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MINOR LIMONOIDS FROM *TRICHILIA RUBRA*

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Key Word Index—*Trichilia rubra*; Meliaceae; root; limonoids; rubralins A–C.

Abstract—Three minor limonoid components, rubralins A–C, were isolated from the root of *Trichilia rubra*, whose structures were determined by extensive spectroscopic studies. They showed moderate inhibitory activity in a β_2-integrin mediated cell adhesion assay.

INTRODUCTION

Trichilia rubra C. DC., a small tree native to the Amazon basin, generally prefers river banks and permanently flooded areas [1]. No phytochemical studies have been published previously on this species, even though related *Trichilia* species have yielded a number of interesting compounds, particularly limonoids [2–4]. Recently, we reported on the isolation and characterization of a series of A, B-seco-limonoids from the root of *Trichilia rubra* [5] possessing potent cell adhesion inhibitory activity. These included five new compounds, rubrins A–E, hispidin A [4] and nymania I [6]. Further investigation of the root material provided three minor tetracyclic limonoids exhibiting moderate bioactivity. These compounds, which we now name rubralins A (**1**), B (**2**) and C (**3**), are possible biosynthetic precursors of the A, B-seco limonoid rubrins [7, 8].

RESULTS AND DISCUSSION

The root material of *Trichilia rubra* was ground and subjected to Soxhlet extraction with dichloromethane for 24 hr. Subsequent steps of silica flash chromatography, normal phase and reversed phase HPLC led to the isolation of three pure compounds, rubralins A (**1**), B (**2**) and C (**3**).

The high resolution FAB-mass spectral data of rubralin A (**1**) indicated a molecular formula of $C_{42}H_{60}O_{13}$. The ^1H and ^{13}C NMR spectra suggested a tetracyclic triterpenoid skeleton which was found in a group of compounds, reported by Mulholland and Taylor [9], in *Trichilia dregeana*. Eleven of the 13 oxygens were accounted for by a furan, two acetyl and two α-hydroxy-ester substitutions, the remaining two oxygens were found to be part of a lactone ring, which was supported by the presence of five carbonyl carbon peaks between δ168.6 and 174.7 in the ^{13}C spectrum. Data from DQF-COSY and HMBC experiments provided further evidence of the tetracyclic skeleton with a lactone A-ring and helped to establish the two ester groups as 2-hydroxy-3-methyl-valerates. HMBC data also confirmed the site of attachments of the individual ester subsititutions: correlations were observed from H-1 and H-2 to C-3 (δ168.6), from H-1 and H-2′ to C-1′ (δ169.4), from H-12 and H-2‴ to C-1‴ (δ170.8), from H-7 and H-2″ to C-1″ (δ173.9) and from H-29α, H-29β and H-2⁗ to C-1⁗ (δ174.7). The relative stereochemistry of the chiral carbons in the skeleton of **1** was based on coupling constant data and through-space correlations from a NOESY experiment.

The molecular formula of **2** was established as $C_{41}H_{58}O_{13}$ based on HRFAB-mass spectroscopy. The NMR spectra of **2** were very similar to those of **1**, with the exception of some of the aliphatic proton and carbon chemical shifts. DQF-COSY and HMBC spectra revealed that instead of two 2-hydroxy-3-methyl-valerate groups, one of the ester substituents is 2-hydroxy-3-methyl-butyrate in **2**. The positions of the individual ester groups were unambiguously determined by HMBC spectroscopy. The molecular formula of **2** is identical to the formula of dregeana-4 (**4**) [9]. The spectral data of **4**, reported by Mulholland and Taylor, are also very similar to the data obtained for **2**, but a different arrangement of ester substitutions was assigned to **4** based on chemical shift arguments [9]. NOESY spectroscopy provided further supporting evidence for our assignments. Cross-peaks were found between H-4″ and H-15, H-5″ and H-15, H-2‴ and H-21 and H-2⁗ and H-22.

$C_{35}H_{46}O_9$ was established by HRFAB-mass spectroscopy as the molecular formula of **3**. The ^1H and ^{13}C NMR spectra suggested the same tetracyclic skeleton

