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The First Limonoid Peroxide in the Meliaceae Family: Walsuronoid A from *Walsura robusta*

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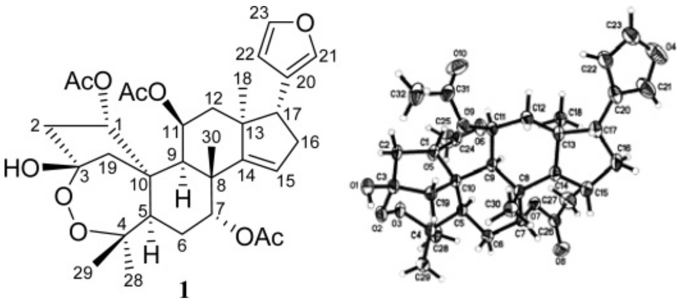
ABSTRACT



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Walsuronoid A (1), a limonoid featuring an unprecedented 3,4-peroxide-bridged A-*seco* skeleton, together with walsuronoids B (2) and C (3) possessing a rare 18(13→14)-*abeo*-limonoid skeleton, was isolated from *Walsura robusta*. The structures were elucidated by spectroscopic analysis and chemical correlation, and that of 1 was confirmed by single-crystal X-ray diffraction. Biosynthetic pathway of 2 and 3 was proposed and was chemically mimicked. Compounds 1 and 2 showed weak antimalarial activity.

The genus *Walsura* (Meliaceae) comprising about 40 species and varieties is mainly distributed in China, India, and Indonesia.[1] Previous investigations on this genus have led to the isolation of several tetranortriterpenoids and triterpenoids.[2−5] *Walsura robusta* Roxb., an arbor tree mainly growing in Yunnan and Guangdong provinces of China, has not been chemically studied previously. In the current study, three novel limonoids, walsuronoids A−C (**1**−**3**), were isolated from the leaves and twigs of *W. robusta*. Walsuronoid A (**1**) is the first limonoid peroxide isolated from the Meliaceae family, which features an unprecedented A-*seco* limonoid skeleton incorporating a 3,4-peroxide bridge. Walsuronoids B (**2**) and C (**3**) possess the rare 18(13→14)-*abeo*-limonoid skeleton. Their structures were elucidated by spectroscopic analysis and chemical correlation, and the structure of **1** was further verified by a single-crystal X-ray study. Biosynthetic origin of **2** and **3** was proposed and was chemically mimicked. The antimalarial activity of **1**−**3** was also tested.

Walsuronoid A (**1**) was obtained as a colorless crystal. The HRESIMS displayed a pseudo-molecular ion at *m*/*z* 609.2675 [M + Na]$^+$ (calcd for $C_{32}H_{42}O_{10}Na$, 609.2676) consistent with a molecular formula of $C_{32}H_{42}O_{10}$, requiring 12 double-bond equivalents. This was supported by the EIMS at *m*/*z* 586 [M]$^+$. The IR absorption bands at 3485, 1728, and 1709 cm^{-1} indicated the presence of hydroxyl and ester functionalities. The ^{13}C NMR spectrum of **1** revealed that 6 of the 12 double-bond equivalents were occupied by three

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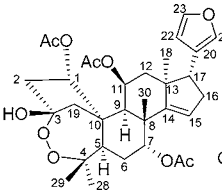
Table 1. ^{1}H and ^{13}C NMR Assignments of **1**−**3**[a]

