ORGANIC LETTERS

2006
Vol. 8, No. 17
3845−3848

Turrapubesins A and B, First Examples of Halogenated and Maleimide-Bearing Limonoids in Nature from *Turraea pubescens*

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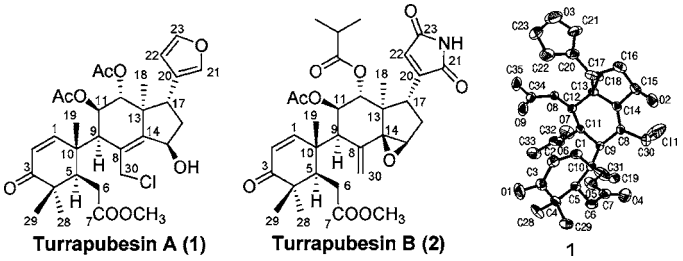
Received June 15, 2006

ABSTRACT



Two novel tetranortriterpenoids, turrapubesins A (1) and B (2), representing the first examples of halogenated and maleimide-bearing limonoids, were isolated from the twigs and leaves of *Turraea pubescens*. The structures of 1 and 2 were elucidated by extensive spectroscopic analysis. Their absolute configurations were determined by X-ray crystallography of 1 and by CD analysis of a dihydrogenated derivative of 2. Turrapubesin A (1) exhibited weak cytotoxicity against the P-388 tumor cell line.

Limonoids are a class of highly oxygenated nortriterpenoids, either containing or derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton, and present a wide range of biological activities, such as insect antifeeding, antibacterial, antifungal, antiviral, antimalarial, and anticancer properties.[1] The plants belonging to the families of Meliaceae and Rutaceae are rich sources of these fascinating metabolites.[1] Previous studies on the genus of *Turraea* have afforded a series of protolimonoids and limonoids.[2] The titled plant material of *T. pubescens* has been used in the remedies of dysentery, pharyngolaryngitis, and traumatic hemorrhage.[3] In this study, turrapubesins A (**1**) and B (**2**), the first examples of halogenated and maleimide-bearing limonoids in nature, were isolated from the twigs and leaves of *Turraea pubescens* Hellen (Meliaceae). We report herein the isolation, structural elucidation, and biological activities of the two compounds.

The air-dried powder of the plant material (5 kg) was percolated with 95% EtOH to give 600 g of crude extract, which was then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction (211 g) was

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(2) (a) Mulholland, D. A.; Parel, B.; Coombes, P. H. *Curr. Org. Chem.* **2000**, *4*, 1011−1054. (b) McFarland, K.; Mulholland, D. A.; Fraser, L. A. *Phytochemistry* **2004**, *65*, 2031−2037. (c) Ndung'u, M.; Hassanali, A.; Hooper, A. M.; Chhabra, S.; Miller, T. A.; Paul, R. L.; Torto, B. *Phytochemistry* **2003**, *64*, 817−823. (d) Cheplogoi, P. K.; Mulholland, D. A. *Phytochemistry* **2003**, *62*, 1173−1178. (e) Cheplogoi, P. K.; Mulholland, D. A. *Biochem. Syst. Ecol.* **2003**, *31*, 799−803. (f) Ndung'u, M. W.; Kaoneka, B.; Hassanali, A.; Lwande, W.; Hooper, A. M.; Tayman, F.; Zerbe, O.; Torto, B. *J. Agric. Food. Chem.* **2004**, *52*, 5027−5031.

