1.31(m)	1.90 (m), 1.84 (m)	1.95 (m), 1.56 (m)	1.96 (m) 1.50 (m)	5.35 (m)	1.85 (m), 1.78 (m)
16	2.07 (m), 1.32 (m)	1.61 (m), 1.10 (m)	2.07 (m), 1.19 (m)	2.00 (m), 1.09 (m)	2.51 (m) 2.39 (dddd, 15.3, 10.7, 7.1, 3.5)	1.65 (m), 0.90 (m)
17	1.57 (m)	2.55 (dd, 11.0, 7.5)	2.90 (dd, 10.5, 7.9)	2.85 (m)	2.84 (m)	⟨1.85⟩[a] (m)
18	0.75 (s)	0.74 (d, 6.7), 0.36 (d, 6.7)	0.99 (d, 6.1), 0.70 (d, 6.1)	0.64 (d, 5.8), 0.59 (d, 5.8)	0.83 (s)	0.65 (d, 6.0), 0.34 (d, 6.0)
19	1.00 (s)	1.47 (s)	1.32 (s)	1.17(s)	1.16 (s)	1.46 (s)
20	1.43 (s)					
21	0.99 (d, 6.0)	6.13 (m)				
22	1.67 (m), 1.44 (m)	2.71 (dddd, 15.0, 10.5, 2.0, 0.9) 2.34 (dddd, 15.0, 10.5, 2.5, 1.0)	6.88 (t, 1.5)	6.90 (dd, 2.0, 1.0)	6.97 (dd, 1.5, 1.1)	
23	3.58 (m)	4.32 (dt, 10.5, 8.0)	4.76 (dt, 7.5, 1.5)	4.76 (m)	4.78 (m)	
24	2.67 (d, 8.5)	2.94 (d, 8.0)	2.58 (d, 7.5)	2.58 (d, 7.4)	2.61 (d, 7.4)	
25						
26	1.34 (s)	1.32 (s)	1.42 (s)	1.37 (s)	1.37 (s)	
27	1.32 (s)	1.35 (s)	1.36 (s)	1.45 (s)	1.45 (s)	
28	1.17 (s)	1.44 (s)	1.44 (s)	1.48 (s)	1.41 (s)	1.43 (s)
29	1.09 (s)	1.46 (s)	1.36 (s)	1.49 (s)	1.50 (s)	1.46 (s)
30	0.92 (s)	1.43 (s)	1.20 (s)	1.18 (s)	1.21 (s)	1.41 (s)
31						
32			2.13 (s)	2.09 (s)	2.09 (s)	
33						
34			6.80 (dq, 7.1, 1.9)	2.13 (s)	2.13 (s)	
35						
36			1.81 (d, 7.1)			
37			1.80 (d, 1.9)			

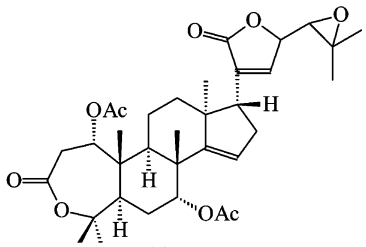
[a] Average value for an incompletely resolved CH_2 group.

HMBC correlations to the C-6 methylene protons at δ 2.86 and 2.15 (H-6), as well as the C-30 methyl group at δ 1.43. The configuration at C-23 and C-24 remains undefined. Simaroubin A (**4**), 21,23:24,25-diepoxy-7-oxo-14,18-cycloapotirucalla-1,20-dien-3,4-olide, is thus a new apotirucallane triterpene possessing both dihydrofuran and epoxide moieties in the side chain.

