Tetranortriterpenoids from *Ruagea glabra*

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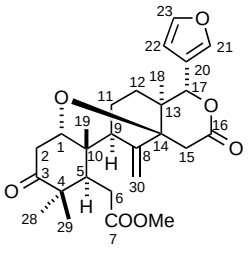
Received December 8, 1995[⊗]

Six tetranortriterpenoids including four new compounds, ruageanins A (**3**), B (**4**), C (**5**), and D (**6**), were isolated from fruits of *Ruagea glabra* (Meliaceae). Structures of the new compounds were established by analysis of the high-field NMR data. Methyl angolensate (**1**), xylocarpin (**2**), and ruageanins A (**3**) and B (**4**) showed significant antifeedant activity to the final instar larvae of *Spodoptera frugiperda*.

In our continuing studies[1] on the Meliaceae of tropical and semitropical America, we have investigated *Ruagea glabra* Triana and Planchon, a member of the Meliaceae which is closely related to *Guarea* species. The plant material was collected at an altitude of 2060 m in Cartago Costa Rica. We obtained no limonoids from the ground heartwood. However, six compounds of this type were isolated and characterized from the seeds, viz. the known compounds methyl angolensate (**1**)[2,3] and xylocarpin (**2**)[4] and the new bicyclononalides ruageanins A (**3**), B (**4**), C (**5**), and D (**6**). We report here the identification of these compounds and the structure elucidation of **3**−**6**. The latter three compounds are members of the mexicanolide group of limonoids possessing the relatively rare −OH or *O*-acyl C-2 substituent.



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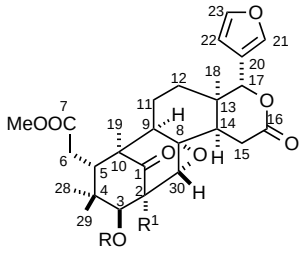
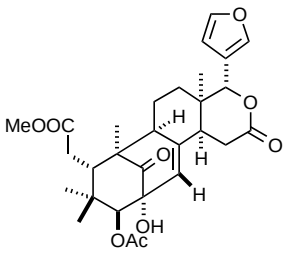


2: R = Ac, R^1 = H
3: R = iPrCO, R^1 = H
4: R = $COCCH_3{=}CHCH_3$, R^1 = OH
5: R = Ac, R^1 = OH



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Methyl angolensate (**1**) and xylocarpin (**2**) were identified by a comparison of their mp, IR, and ^{1}H-NMR data with the reported values,[2−4] as well as by a detailed analysis of the ^{1}H and ^{13}C spectral data (Tables 1 and 2). In both cases the bond connectivities were clearly established.

The ^{1}H and ^{13}C NMR data for the new compounds ruageanin A−D also allowed unambiguous assignment of the signals (Tables 1 and 2), and several $^{2,3}J_{CH}$ bond connectivities could be established for most of the carbon atoms.

Ruageanin A (**3**), $C_{31}H_{40}O_9$ (HRMS), showed absorption maxima in the IR spectrum for carbonyl (1730 cm^{-1}) and furan (875 cm^{-1}). The ^{1}H and ^{13}C NMR data were almost identical to those of xylocarpin,[4] including the characteristic proton on the epoxide ring (δ_H 3.31, d, 2.0 Hz). The substituent at C-3 now present as an isobutyryl moiety showed δ_H 1.26 (3H, d, 7.0 Hz), δ_H 1.28 (3H, d, 7.0 Hz), and δ_H 2.75 (1H, m) with the corresponding δ_C signals at 18.94, 19.45, 34.17, and 176.02. The detailed bond connectivities and assignment of all the NMR signals (^{1}H and ^{13}C) were obtained from the COSY, HMBC, and HETCOR data.