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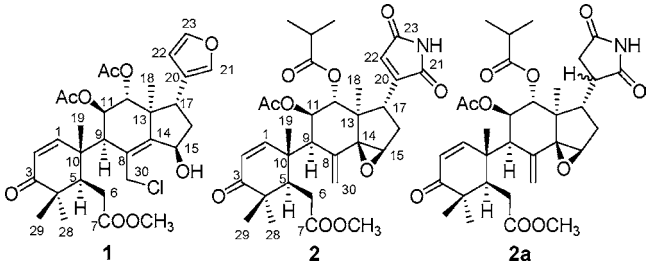
10.1021/ol061466a CCC: $33.50
Published on Web 07/19/2006

chromatographed on a silica gel column (petroleum ether/Me_2CO, 10:1 to 0:1) to give six fractions 1−6. Fraction 5 (50 g) was separated on an MCI gel column (MeOH/H_2O, 5:5 to 9:1) to give six subfractions 5a−5f. Fraction 5b (4 g) was extensively separated over silica gel, RP-18 silica gel, and Sephadex LH-20 to obtain the major components, two of which were further purified on preparative HPLC (Waters 515 pump and Waters 2487 detector, YMC-Pack ODS-A column, 250 × 10 mm, CH_3CN/H_2O 60:40) to yield **1** (60 mg) and **2** (45 mg).



Turrapubesin A (**1**),[4] a colorless crystal (in MeOH), showed the molecular formula $C_{31}H_{39}ClO_9$ as determined by HREIMS at *m*/*z* 572.2163 $[M - H_2O]^+$ (calcd 572.2177), requiring 12 double bond equivalents. The positive mode of ESIMS at *m*/*z* 613 $[M + Na]^+$ and an isotopic ion at *m*/*z* 615 with ca. 30% intensity further secured the molecular formula. The IR absorptions revealed the presence of hydroxyl (3492 cm^{-1}), carbonyl (1753, 1743, and 1722 cm^{-1}), and conjugated carbonyl (1682 cm^{-1}) functionalities. The ^{13}C NMR resolved 31 carbon signals, which were classified by chemical shifts and HMQC spectrum as 7 methyls, 3 methylenes, 11 methines (three oxygenated and five olefinic ones), and 10 quaternary carbons (one ketone, three ester, and three olefinic carbons). In addition, four tertiary methyls (δ_H 1.04, 1.12, 1.14, and 1.19), one methoxy group (δ_H 3.70; δ_C 52.2), two acetyls, a typical chlorinated methylene[5] (δ_H 4.38 and 4.92, d, $J = 12.3$ Hz; δ_C 46.3), and a β-substituted furyl ring were distinguished by analysis of its NMR data (Table 1). The spectral data aforementioned implied a limonoid feature of **1**.

The 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) experiments further revealed the planar structure of **1** with a unique chlorinated limonoid feature (Supporting Information). The relative stereochemistry of **1** was mainly deduced by NOESY spectrum (Supporting Information) and comparison of the ^1H NMR data with those of the reported B-*seco* limonoids.[6] Single-crystal X-ray diffraction analysis[7] confirmed the above conclusion. The anomalous dispersion of the chlorine atom of **1** also allowed the determination of its absolute configuration as depicted in Figure 1 with the absolute parameter of 0.04(13).[8] This is the first report on the determination of the absolute configuration of a limonoid by a chlorine-based X-ray crystallography.

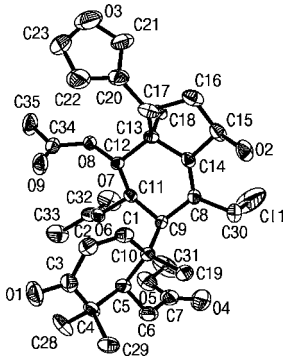


Figure 1. X-ray structure of **1** showing the absolute configuration.