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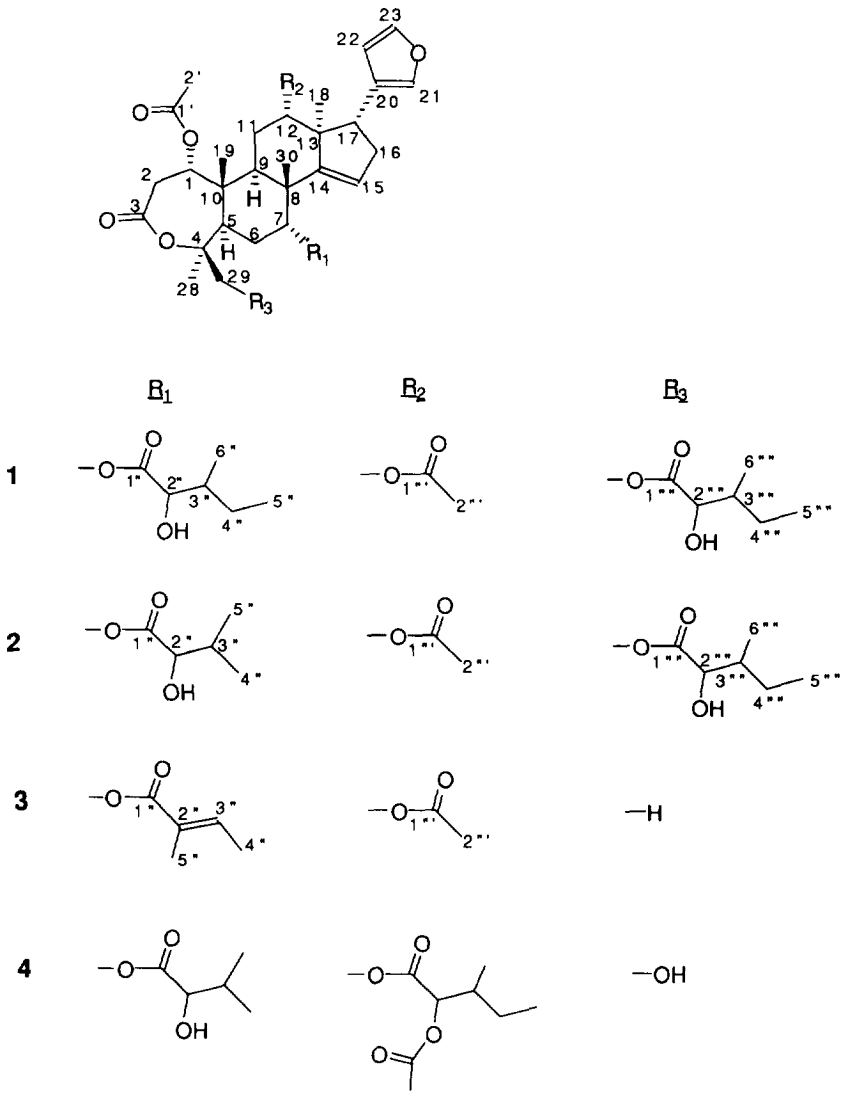

as in **1** and **2**, but without the esterification of the C-29 methyl group. COSY spectroscopy revealed tiglate substitution at C7 and acetates at C1 and C12. Decomposition of **3** prevented the application of heteronuclear correlation spectroscopy, and the proposed, structure was based on spectral similarity to **1** and **2**.

Compounds **1–3** may be biosynthetic precursors of the more abundant A, B-seco-limonoid rubrins [5, 8]. They were tested in the LFA-1: ICAM-1 mediated cell adhesion assay [5]. Compounds **1** and **2** showed moderate inhibitory activity with IC_{50} values of 30–50 μM, three orders of magnitude less than the rubrins. This difference in bioactivities may be due to the structural features of the differing A- and B-rings.

EXPERIMENTAL

General. Low and high resolution mass spectral data were obtained in positive fast atom bombardment (FAB) mode using a Cs^+ ion gun at 30 kV. The samples were dissolved in methanol and meta-nitrobenzyl alcohol (mNBA) was used as the matrix. NMR spectra were recorded on Varian Gemini 300, Bruker AMX-360 or Bruker AMX-500 spectrometers at 27°. The samples were dissolved in $CDCl_3$. The chemical shifts are reported as δ values in ppm relative to the signal of TMS, solvent peaks were used as reference. COSY [10, 11], DQF-COSY [12], NOESY [13], HMQC [14] and HMBC [15] spectra were obtained using standard pulse sequences. NOESY and HMQC spectra were recorded in phase-sensitive mode using time proportional phase incrementation (TPPI) [16, 17]. Mixing times of 400 msec were used in the NOESY spectra. The HMQC and HMBC experiments were optimized for one-bond proton–carbon couplings of about 140 Hz and for long range proton–carbon couplings of about 6.5 Hz, respectively. Typically 1024 × 512 data points were acquired and linear prediction was used in the t1 domain to 1024 points. The time domain data were Fourier transformed

after multiplication with appropriate window functions in both dimensions.