Ruageanin B (**4**) was obtained as a crystalline solid by repeated recrystallization from a mixture with humulin B.[7] The molecular formula, $C_{32}H_{40}O_{10}$, was obtained from HRMS data. Ruageanin C (**5**) was also crystalline and showed a molecular formula $C_{29}H_{36}O_{10}$. The ^{1}H NMR spectra of these two compounds are virtually identical except that ruageanin B bears an *O*-tigloyl group at C-3 which is replaced in compound **5** by an acetate. H-3 and H-30 are characteristic singlets in these molecules (for compound **5** at δ 5.09 and 3.52, respectively), and all the ^{1}H and ^{13}C assignments could be made from the NMR data. These compounds are closely related to humilinolides A−D recently reported from *Swietenia humilis*[8] and are 6-deoxy analogues of humilinolide A. The structure and stereochemistry of the latter was confirmed by single-crystal X-ray diffraction studies.[8]

Ruageanin D (**6**), the least abundant of the compounds isolated, was obtained as an amorphous solid. The molecular formula, $C_{29}H_{36}O_9$, was deduced from the ^{13}C and ^{1}H NMR spectra and confirmed by HRMS. The

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[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0163-3864(96)00139-5 CCC: $12.00

Table 1. ^{1}H-NMR Data for Compounds **2**−**4**, **6** (500 MHz), and **5** (400 MHz)[a]

	compound				
proton	**2**	**3**	**4**	**5**	**6**
H-2	3.55 (8, 2)	3.56 (12, 2)			
H-3	5.10 (8)	5.09 (12)	5.14 s	5.09 s	4.87 s
H-5	3.23 (8, 3)	3.24 (12, 5)	3.24 (10, 5)	3.15 (9, 3.5)	3.28 (9, 4)
H-6	2.34 m	2.34 m	2.35 m	2.31 m	2.36 m
H-9	1.92 m	1.90 m	1.86 (13, 3.7)	1.90 m	2.27 m
H-11	1.82 m	1.78 m	1.81 m	1.77 m	1.17 m
	1.92 m	1.90 m	1.90 m	1.90 m	1.62 m
H-12	1.20 m	1.18 m	1.22 m	1.20 m	1.42 m
	1.95 m	1.96 m	1.97 m	1.95 m	1.67 m
H-14	1.62 m	1.58 (12.5, 6)	1.61 (13, 6)	1.65 (13, 5)	2.25 m
H-15	2.78 (16, 6)	2.80 (18, 6)	2.83 (16, 6)	2.81 (16, 5)	2.84 m
	3.63 (16, 4)	3.64 (18, 12.5)	3.50 (16, 13)	3.49 (16, 13)	2.90 m
H-17	5.23 s	5.18 s	5.18 s	5.25 s	5.69 s
H-18	1.02 s	1.01 s	1.00 s	1.00 s	1.09 s
H-19	1.07 s	1.06 s	1.17 s	1.15 s	1.23 s
H-21	7.51 (1.5, 0.7)	7.48 m	7.49 (1.8, 0.8)	7.53 bs	7.81 (1.7)
H-22	6.46 (1.5, 0.7)	6.45 m	6.44 (1.8, 0.8)	6.47 (2)	6.46 (1.7)
H-23	7.43 t (2)	7.43 m	7.43 t (1.8)	7.45 t (2)	7.42 t (1.7)
H-28	0.81 s	0.81 s	0.81 s	0.79 s	0.81 s
H-29	0.78 s	0.78 s	0.79 s	0.75 s	0.76 s
H-30	3.31 (2)	3.31 (2)	3.51 s	3.52 s	5.38 t (2)
−O*CH*$_3$	3.73 s	3.72 s	3.74 s	3.72 s	3.72 s
H-2′		2.75 m			
H-3′		1.28 (7)[b]	7.04 qq (6.5, 1.5)		
2′-Me		1.26 (7)[b]	1.97 t (1)		
3′-Me			1.93 (6.5, 1)		
−OH			4.01 s	4.15 s	
−CO*CH*$_3$	2.24 s			2.28 s	2.13 s

[a] Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz. [b] Assignments interchangeable. Where signal multiplicity is not noted, d is implied. The assignments were made by a combination of COSY, HETCOR, and HMBC.