Turrapubesin B (**2**),[9] a white amorphous solid, presented a molecular formula of $C_{33}H_{41}NO_{10}$ as determined by HREIMS at *m*/*z* 611.2739 $[M]^+$ (calcd 611.2730). The IR absorptions at 1728 and 1678 cm^{-1} were ascribable to the carbonyl and conjugated carbonyls, respectively. The NMR data (Table 1) revealed typical characteristics of a ring B-*seco* limonoid[2d] for **2** with an α,β-unsaturated ketone, the C-8/C-30 double bond, and the 14,15-epoxide, which were verified by HSQC, $^1H-^1H$ COSY, and HMBC spectra. The most striking feature of **2** was the absence of the β-substituted furan ring and the presence of a maleimide ring, and the latter was deduced from the ^{13}C NMR data showing two carbonyls at δ 170.5 (C-21) and 169.4 (C-23) and two sp^2 carbons at δ 148.1 (C-20) and 130.4 (C-22). Correspondingly, a singlet proton signal at δ 6.29 in the ^1H NMR was assigned to H-22, which correlated with C-17, C-20, and C-23 in the HMBC spectrum. The NH proton signal in the maleimide ring was not observed due likely to its exchangeable nature.[10] In addition, the HMBC correlations also allowed the attachment of acetoxyl and the isobutanoyloxyl at C-11 and C-12, respectively.

(4) Turrapubesin A (**1**): Colorless crystals (MeOH), mp 181−182 °C; $[\alpha]_D^{20}$ +74.3° (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.06) nm; CD (MeOH) 210 ($\Delta\epsilon$ −3.04), 244 ($\Delta\epsilon$ +8.63), 328 ($\Delta\epsilon$ +2.40) nm; IR (KBr) ν_{max} 3492, 2962, 1753, 1743, 1722, 1682, 1369, 1244 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS 70 eV *m*/*z* (relative intensity) 574 (4), 572 $[M - H_2O]^+$ (12), 554 (12), 494 (10), 434 (22), 297 (42), 210 (76), 149 (100), 121 (36); positive ESIMS *m*/*z* (relative intensity) 615 (37), 613 $[M + Na]^+$ (100), 577 $[M + Na - HCl]^+$ (33); HREIMS *m*/*z* 572.2163 $[M - H_2O]^+$ (calcd for $C_{31}H_{37}ClO_8$, 572.2177).

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(7) Crystallographic data for turrapubesin A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-608783). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223−336−033; or email: deposit@ccdc.cam.ac.uk].

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(9) Turrapubesin B (**2**): White amorphous solid; $[\alpha]_D^{20}$ +96.1° (*c* 0.230, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.16) nm; CD (MeOH) 205 ($\Delta\epsilon$ +2.70), 237 ($\Delta\epsilon$ −5.86), 293 ($\Delta\epsilon$ +2.36), 325 ($\Delta\epsilon$ +2.29) nm; IR (KBr) ν_{max} 3435, 2925, 1728, 1678, 1460, 1384, 1226 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS 70 eV *m*/*z* (relative intensity) 611 $[M]^+$ (6), 551 (4), 463 (13), 388 (20), 254 (37), 208 (100), 149 (82), 105 (32), 71 (56); positive ESIMS *m*/*z* (relative intensity) 634 $[M + Na]^+$ (100), 1245 $[2M + Na]^+$ (10); negative ESIMS *m*/*z* (relative intensity) 610 $[M - H]^-$ (100); HREIMS *m*/*z* 611.2739 (calcd for $C_{33}H_{41}NO_{10}$, 611.2730).

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** (in $CDCl_3$)