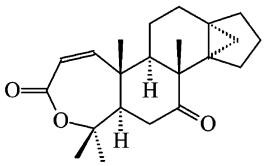
Simaroubin B (**5**) was obtained as a white opaque gum and possessed the molecular formula $C_{37}H_{48}O_9$. The IR spectrum revealed absorbances attributed to α,β-unsaturated γ-lactone (1760 cm^{-1}), saturated ester (1732 cm^{-1}), and unsaturated ester (1701 cm^{-1}) functionalities. The ^{1}H NMR spectrum of compound **5** was similar to that of **4** in that the seven-membered ring A lactone and the cyclopropane ring were present. Two olefinic methyl groups at δ 1.81 and 1.80, along with a low-field olefinic proton at δ 6.80 (dq, J = 7.1, 1.9 Hz), indicated the presence of a tiglate ester, while the *O*-acetyl's methyl group resonated at δ 2.13. There were resonances due to six other methyl groups at δ 1.44 (H-28), 1.42 (H-26), 1.36 (H-27 and H-29), 1.32 (H-19), and 1.20 (H-30). Resonances due to four oxymethine protons occurred at δ 5.29 (ddd, J = 8.5, 7.0, 4.1 Hz, H-11), 4.98 (t, J = 3.2 Hz, H-7), 4.76 (dt, J = 7.5, 1.5 Hz, H-23), and 2.58 (d, J = 7.5 Hz, H-24). In addition, there were three olefinic protons with resonances at δ 6.88 (t, J = 1.5 Hz, H-22), 6.30 (d, J = 12.5 Hz, H-1), and 5.71 (d, J = 12.5 Hz, H-2). The resonances for the C-18 methylene protons of the cyclopropane ring had doublets at δ 0.99 and 0.70 (6.1 Hz). The COSY spectrum showed cross-peaks between the oxymethine proton at δ 5.29 (H-11) and the C-12 methylene protons at δ 2.38 (dd, J = 15.0, 7.0 Hz) and 1.83 (dd, J = 15.0, 4.1 Hz) as well as a methine proton at 2.05 (d, J = 8.5 Hz, H-9). The ^{13}C NMR spectra showed resonances for 37 carbons. There were carbon signals at δ 62.8 (C-24) and 57.6 (C-25) that inferred the presence of an epoxide. In addition, there were four ester carbonyl carbons at δ 172.2 (C-21), 169.9 (C-31), 167.5 (C-3), and 167.1 (C-33). The carbon at δ 167.5 (C-3) had long-range correlations with the two olefinic protons of the α,β-unsaturated ϵ-lactone at δ 6.30 (H-1) and 5.71 (H-2) as in compound **4**. An oxymethine carbon at δ 74.1 (C-7) had HMBC correlations to methyl protons at δ 1.20 (H_3-30), while the quaternary carbon at δ 48.9 (C-9) had correlations to H_3-10 and H_3-30. The acetate group was placed at C-7 since the carbonyl carbon showed HMBC correlations to H-7. The γ-lactone carbon at δ 167.1 (C-33) exhibited long-range correlation to H-11 at δ 5.29, thereby establishing the position of the tiglate moiety. The orientation of H-7 and H-11 followed from their coupling constants and from their NOE interactions. Thus, simaroubin B (**5**), 24,25-epoxy-7-acetoxy-11-tigloloxy-7α,11α-dihydroxy-14,18-cycloapotirucalla-1,20(22)-dien-3,4:20,23-diolide, is a new apotirucallane triterpene with an α,β-unsaturated γ-lactone in the side chain, instead of a dihydrofuran as in compound **4**.



(6)



(7)



(8)

Simaroubins C (**6**) and D (**7**) could not be separated and were isolated as a mixture (ca. 2:3) of closely related isomers of molecular formula $C_{34}H_{46}O_9$. The difference between these two compounds is that in **7** the cyclopropane ring has been opened to give a methyl group at C-13 and a double bond at C-14/C-15. The seven-membered ring A lactone was saturated in both **6** and **7** and contained an acetate group at C-1, as is evident from the oxymethine proton at δ 4.93 (d, $J = 7.2$ Hz) in **6** and at 4.81 (t, $J = 4.1$ Hz) in **7**. In compound **6** the cyclopropyl protons had resonances at δ 0.64 and 0.59, while **7** had an olefinic proton at δ 5.35 and a methyl group at δ 0.83 (s). In the T-ROESY spectrum, the C-1 proton of both compounds **6** and **7** showed correlation to their respective C-19 methyl group, and this indicated that they both had the β-orientation at C-1. Thus compound **6** is characterized as 24,25-epoxy-1,7-diacetoxy-1α,7α-dihydroxy-14,18-cycloapotirucalla-20(22)-en-3,4:20,23-diolide and compound **7** as 24,25-epoxy-1,7-diacetoxy-1α,7α-dihydroxyapotirucalla-14,20(22)-dien-3,4:20,23-diolide.