Table 2. ^{13}C-NMR Data for Compounds **2**−**4**, **6** (125 MHz), and **5** (100 MHz)[a]

	compound				
carbon	**2**	**3**	**4**	**5**	**6**
C-1	214.04	214.17	213.00	213.15	215.40
C-2	48.61	48.86	78.37	77.95	76.71
C-3	77.21	76.74	84.79	84.70	84.80
C-4	39.18	39.39	40.12	39.73	38.42
C-5	42.48	42.57	42.32	42.31	41.02
C-6	33.06	33.14	32.94	32.77	32.56
C-7	174.19	174.16	173.86	174.02	174.30
C-8	60.46	60.65	63.14	62.89	136.46
C-9	55.81	55.82	55.14	55.23	56.25
C-10	48.18	48.25	49.11	48.95	48.81
C-11	19.35	19.40	19.44	19.27	19.10
C-12	33.42	33.38	33.20	33.31	33.74
C-13	36.42	36.40	36.23	36.31	36.08
C-14	45.93	45.84	45.24	45.56	44.80
C-15	33.74	33.92	33.49	33.29	29.90
C-16	172.06	171.94	171.21	171.74	169.25
C-17	78.76	78.84	78.89	78.71	77.10
C-18	26.27	26.42	26.26	26.04	21.90
C-19	16.02	15.88	16.12	16.21	15.70
C-20	120.05	120.07	120.17	120.05	120.35
C-21	141.00	140.94	140.89	141.06	141.88
C-22	110.24	110.24	110.10	110.19	109.70
C-23	143.07	143.09	143.11	143.14	142.94
C-28	20.67	20.96	20.53	20.00	19.52
C-29	22.50	22.45	22.00	21.79	21.80
C-30	63.57	63.33	67.38	67.72	129.10
−O*CH*$_3$	52.34	52.32	52.39	52.43	52.20
C-1′		176.02	166.92		
C-2′		34.17	127.77		
C-3′		19.45[b]	139.75		
2′-*C*H$_3$		18.94[b]	12.60		
3′-*C*H$_3$			14.63		
*C*OCH$_3$	169.82			169.54	171.20
CO*C*H$_3$	20.80			20.80	20.32

[a] Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz. [b] Assignments interchangeable. Where signal multiplicity is not noted, d is implied. The assignments were made by a combination of COSY, HETCOR, and HMBC.

IR spectrum indicated hydroxyl (3450 cm^{-1}), carbonyl (1730 cm^{-1}), and furan (875 cm^{-1}) moieties.

Both the ^{13}C and ^{1}H spectra were closely similar to those of humilinolide D except that H-5 was now a

Table 3. Antifeedant Activity of Compounds **1**−**4** Bioassayed with *S. frugiperda* (Final Instar Larvae)[5]

compound	AI[a] SEM
methyl angolensate (**1**)	66.4 ± 10.63
xylocarpin (**2**)	77.8 ± 6.90
ruageanin A (**3**)	72.6 ± 19.60
ruageanin B (**4**)	86.3 ± 6.41

[a] AI represents the antifeedant index calculated from AI = [(*C* − *T*)/(*C* + *T*)]100. *C* and *T* represent the amount eaten by the larvae of the control and treatment disks, respectively (Wilcoxon's matched pairs test $p < 0.05$).

double doublet (δ_H 3.28 J = 9, 4 Hz) and C-6 was present as a methylene (δ_H 2.36, m). The C-8/C-30 trisubstituted olefin was apparent (δ_C 136.46, 129.10; δ_H 5.38, t, 2.0 Hz), and typically H-30 showed allylic coupling with both H-9 and H-14. The carbonyl of the −OAc group (δ_C 171.2) and the quaternary carbinol at C-2 (δ_C 76.71) showed HMBC with H-3 (δ_H 4.87, s). C-3 (δ_C 84.40) in turn showed $^3J_{CH}$ correlation with the protons of the *gem*-dimethyl protons at C-4. The stereochemistry was assigned by analogy with humilinolide D.[8]

Five of these compounds were subjected to an antifeedant bioassay[5,6] on the final instar larvae of *Spodoptera frugiperda* at concentrations of 1000 ppm. Compounds **1**−**4** showed comparable antifeedant activity (Table 3) which was lower than that for azadiractin[9] but more active than a model compound based on jodrellin A.[6] Compound **5** exhibited low phagostimulation.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Pye Unicam SP3-200 spectrophotometer in Nujol mulls; optical rotations were measured with a Schmidt and Haensch Polartronic-D polarimeter in $CHCl_3$ solutions. ^1H and ^{13}C spectra were recorded on a Varian XL 400 or a Varian Unity 500 spectrometer in $CDCl_3$ solutions with TMS as internal standard.