	1		**2**	
no.	δ_H (mult, *J*, Hz)[a]	δ_C[b]	δ_H (mult, *J*, Hz)[c]	δ_C[d]
1	6.83 (d, 10.4)	152.8 d	7.39 (d, 10.6)	151.9 d
2	6.02 (d, 10.4)	123.9 d	6.21 (d, 10.6)	126.0 d
3		203.5 s		203.8 s
4		46.2 s		46.2 s
5	3.09 (dd, 6.7, 4.2)	45.0 d	2.90 (dd, 7.6, 2.1)	45.2 d
6α	2.58 (dd, 17.0, 4.2)	32.5 t	2.32 (dd, 17.0, 2.1)	31.3 t
6*β*	2.52 (dd, 17.0, 6.7)		2.45 (dd, 17.0, 7.6)	
7		174.3 s		174.1 s
8		128.7 s		136.1 s
9	3.42 (d, 6.1)	46.7 d	2.97 (d, 7.1)	52.8 d
10		44.5 s		42.0 s
11	5.56 (dd, 11.3, 6.1)	70.5 d	5.55 (dd, 10.6, 7.1)	71.2 d
12	5.68 (d, 11.3)	73.2 d	5.69 (d, 10.6)	74.6 d
13		50.2 s		46.8 s
14		154.7 s		71.0 s
15	5.08 (br d, 7.3)	69.7 d	3.92 (s)	59.5 d
16α	2.47 (m)	40.8 t	2.14 (m)	31.6 t
16*β*	1.97 (m)		2.29 (m)	
17	3.20 (dd, 12.6, 7.4)	43.2 d	3.13 (dd, 10.6, 7.0)	38.7 d
18	1.04 (3H, s)	17.0 q	1.02 (3H, s)	13.8 q
19	1.19 (3H, s)	23.4 q	0.96 (3H, s)	21.2 q
20		122.5 s		148.1 s
21	7.22 (s)	140.2 d		170.5 s
22	6.24 (s)	110.6 d	6.29 (s)	130.4 d
23	7.33 (s)	142.5 d		169.4 s
28	1.12 (3H, s)	23.2 q	0.98 (3H, s)	23.0 q
29	1.14 (3H, s)	25.0 q	1.09 (3H, s)	22.7 q
30a	4.92 (d, 12.3)	46.3 t	5.35 (s)	121.5 t
30b	4.38 (d, 12.3)		5.26 (s)	
OMe	3.70 (3H, s)	52.2 q	3.67 (3H, s)	52.1 q
11-OAc	1.93 (3H, s)	20.8 q	1.90 (3H, s)	20.8 q
		169.6 s		169.8 s
12-OAc	1.66 (3H, s)	20.3 q		
		170.2 s		
isobutanoyl				
1′				175.1 s
2′			2.22 (m)	33.9 d
3′			1.00 (3H, d, 7.1)	18.3 q
4′			0.98 (3H, d, 7.1)	18.9 q

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Recorded at 400 MHz. [d] Recorded at 100 MHz. ^{13}C multiplicities were determined by DEPT or by HMQC experiments.

Turrapubesin B (**2**) also possessed an 11*β*,12α-substitution pattern as deduced by coupling constants of $J_{9,11}$ and $J_{11,12}$.[6] In the ROESY spectrum of **2** (Figure 2), the observed correlations of H_3-18/H-11, H_3-18/H-15, H-11/H-9, H-12/H-17, H-12/H-1, H-9/H-5, H-5/H_3-28, H-30b/H_3-19, and H-30a/H-15 were fully consistent with the relative configuration of **2** as depicted.

An attempt to assign the absolute configuration of **2** directly by CD exciton chirality method or by correlating its CD spectrum with that of **1** failed since both compounds possessed multiple chromophores (more than three), which resulted in complex CD curves with different split patterns. The $\pi \rightarrow \pi^*$ electric transition moments of the enone and furan of **1** adopted similar directions with those of the enone and maleimide of **2**, while the $\Delta^{8(14)}$ double bond of **1** and the $\Delta^{8(30)}$ double bond of **2** were differently oriented in space. The summation of CD exciton couplets[11] for each compound would definitely exhibit complex CD curves with different split patterns (Supporting Information), respectively. Turrapubesin B (**2**) was thus hydrogenated to give 20,22-dihydroturrapubesin B (**2a**).[12] With the reduction of the maleimide to succinimide in **2a**, two easily distinguishable chromophores (enone and $\Delta^{8(30)}$ double bond) allowed the rational application of the CD exciton chirality method.[11] The CD

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(12) 20,22-Dihydroturrapubesin B (**2a**): White amorphous solid; $[\alpha]_D^{20}$ +82.0° (*c* 0.100, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (4.01) nm; CD (MeOH) 197 ($\Delta\epsilon$ +9.12), 242 ($\Delta\epsilon$ −2.38), 328 ($\Delta\epsilon$ +2.47) nm; ^{1}H NMR and ^{13}C NMR, EIMS, see Supporting Information.