Plant material. Twigs, leaves, stem, bark and root material of *Trichilia rubra* were collected in the Iquitos area in Peru and taxonomically identified by Professor S. McDaniel (Mississippi State University). Voucher specimens are deposited in the herbarium of the Mississippi State University.

Isolation procedure. Dry root material (340 g) was ground and subjected to Soxhlet extraction with CH_2Cl_2 for 24 hr. Silica flash CC was used with a hexane–isopropanol gradient to fractionate the extract. Twenty-five fractions were collected and, based on normal and reversed phase TLC, 7 fractions were pooled. The components of fr. 4 were sepd by semi-prep. normal phase HPLC on a Waters system using a YMC A-023 silica column, with hexane–isopropanol gradient and photodiode array detection. The compounds were purified by semi-preparative reversed phase HPLC using a YMC ODS A-323 column with a methanol–water gradient and with photodiode array detection, yielding 5, 5 and 2 mg for **1**, **2** and **3**, respectively, after drying under N_2. All 3 compounds were obtained as non-crystalline solids.

Rubralin A (**1**). $[\alpha]_D^{25}$ −4.9° (MeOH; *c* 2.0); UV λ_{max}^{MeOH} nm (log ε): 215 (3.75); FTIR ν_{max}^{KBr} cm^{-1} 3540, 2998, 1730, 1255, 1140, 1010; HRFAB-MS *m/z* 773.4086 $[M + H]^+$, $C_{42}H_{61}O_{13}$ requires 773.41122; ^1H NMR data in Table 1, ^{13}C NMR data in Table 2.

Rubralin B (**2**). $[\alpha]_D^{25}$ −6.1° (MeOH; *c* 1.1); UV λ_{max}^{MeOH} nm (log ε): 215 (3.72); FTIR ν_{max}^{KBr} cm^{-1}: 3550, 2995, 1725, 1640, 1260, 1145, 1010; HRFAB-MS *m/z* 759.3968 $[M + H]^+$, $C_{41}H_{59}O_{13}$ requires 759.3956; ^1H NMR data in Table 1, ^{13}C NMR data in Table 2.

Rubralin C (**3**). $[\alpha]_D^{25}$ −8.1° (MeOH; *c* 0.8); UV λ_{max}^{MeOH} nm (log ε): 215 (3.68); FTIR ν_{max}^{KBr} cm^{-1}: 3550, 2995, 1730, 1262, 1140; HRFAB-MS *m/z* 611.3219 $[M + H]^+$, $C_{35}H_{47}O_9$ requires 611.3220; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2.

Cell adhesion assay. For the description of the bioassay see ref. [5].

Table 1. ^1H NMR spectral data for compounds **1–3** (chemical shifts in ppm and coupling constants in Hz in parentheses, in $CDCl_3$, 360 MHz)

H	1	2	3
1	4.73 *dd* (3.1, 4.8)	4.75 *dd* (3.5, 4.5)	4.75 *dd* (2.7, 4.6)
2	3.19 *m*	3.20 *m*	3.16 *m*
5	2.52 *m*	2.56 *d* (10.9)	2.55 *dd* (2.4, 12.8)
6α	1.99 *m*	1.95 *m*	2.06 *m*
6β	2.13 *m*	2.10 *m*	2.11 *m*
7	5.27 *m*	5.29 *m*	5.26 *m* (1.7)
9	2.70 *dd* (8.1, 11.8)	2.73 *dd* (8.1, 11.7)	2.83 *dd* (8.2, 11.7)
11α	1.32 *m*	1.30 *m*	1.20 *m*
11β	2.07 *m*	2.07 *m*	2.10 *m*
12	5.04 *t* (8.2)	5.06 *t* (8.3)	5.06 *t* (8.3)
15	5.48 *t* (2.4)	5.51 *br s*	5.50 *d* (2.5)
16	2.40 *m*	2.41 *m*	2.39 *dd* (2.5, 9.2)
17	3.00 *dd* (8.4, 10.2)	3.03 *dd* (7.9, 10.6)	2.98 *d* (9.3)
18	0.94 *s*	0.95 *s*	0.95 *s*
19	1.17 *s*	1.20 *s*	1.20 *s*
21	7.16 *s*	7.18 *s*	7.19 *s*
22	6.20 *dd* (0.8, 1.7)	6.22 *s*	6.23 *s*
23	7.32 *d* (1.7)	7.34 *s*	7.34 *d* (1.6)
28	1.41 *s*	1.43 *s*	1.51 *s*
29α	4.02 *d* (12.5)	4.05 *d* (12.3)	1.37 *s*
29β	4.91 *d* (12.4)	4.93 *d* (12.3)	
30	1.23 *s*	1.25 *s*	1.24 *s*
2′	2.05 *s*	2.07 *s*	2.07 *s*
2″	4.03 *d* (3.5)	4.03 *m*	
3″	1.79 *m*	2.06 *m*	6.88 *q* (7.1)
4″	1.31 *m*	1.09 *d* (7.0)	1.82 *d* (7.0)
5″	0.87 *t* (7.4)	0.87 *d* (6.9)	1.86 *s*
6″	0.98 *d* (6.9)		
2‴	1.90 *s*	1.93 *s*	1.93 *s*
2″″	4.12 *d* (3.7)	4.14 *d* (3.8)	
3″″	1.79 *m*	1.83 *m*	
4″″	1.31 *m*	1.28 *m*	
5″″	0.92 *t* (7.6)	0.90 *t* (7.4)	
6″″	1.03 *d* (8.0)	1.00 *d* (7.6)	