Mass spectra data were recorded on a VG 70-250S mass spectrometer operating at 70 eV (direct insertion). Silica gel 60 $PF_{254+366}$ (Merck) was used for vacuum liquid chromatography (VLC), TLC, and PTLC. Flash chromatography was done on silica gel 60 (230−400 mesh, Merck).

Plant Material. The plant material was collected in February 1992 at 2060 m in Cartago Costa Rica by Dr. T. D. Pennington and P. E. Owen of The Royal Botanic Gardens, Kew, England where a voucher specimen has been deposited (Pennington 13605).

Extraction and Isolation. The dried ground fruits (341.1 g) were exhaustively extracted with benzene to yield a dark-green viscous oil (91.9 g). The latter was washed with petroleum ether (60−80 °C) and triturated with EtOAc to yield a brown gum on evaporation of the solvent (26.6 g). The EtOAc-soluble fraction was subjected to extensive VLC, flash chromatography, and preparative TLC to yield the reported compounds. Yields are quoted as percentages based on the dry weight of ground plant material.

The EtOAc extract was subjected to VLC using $CHCl_3$ with increasing quantities of EtOAc as eluent. Fractions were grouped on the basis of their TLC profiles into three major fractions (F1−F3). F1 (90% $CHCl_3$/10% EtOAc) and F2 (90% $CHCl_3$/10% EtOAc) were subjected to flash chromatography using the same solvent system as used for VLC. F1 yielded subfractions containing a mixture of two compounds which were further separated by PTLC ($CHCl_3$−EtOAc, 10:1, ×2) into methyl angolensate (**1**) (0.01%) and xylocarpin (**2**) (0.008%). A later fraction from the flash column of F1 yielded ruageanin A (**3**) (0.01%) which was finally purified by PTLC ($CHCl_3$−EtOAc, 8:1, ×3). F2 yielded ruageanin B (**4**) (0.03%) purified by PTLC (petroleum ether−EtOAc, 2:1, ×2) and repeated recrystallization from EtOAc/MeOH. F3 (85% $CHCl_3$/15% EtOAc) was subjected to PTLC ($CHCl_3$−EtOAc, 8:1, ×3) to yield ruageanin C (**5**) (0.06%) and a more polar band which on further PTLC (hexane−EtOAc, 2:1 ×4) gave ruageanin D (**6**) (0.004%).

Methyl angolensate (1): crystalline solid (MeOH); mp 201−203.5 °C (lit.[2] mp 205 °C); $[\alpha]^{25}_D$ −7.3° (c = 0.15) (lit.[3] $[\alpha]^{25}_D$ −4.2° (c = 2.14)); IR ν_{max} 1735, 1715, 875 cm^{-1}; EIMS m/z [M$^+$] 470 (100), 455 (5), 439 (4), 397 (4), 375 (25), 359 (33), 332 (26), 243 (17), 227 (9), 210 (28); HREIMS m/z 470.2292, calcd for $C_{27}H_{34}O_7$ 470.2305.

Xylocarpin (2): amorphous solid; $[\alpha]^{25}_D$ −79.8° (c = 0.25) (lit.[4] $[\alpha]^{25}_D$ −88°); IR ν_{max} 1730, 878 cm^{-1}; EIMS m/z [M$^+$] 528 (14), 510 (3), 466 (3), 435 (3), 414 (2), 390 (22), 330 (8), 302 (20), 281 (25), 239 (26), 221 (100), 149 (46), 69 (67); HREIMS m/z 528.2878, calcd for $C_{29}H_{36}O_9$ 528.2859.