	1[b]		**2**[c]		**3**[c]	
no.	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C
1	5.76 (dd, 11.1, 2.2)	77.8	7.10 (d, 10.2)	151.7	7.19 (d, 10.2)	152.1
2α	2.07 (m)	43.5	6.13 (d, 10.2)	126.8	6.13 (d, 10.2)	126.6
2β	2.26 (ddd, 11.2, 2.2, 2.9)					
3		111.5		202.6		202.5
4		84.7		48.1		48.0
5	2.11 (m)	45.6		139.3		140.4
6a	1.59 (m)	27.8		141.6		141.6
6b	2.06 (m)					
7	5.03 (br s)	76.3		201.5		201.7
8		41.9		55.3		55.6
9	2.77 (d, 5.5)	40.4	2.35 (d, 1.7)	46.2	2.31 (m)	46.8
10		52.8		40.9		41.3
11	6.05 (ddd, 12.5, 5.5, 1.6)	71.0	4.70 (br s)	67.9	4.75 (br s)	67.0
12α	2.46 (m)	43.6	2.45 (dq, 14.2, 2.8)	34.1	2.30 (m)	33.4
12β	1.57 (m)		2.97 (dd, 14.2, 2.8)		2.95 (m)	
13		46.4		134.0		142.0
14		158.4		58.4		59.4
15	5.47 (d, 1.9)	120.5	4.59 (t, 8.5)	81.2	4.63 (t, 8.4)	81.3
16α	2.50 (m)	34.9	2.92 (ddd, 15.6, 8.2, 2.7)	43.6	3.01 (m)	45.0
16β	2.32 (m)		2.76 (ddd, 15.6, 8.2, 2.7)		2.64 (ddd, 15.9, 7.9, 2.5)	
17	2.86 (dd, 12.2, 7.4)	52.2		127.6		123.7
18	0.79 (3H, s)	20.4	1.28 (3H, s)	24.1	1.27 (3H, s)	24.6
19α	2.45 (d, 15.0)	44.3	1.61 (3H, s)	29.1	1.68 (3H, s)	29.4
19β	2.85 (d, 15.0)					
20		125.2		121.2		130.1
21	7.39 (br s)	140.8	7.50 (br s)	140.6		173.7
22	6.37 (br s)	111.9	6.54 (br s)	109.5	7.45 (dd, 1.8, 1.8)	149.6
23	7.48 (t, 1.6)	143.6	7.43 (t, 1.5)	143.3	4.96 (2H, br s)	70.8
28	1.40 (3H, s)	25.2	1.55 (3H, s)	26.3	1.55 (3H, s)	26.4
29	1.15 (3H, s)	29.5	1.56 (3H, s)	21.1	1.58 (3H, s)	21.2
30	1.36 (3H, s)	27.8	1.84 (3H, s)	19.9	1.92 (3H, s)	19.5
1-OAc		171.5	6-OH 6.79 (s)		6-OH 6.75 (s)	
	2.05 (3H, s)	21.4	15-OH 5.69 (s)		11-OH 4.21 (s)	
7-OAc		169.8			15-OH 5.71 (s)	
	1.87 (3H, s)	21.0				
11-OAc		170.2				
	2.07 (3H, s)	21.1				

[a] Data were recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. [b] Recorded in acetone-d_6. [c] Recorded in $CDCl_3$.

double bonds and three carbonyls. The remaining six double-bond equivalents therefore required compound **1** to be hexacyclic. In addition, the NMR and HSQC spectra (Table 1) showed the presence of four methyls [δ_H 0.79 (s), 1.40 (s), 1.15 (s), and 1.36 (s); δ_C 20.4, 25.2, 29.5, and 27.8], three acetyls [δ_H 2.05 (s), 1.87 (s), and 2.07 (s); δ_C 21.4, 21.0, and 21.1], and a β-furyl ring [δ_H 7.39 (br s), 6.37 (br s), and 7.48 (t, J = 1.6 Hz); δ_C 125.2, 140.8, 111.9, and 143.6]. The aforementioned data implied that compound **1** possesses limonoid features. A detailed account of the structural assignment of **1** is given below.



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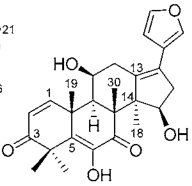

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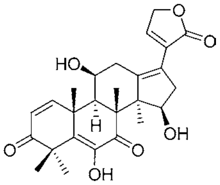

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Several structural fragments (C-1−C-2, C-5−C-7, C-9−C-11−C-12, and C-15−C-17) were first established by the correlations observed in the $^{1}H-^{1}H$ COSY spectrum (Figure 1). The connectivities of the structural fragments, the quaternary carbons, and the incorporated oxygens were achieved by analysis of the HMBC correlations. The B, C, and D rings were readily established by comparison with those of several known limonoids.[6−8] The β-furyl ring was attached to C-17 in the D ring by the HMBC correlations of H-17/C-20, C-21, and C-22. Two acetoxyls were located at C-7 and C-11 by the HMBC correlations from H-7 and H-11 to the corresponding carbonyls at δ_C 169.8 and 170.2, respectively. One proton resonance at δ_H 5.76 was assigned

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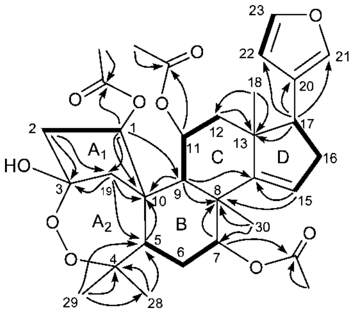

Figure 1. Key $^1H-^1H$ COSY (—) and HMBC (H→C) correlations of **1**.

to H-1 on the basis of the HMBC correlations from H-1 to C-10, C-9, and the remaining carbonyl at δ_C 171.5, suggesting that C-1 bearing an acetoxyl was attached to C-10. The connection of C-2 to the hemiketal C-3 (δ_C 111.5) was revealed by the HMBC correlations of H-2/C-3 and C-19. Two quaternary carbons C-3 and C-10 were readily linked through C-19 by the HMBC correlations of H-19/C-3 and C-10 to construct the five-membered ring A_1. The HMBC correlations from both H_3-28 and H_3-29 to C-4 (δ_C 84.7) and C-5 (δ_C 45.6) allowed a reasonable connection of Me-28, Me-29, and C-5 to C-4. Taken together, the hexacyclic feature, the still "loose ends" of two oxygenated quaternary carbons C-3 and C-4, as well as the remaining two unassigned oxygen atoms in the structure of **1** suggested that a peroxide bridge is located between C-3 and C-4 to tentatively establish the gross structure of **1**.