Compound **8**, designated octanorsimaroubin A, had the molecular formula $C_{22}H_{30}O_3$, compared to compound **4**. The IR spectrum had absorptions at 1758 and 1703 cm^{-1} due to α,β-unsaturated ϵ-lactone and saturated ketone functionalities, respectively. The presence of the α,β-unsaturated ϵ-lactone as in **4** and **5** was confirmed by ^{1}H NMR resonances at δ 6.42 (1H, $J = 12.1$ Hz, H-1) and 5.92 (1H, $J = 12.1$ Hz, H-2) and ^{13}C NMR resonances at δ 157.3 (C-1), 121.9 (C-2), 167.3 (C-3), and 84.2 (C-4). The cyclopropane ring was evident from high-field resonances at δ 0.65 (1H, d, $J = 6.0$ Hz) and 0.34 (1H, d, $J = 6.0$ Hz). The ketone was assigned to C-7 since it showed HMBC correlations to the C-6 methylene protons at δ 2.85 and 2.14 as well as the C-30 methyl group at δ 1.41. Compound **8**, 7-oxo-20,21,22,23,24,25,26,27-octanor-14,18-cycloapotirucalla-1-en-3,4-olide, is a degraded apotirucallane triterpene lacking the C_8 side chain. This is the first report on the occurrence of tirucallane/apotiurcallane triterpenes containing the ring A ϵ-lactone from *S. amara*.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on an HP8452A diode array spectrophotometer, while IR spectra were recorded on a Nexus 670 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Unity 500 MHz spectrometer in $CDCl_3$ with TMS as internal standard. The high- and low-resolution EIMS were recorded on a Micromass 70-250S mass spectrometer at an ionizing voltage of 70 eV. Flash chromatography was performed using Merck silica gel.

Table 2. ^{13}C NMR Data of Compounds **3**−**8**[a]

C	3	4	5	6	7	8
1	34.7	157.30	156.3	70.7	71.0	157.3
2	34.2	122.0	117.8	35.4	34.8	121.9
3	217.6	167.3	167.5	170.3	170.3	167.3
4	46.6	84.2	84.2	85.6	85.4	84.2
5	44.3	55.9	48.7	43.4	44.2	56.0
6	79.2	39.1	26.9	26.3	26.3	39.1
7	24.1	212.4	74.1	74.8	74.3	212.5
8	131.1	49.8	39.1	37.9	42.6	49.9
9	148.9	48.9	48.9	37.0	35.7	48.7
10	37.9	43.1	43.6	44.3	44.3	43.1
11	22.0	19.9	68.4	16.8	16.2	19.8
12	30.6	28.2	34.1	25.9	32.7	27.5
13	49.9	29.2	27.8	28.7	47.4	44.8
14	44.1	34.0	35.1	35.6	158.6	36.2
15	29.6	28.0	26.9	27.1	118.5	29.3
16	28.7	26.9	28.2	27.9	34.0	28.0
17	50.6	44.2	42.9	43.2	50.7	29.7
18	15.6	15.5	13.7	15.0	20.9	15.1
19	18.7	15.7	17.7	15.5	15.2	15.6
20	33.8	139.4	138.3	138.8	136.9	
21	20.3	114.5	172.2	173.0	173.0	
22	40.8	33.7	141.9	141.6	144.0	
23	69.2	81.0	81.2	81.3	81.3	
24	68.4	65.7	62.8	62.9	63.0	
25	60.3	57.4	57.6	57.6	57.4	
26	24.9	19.4	19.5	24.7	24.7	
27	19.8	24.9	24.7	19.5	19.5	
28	27.0	32.1	26.0	34.4	34.4	32.1
29	21.2	26.8	24.7	23.8	23.6	26.8
30	25.7	19.5	20.1	19.9	27.4	19.4
31			169.9	169.8	169.8	
32			21.4	21.4	21.4	
33			167.1	169.6	169.6	
34			138.6	20.9	20.9	
35			128.4			
36			14.3			
37			12.2			

[a] Recorded at 125 MHz in $CDCl_3$.

Plant Material. The plant material was collected from Turner's Hall woods, St. Thomas, Barbados, in March 2002, and identified as *S. amara* Aubl. (Simaroubaceae) by Prof. Sean Carrington of this department. A voucher specimen (SG 2) was deposited in the National Herbarium, Barbados.

Extraction and Isolation. The air-dried bark (3.65 kg) of *S. amara* was extracted with MeOH (8.5 L) and the solvent evaporated in vacuo to give a dark green gum (21.3 g). The extract was triturated with CH_2Cl_2 (4 × 100 mL) to give a CH_2Cl_2-soluble fraction (11.7 g). The crude CH_2Cl_2 extract (11.7 g) was chromatographed on Si gel with a hexane/acetone (3:1) solvent system, followed by EtOAc. This produced 29 fractions, which were subsequently pooled to give nine major fractions, A−I. Fraction C was separated by preparative TLC using hexane/acetone (4:1), to give compound **5** (30 mg) and a mixture of compounds **6** and **7** (11 mg). Fraction D was separated by preparative TLC using hexane/acetone (4:1), to give compounds **1** (32 mg) and **2** (210 mg). Fraction E was separated by preparative TLC using hexane/acetone (3:1), to give compounds **4** (110 mg) and **8** (4 mg). Fraction G was purified by preparative TLC with hexane/acetone (3:1), to give compound **3** (19 mg).