Ruageanin A (3): amorphous solid; $[\alpha]^{25}_D$ −63.9° (c = 0.74); IR ν_{max} 1730, 875 cm^{-1}; EIMS m/z [M$^+$] 556 (23), 468 (7), 437 (4), 418 (28), 390 (7), 348 (8), 330 (25), 309 (52), 286 (8), 257 (8), 239 (30), 221 (100), 71 (33); HREIMS m/z [M$^+$] 556.2681, calcd for $C_{31}H_{40}O_9$ 556.2672.

Ruageanin B (4): crystalline solid (EtOAc/MeOH); mp 227−229 °C; $[\alpha]^{25}_D$ −17.7 (c = 0.52); IR ν_{max} 3500, 1735, 1645, 880 cm^{-1}; EIMS m/z [M$^+$] 584 (5), 566 (1), 502 (1), 485 (2), 467 (6), 425 (3), 407 (2), 289 (2), 224 (6), 196 (6), 164 (10), 141 (10), 121 (9), 95 (24), 83 (100), 67 (7), 55 (66); HREIMS m/z [M$^+$] 584.2629, calcd for $C_{32}H_{40}O_{10}$ 584.2621.

Ruageanin C (5): crystalline solid (MeOH); mp 217.5−219 °C; $[\alpha]^{25}_D$ −15.9° (c = 0.64); IR ν_{max} 3510, 1735, 1645, 875 cm^{-1}; EIMS m/z [M$^+$] 544 (49), 529 (1), 502 (2), 484 (16), 453 (2), 406 (34), 389 (3), 297 (14), 243 (26), 224 (46), 196 (47), 164 (67), 137 (86), 122 (100), 95 (84); HREIMS m/z [M$^+$] 544.2311, calcd for $C_{29}H_{36}O_{10}$ 544.2308.

Ruageanin D (6): amorphous solid; $[\alpha]^{25}_D$ −55.9° (c = 0.15); IR ν_{max} 3450, 1730, 875 cm^{-1}; EIMS m/z [M$^+$] 528 (9), 486 (6), 447 (12), 429 (11), 411 (23), 379 (19), 343 (6), 245 (11), 196 (17), 173 (14), 149 (31), 91 (47), 83 (100), 89 (63), 55 (90); HREIMS 528.2347, calcd for $C_{29}H_{36}O_9$ 528.2359.

Feeding Inhibition Assay on the Final Instar Larvae of *S. frugiperda*. The method used was that developed by Simmonds et al.[5] The test insects were final instar larvae of *S. frugiperda* (J. E. Smith) (Lepidoptera:Noctuidae) which were reared in the laboratory at 25.0 ± 1.0 °C. Larvae were fed on young leaves of maize (*Zea mays*) and their development monitored daily. The larvae were 24−36 h into the final instar and had been deprived of food for 4 h prior to being individually placed in Petri dishes.

Compounds were assayed for antifeedant activity by presentation on glass fiber disks (Whatman GF/A or GF/

C, 2.1 cm diameter) to final instar *S. frugiperda* larvae. The test compounds were dissolved in ethanol and assayed at 1000 ppm. Disks were made palatable by the addition of 100 μL of sucrose (0.05 M).

Ten Petri dishes each containing one larva and two glass fiber disks [control (C) and treatment (T)] were used for every compound. The control disk (C) had 100 μL of sucrose and 100 μL of ethanol, while the treatment disk (T) had sucrose and a 100 μL aliquot of one of the test solutions (compound + ethanol). Both disks were dried and weighed before being presented to the larva. The duration of the bioassay was 18 h after which the disks were reweighed and the amount of each disk consumed noted.

The antifeedant index (AI) was calculated from

$$[(C - T)/(C + T)]100$$

where C and T are the amounts of the control and treatment discs eaten, respectively. This index identified both phagostimulants (−AI values) and antifeedants (+AI values).

Acknowledgment. We thank Dr. T. D. Pennington, Kew Gardens, England, for collection of plant material. Research at the University of Toronto was supported by grants from the Natural Sciences and Engineering Research Council of Canada. The Canadian International Development Agency−University of the West Indies Institutional Strengthening Project−Sustainable Development No. 13 generously supported the Toronto/University of the West Indies collaboration. One of us (R.R.) acknowledges the award of a University of the West Indies Postgraduate Scholarship.

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NP960139G