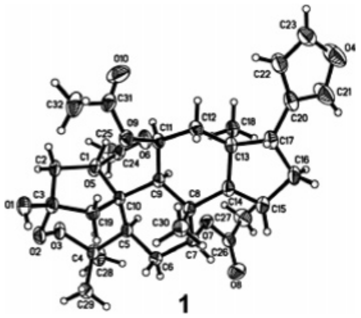

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Figure 2. Single-crystal X-ray structure of **1**.

A single-crystal X-ray diffraction study[9] (Figure 2) finally confirmed the planar structure of **1** and allowed the determination of its relative configuration, which is in good accordance with its relative configuration in solution as assigned by the ROESY spectrum. Unlike other reported A-*seco* limonoids having an ether bridge either between C-4 and C-19 or between C-4 and C-1 to form a five-membered A_2 ring,[6−8] compound **1** bears a peroxide bridge between C-4 and C-3 to furnish a seven-membered A_2 ring. To the best of our knowledge, compound **1** is the first limonoid peroxide ever found in the Meliaceae family.

Walsuronoid B (**2**), a white amorphous powder, displayed a molecular ion peak at *m/z* 438.2034 (calcd 438.2042) in the HREIMS corresponding to the molecular formula of $C_{26}H_{30}O_6$. The IR absorption bands at 3404 and 1743 cm^{-1} indicated the presence of hydroxyl and ester carbonyl groups, respectively. The ^{13}C NMR data of compound **2** resolved 26 resonances attributable to two conjugated carbonyls, five sp^2 quaternary carbons, four sp^3 quaternary carbons, five sp^2 methine, three sp^3 methines (two oxygenated), two sp^3 methylenes, and five methyls. The characteristic features of a β-furyl ring and an α,β-unsaturated ketone moiety were also observed in its ^1H and ^{13}C NMR spectra (Table 1). The aforementioned data indicated that **2** is also a limonoid and bears a high resemblance to those of a coexisting known limonoid 11β-hydroxycedrelone (**4**),[5] except for differences in the C/D rings. In comparison with **4**, the NMR spectroscopic data for **2** differed significantly in the D ring, featuring replacement of the 14,15-epoxide by a methyl and a hydroxy at C-14 and C-15, respectively, and the absence of the methyl at C-13 and the presence of a $\Delta^{13(17)}$ double bond (δ_C 134.0 and 127.6). The HMBC correlations from both H-12 and H-16 to the olefinic carbons indicated the existence of a $\Delta^{13(17)}$ double bond (Figure 3a). The HMBC correlations from Me-18 to C-8, C-13, C-14, and C-15 located the Me-18 to C-14. Detailed 2D NMR analysis (HSQC, $^1H-^1H$ COSY, and HMBC experiments) further accomplished the planar structure of **2**, a rare 18(13→14)-*abeo*-limonoid.

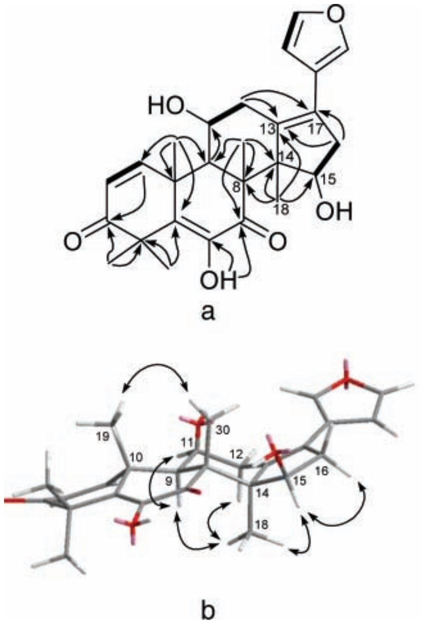

Figure 3. (a) Key $^1H-^1H$ COSY (—) and HMBC (H→C) correlations of **2**; (b) selected ROESY correlations of **2**.