Compound 3: colorless gum; $[\alpha]^{25}_D$ +17.2 (*c* 0.38, $CHCl_3$); IR ν_{max} (film) 3383, 1700, 1020 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m/z* (rel int) $[M]^+$ 472 (27), 439 (40), 383 (18), 367 (57), 339 (26), 243 (15), 190 (41), 91 (100); HREIMS 472.3522 (calcd for $C_{30}H_{48}O_4$, 472.3553).

Simaroubin A (4): pale yellow gum; $[\alpha]^{25}_D$ −3.3 (*c* 0.33, $CHCl_3$); UV λ_{max} (MeOH) (log ϵ) 222 nm (4.21); IR (film) ν_{max} 1755, 1704, 1053, 754 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS

m/*z* (rel int) $[M]^+$ 480 (11), 449 (26), 395 (41), 341 (100), 311 (62), 281 (40); HREIMS 480.2865 (calcd for $C_{30}H_{40}O_5$, 480.2876).

Simaroubin B (5): pale yellow gum; $[\alpha]^{25}_D$ +18.3 (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 226 nm (4.10); IR (film) ν_{max} 1760, 1732, 1701, 1070, 754 cm^{-1}; ^1H NMR and ^{13}C NMR data, 1 and Tables 2; EIMS *m*/*z* (rel int) $[M]^+$ 636 (1), 618 (2), 494 (12), 476 (64), 458 (28), 404 (19), 363 (23), 83 (100); HREIMS 636.3304 (calcd for $C_{37}H_{48}O_9$, 636.3298).

Simaroubins C and D (6/7): colorless gum; IR (film) ν_{max} 1756, 1732, 1025, 754 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* (rel int) $[M]^+$ 598 (5), 583 (5), 538 (8), 523 (10), 469 (70), 409(100), 105 (80); HREIMS 598.3138 (calcd for $C_{34}H_{46}O_9$, 598.3142).

Simaroubin E (8): colorless gum; $[\alpha]^{25}_D$ −1.8 (*c* 0.40, $CHCl_3$); UV λ_{max} (MeOH) (log ϵ) 223 nm (4.08); IR (film) ν_{max} 1758, 1703, 1070, 754 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* (rel int) $[M]^+$ 342 (5), 311 (15), 299 (8), 281 (10), 213 (28), 145 (24), 105 (100); HREIMS 342.2189 (calcd for $C_{22}H_{30}O_3$, 342.2195).

Acknowledgment. We wish to thank Dr. A. Young, University of Toronto, and Prof. J. D. Connolly, Glasgow University, for providing the MS data. One of us (S.N.J.G.) acknowledges the award of a postgraduate scholarship by the University of the West Indies.

References and Notes

(1) O'Neill, M. J; Bray, D. H; Boardman, P.; Wright, C. W.; Phillipson, J. D.; Warhurst, D. C.; Gupta, M. P.; Correya, M.; Solis, P. *J. Ethnopharmacol.* **1988**, *22*, 183−190.
(2) Carrington, S. *Wild Plants of Barbados*; The Macmillan Press Ltd: London and Basingstoke, 1993; p 53.
(3) Polonsky, J.; Varon, Z.; Jacquemin, H.; Pettit, G. R. *Experientia* **1978**, *34*, 1122−1123.
(4) Grandolini, G.; Cassinovi, G.; Barbetti, P.; Fardella, G. *Phytochemistry* **1987**, *26*, 3085−3087.
(5) Barbetti, P.; Grandolini, G.; Fardella, G.; Chiappini, I. *Phytochemistry* **1993**, *32*, 1007−1013.
(6) Polonsky, J.; Baskevitch-Varon, Z.; Das, B. C. *Phytochemistry* **1976**, *15*, 337−339.
(7) Lassak, E. V; Polonsky, J.; Jacquemin, H. *Phytochemistry* **1977**, *16*, 1126−1127.
(8) Bhatnagar, S.; Polonsky, J.; Prange, T.; Pascard, C. *Tetrahedron Lett.* **1984**, *25*, 299−302.

NP060214F