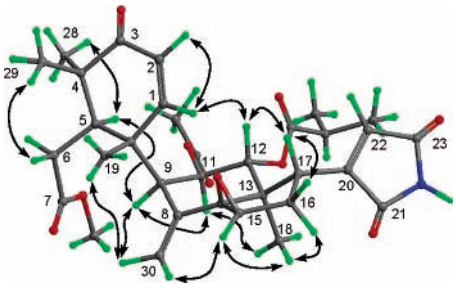

Figure 2. Key ROESY correlations of **2**.

spectrum of **2a** exhibited negative chirality resulting from the exciton coupling of a nondegenerate system comprising two different chromophores of the enone at 242 nm ($\Delta\epsilon$ −2.38, $\pi \rightarrow \pi^*$ transition)[13] and the $\Delta^{8(30)}$ double bond at 197 nm ($\Delta\epsilon$ +9.12, $\pi \rightarrow \pi^*$ transition).[14] The negative chirality of **2a** revealed that the transition dipole moments of two chromophores were oriented in a counterclockwise manner (Figure 3), and the absolute stereochemistry of the

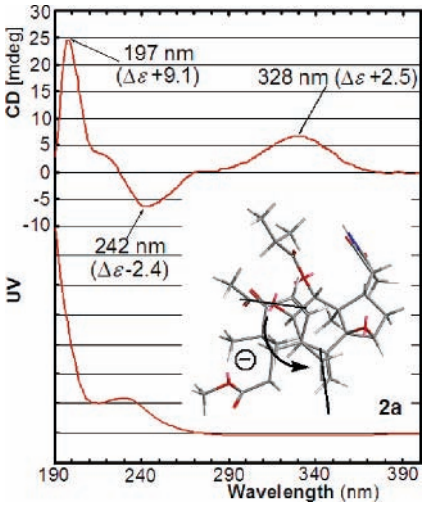

Figure 3. CD and UV spectra of **2a**. Bold lines denote the electric transition dipole of the chromophores.

limonoid core in **2a** was thus assigned. Accordingly, the absolute stereochemistry of **2** was determined as depicted.

Both compounds **1** and **2** are genuine natural products and major components in this plant, which were confirmed to exist in the ethanolic crude extract by TLC check. The absolute configurations of **1** and **2** assigned, respectively, by X-ray crystallography and CD exciton chirality methods were consistent with all the limonoids reported in the literature with only one exception.[15] Turrapubesins A (**1**) and B (**2**) are first examples of halogenated and maleimide-bearing limonoids, especially compound **2** with a maleimide ring, which is very rare in nature, and only limited examples were encountered in microbes and ascidians,[10,16] suggesting that the biosynthesis of **2** may involve the contribution of microbes, such as endophytic fungi.[17]

Turrapubesins A (**1**) and B (**2**) were tested for the in vitro cytotoxicity against the P-388 (murine leukemia) and A-549 (human lung adenocarcinoma) cell lines by using the MTT[18] and SRB[19] methods, respectively, and with pseudolaric acid B[20] as a positive control (IC_{50} = 0.74 μM against P-388). Only **1** showed weak activity (IC_{50} = 12.14 μM) against the P-388 cell line.

Acknowledgment. Shanghai Municipal Scientific Foundation (Grant No. 04XD14019) and the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of P. R. China are gratefully acknowledged. We thank professor S. M. Huang, Department of Biology, Hainan University, for the collection and identification of the plant material.

Supporting Information Available: Experimental procedures; detailed HMBC correlations (in tables and figures), 1D and 2D NMR, EIMS, ESIMS, IR, UV, and CD spectra of turrapubesins A (**1**) and B (**2**); NOESY correlations of **1**; CIF data for crystal structure of **1**; preparation and spectroscopic data of **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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