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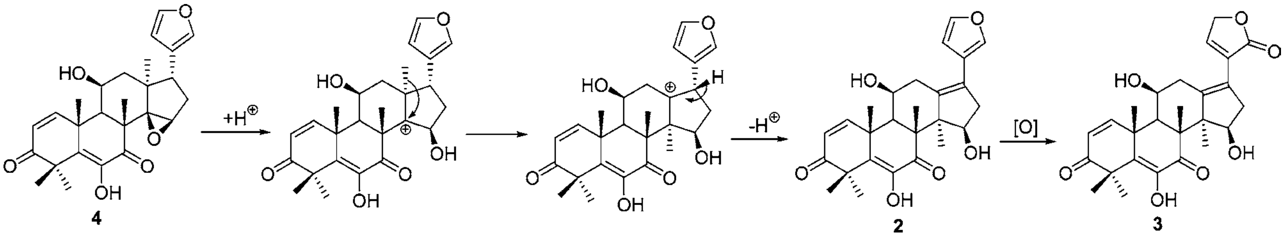
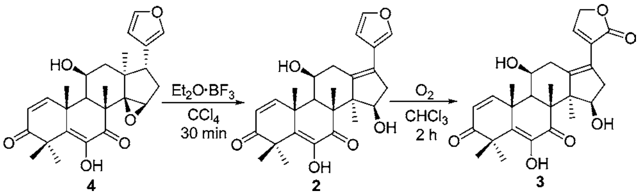
[9] Crystallographic data for walsuronoid A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-634341). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm.

Scheme 1. Hypothetical Biosynthesis Route of **2** and **3**



Its relative stereochemistry was established by analysis of the ROESY spectrum (Figure 3b). In particular, ROESY correlation between H-9 and H_3-18 indicated that they were cofacial and was arbitrarily assigned as α-oriented. A 11β-hydroxy and a 15β-hydroxy were consequently assigned by the ROESY correlations of H-9/H-11, H_3-18/H-12α, and H_3-18/H-15. Naturally rearranged 14-methyl limonoids are very rare,[10,11] and the isolation of **2** and the major component **4** from the same origin strongly suggested a possible biogenetic transformation of **4** into **2** (Scheme 1) via a Wagner–Meerwein rearrangement,[12] in which Me-18 migrated from C-13 to C-14 to form a rare 18(13→14)-*abeo*-limonoid skeleton. This proposed biogenetic pathway was chemically mimicked by a BF_3·Et_2O-catalyzed transformation of **4** to **2** (Scheme 2).

Scheme 2. Chemical Correlations of Compounds **2**–**4**



Walsuronoid C (**3**), a white amorphous powder, had a molecular formula of $C_{26}H_{30}O_7$ as determined by the HREIMS at *m*/*z* 454.1979 $[M]^+$ (calcd 454.1992). The ^1H NMR spectrum displayed signals due to five tertiary methyls (δ_H 1.27, 1.68, 1.55, 1.58, and 1.92) and two coupled olefinic protons [δ_H 7.19 (d, J = 10.2 Hz) and 6.13 (d, J = 10.2 Hz)]. The ^{13}C NMR spectrum displayed resonances of 26 carbons comprising two conjugated ketone carbonyls, one ester carbonyl, five sp^2 quaternary carbons, four sp^3 quaternary carbons, three sp^2 methine, three sp^3 methines (two oxygenated), three sp^3 methylenes (one oxygenated), and five methyls. In comparison with compound **2**, the ^1H and ^{13}C NMR data revealed that both compounds shared the same A, B, C, and D rings in the limonoid core, with the only difference being the presence of an α,β-unsaturated γ-lactone moiety at C-17. The proton resonances at δ_H 7.45 (dd, J = 1.8, 1.8 Hz, H-22) and 4.96 (br s, H_2-23) in the ^1H NMR spectrum and the carbon resonances at δ_C 130.1, 173.7, 149.6, and 70.8 in the ^{13}C NMR spectrum are characteristic of an α,β-unsaturated γ-lactone. Detailed 2D NMR analysis (HSQC, $^1H-^1H$ COSY, HMBC, and ROESY spectra) further confirmed the structure of **3** (Supporting Information).

The biogenetic precursor of **3** was proposed to be **2**, which was transformed into **3** via an oxidative process. Confirmatory evidence came from the fact that **2** was spontaneously transformed to **3** when exposed to air in $CHCl_3$ (Scheme 2). In other cases without a conjugated double bond, more rigorous conditions were required in the oxidation of a furyl ring to an α,β-unsaturated γ-lactone.[13–15] The presence of the conjugated $\Delta^{13(17)}$ double bond in **2** may render the furyl ring rather labile to oxidation.

Both compounds **2** and **3** are genuine natural products, which were confirmed to exist in the ethanolic crude extract by TLC check.

Compounds **1** and **2** showed 40% inhibition to the malaria parasite *Plasmodium falciparum* at the concentration of 40 μM, and the parasite survivors were not in a good state morphologically.

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Supporting Information Available: Experimental procedures, physical and spectral data of **1**–**3**, and CIF data for the crystal structure of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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