Table 2. ^{13}C NMR spectral data for compounds **1–3** (chemical shifts in ppm, in $CDCl_3$, 90 MHz)

C	1	2	3
1	70.4	70.4	71.0
2	34.9	35.0	34.9
3	168.6	168.5	169.5
4	85.1	85.0	85.5
5	44.4	44.4	44.2
6	26.3	26.4	26.1
7	75.3	75.2	73.2
8	41.4	41.4	41.8
9	37.2	37.2	37.1
10	44.1	44.2	44.0
11	25.4	25.4	25.4
12	76.5	76.5	73.6
13	51.2	51.2	51.2
14	155.1	155.1	155.6
15	122.3	122.4	122.0
16	36.7	36.7	36.7
17	49.9	49.9	50.0
18	15.0	14.7	15.0
19	15.3	15.3	15.3
20	124.1	124.1	124.4
21	140.2	140.3	140.3
22	111.5	111.5	111.7
23	142.2	142.2	142.1
28	29.0	29.1	23.8
29	65.6	65.6	34.5
30	28.2	28.1	27.9
1′	169.4	169.4	170.1
2′	20.7	20.6	20.6
1″	173.9	173.9	166.7
2″	75.0	75.0	128.6
3″	38.4	31.8	137.6
4″	22.8	19.3	12.0
5″	11.7	15.7	14.4
6″	15.3		
1‴	170.8	170.8	170.8
2‴	21.9	21.3	21.3
1⁗	174.7	174.7	
2⁗	75.7	75.5	
3⁗	39.0	39.0	
4⁗	23.7	23.7	
5⁗	11.7	11.7	
6⁗	15.4	15.4	

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REFERENCES

1. Pennington, T. D. (1981) in *Flora Neotropica*, Monograph No. 28. The New York Botanical Garden, New York.
2. Gullo, V. P., Miura, I., Nakanishi, K., Cameron, A. F., Connolly, J. D., Duncanson, F. D., Harding, A. E., McCrindle, R. and Taylor, D. A. H. (1975) *J. Chem. Soc., Chem. Commun.* 345.
3. Connolly, J. D., Okorie, D. A., De Wit, L. D. and Taylor, D. A. H. (1976) *J. Chem. Soc., Chem. Commun.* 909.
4. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J. R., Tempesta, M. S. and Bates, R. B. (1981) *J. Org. Chem.* **46**, 641.
5. Musza, L. L., Killar, L. M., Speight, P. McElhiney, S., Barrow, C. J., Gillum, A. M. and Cooper, R. (1994) *Tetrahedron* **50**, 11369.
6. McLachlan, L. K. and Taylor, D. A. H. (1982) *Phytochemistry* **21**, 1701.
7. Taylor, D. A. H. (1984) *Prog. Chem. Org. Nat. Prod.* **45**, 1.
8. Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N. (1992) *Phytochemistry* **31**, 377.
9. Mulholland, D. A., Taylor, D. A. H. (1980) *Phytochemistry* **19**, 2421.
10. Aue, W. P., Bartholdi, E. and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 2229.
11. Bax, A., Freeman, R. and Morris, G. A. (1981) *J. Magn. Res.* **42**, 164.
12. Piantini, U., Sorensen, O. W. and Ernst, R. R. (1982) *J. Am. Chem. Soc.* **104**, 6800.
13. Jeener, J., Meier, B. H., Bachmann, P. and Ernst, R. R. (1979) *J. Chem. Phys.* **71**, 4546.
14. Bax, A. and Subramanian, S. (1986) *J. Magn. Res.* **67**, 565.
15. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.
16. Drobny, G., Pines, A. Sinton, S. Weitekamp, D. and Wemmer, D. (1979) *Soc. Faraday Symp.* **13**, 49.
17. Marion, D. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.* **113**, 967.