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Kadcoccitones A and B, Two New 6/6/5/5-Fused Tetracyclic Triterpenoids from *Kadsura coccinea*

Cheng-Qin Liang,[†,§,∥] Yi-Ming Shi,[†,§] Rong-Hua Luo,[‡,§] Xing-Yao Li,[†,§] Zhong-Hua Gao,[†,§] Xiao-Nian Li,[†] Liu-Meng Yang,[‡] Shan-Zhai Shang,[†,§] Yan Li,[†] Yong-Tang Zheng,[‡] Hong-Bin Zhang,[∥] Wei-Lie Xiao,[*,†] and Han-Dong Sun[*,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, P. R. China, Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, P. R. China, University of Chinese Academy of Sciences, Beijing 100049, P. R. China, and Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, Yunnan, P. R. China

xwl@mail.kib.ac.cn; hdsun@mail.kib.ac.cn

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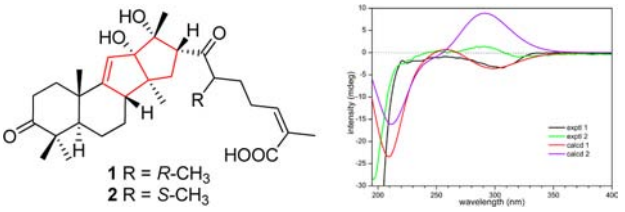
ABSTRACT



A pair of new triterpenoid epimers, kadcoccitones A (1) and B (2), together with a new biogenetically related compound kadcoccitone C (3), were isolated from *Kadsura coccinea*. The epimers featured an unprecedented carbon skeleton with a 6/6/5/5-fused tetracyclic ring system unit and a C_9 side chain. Their structures were determined by spectroscopic data, ECD calculation, and single-crystal X-ray diffraction. Compounds 1 and 3 showed anti-HIV-1 activity with an EC_{50} value of 47.91 and 32.66 μg/mL, respectively.

The Schisandraceae family consists of the genera *Schisandra* and *Kadsura*. In traditional chinese medicine, some plants of this family are used for the treatment of cough, premature ejaculation, chronic dysentery, and insomnia.[1] In the past 15 years, the Schisandraceae family has been the focus of great attention due to the findings of more than 15 kinds of novel skeleton triterpenoids from over 10 species of this family.[1b,2] These triterpenoids characterized by complicated polycyclic rings have aroused herculean challenges and ambitious targets for organic synthesis endeavors.[3]

Kadsura coccinea (Lem.) A. C. Smith is widely distributed throughout southwest China and has been used in Chinese folk medicine for the treatment of cancer and dermatosis and as an anodyne to relieve pain.[4] In this study, kadcoccitones A (**1**) and B (**2**), possessing an unprecedented skeleton featuring a 6/6/5/5-fused tetracyclic ring

[†] Kunming Institute of Botany.
[§] University of Chinese Academy of Sciences.
[∥] Yunnan University.
[‡] Kunming Institute of Zoology.

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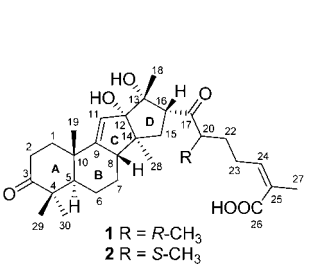
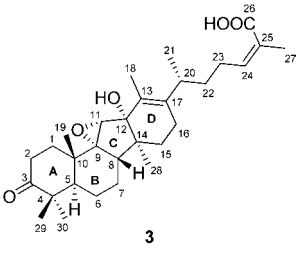
system and a C_9 side chain, as well as kadcoccitone C (**3**), were isolated and identified from the stems of *K. coccinea*. The new compounds were tested for their cytotoxicity against human tumor and inhibitory activity against the HIV virus *in vitro*. Here, we present the isolation, structural elucidation, and biological evaluation of these compounds.





Kadcoccitone A (**1**) was obtained as a colorless solid. The molecular formula of **1** was assigned as $C_{30}H_{44}O_6$ by HREIMS (*m*/*z* 500.3070 $[M]^+$, calcd 500.3138). The IR absorptions at 3441 and 1703 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum of **1** exhibited resonances for one secondary methyl, six tertiary methyls, and two olefinic protons (Table 1). The ^{13}C NMR spectrum with the aid of the DEPT technique resolved 30 carbon signals including seven methyls, seven methylenes, six methines (two olefinic), and ten quaternary carbons (three carbonyl and two olefinic ones). Apart from five degrees of unsaturation occupied by two ketone carbonyls, one carboxyl, and two trisubstituted double bonds, a tetracyclic structure was required for **1** to fulfill the unsaturation requirement.

Two six-membered carbon rings A and B (Figure 1) were assigned by the HMBC correlations of both H_3-29 and H_3-30 with C-3, C-4, and C-5; of both H-1 and H-2 with C-3 and C-10; of H_3-19 with C-1, C-5, C-9, and C-10; and of H-8 to C-9, coupling with the COSY correlations between H-1/H-2 and H-5/H-6/H-7/H-8.

By the HMBC correlations (Figure 1) from H_3-28 to C-8, C-12, C-14, and C-15; from H_3-18 to C-12, C-13, and C-16; and from H-11 to C-8, C-9, C-12, C-13, and C-14, combined with the $^1H-^1H$ COSY correlation of H-15/H-16, allowed the establishment of carbon rings C and D. In addition, there were two hydroxyls in accordance with the molecular formula. They were assigned to the two oxygenated quaternary carbons C-12 and C-13, which were confirmed by the HMBC (recorded in DMSO) correlations from OH-12 (δ_H 4.30) to C-11, C-12, C-13, and C-14 and from OH-13 (δ_H 4.19) to C-12, C-13, and C-16.

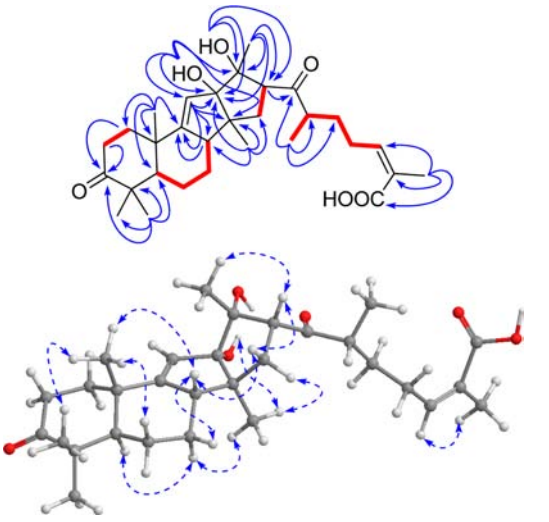


Figure 1. Key $^1H-^1H$ COSY (red —), HMBC (blue →), and ROESY (blue ← - →) correlations of **1**.

The HMBC correlations of H_3-21 to C-17, C-20, and C-22; of H_3-27 to C-24, C-25, and C-26; and of H-24 to C-22 and C-23, together with the $^1H-^1H$ COSY correlations of H_3-21/H-20/H-22/H-23/H-24, enabled the carbon connection of the side chain of **1** (Figure 1). The D ring and side chain connected through the C-16/C-17 bond could be deduced by the HMBC correlations from H-16 to C-17. Thus, the planar structure of **1** was established.

The relative configuration of **1** was fixed by the 600 MHz ROESY spectrum in DMSO. The ROESY correlations of H-5/H-7α, H-7α/H_3-28, and H_3-28/OH-12 showed that they were cofacial and were arbitrarily assigned as α-oriented. In consequence, the ROESY correlations of H_3-29/H_3-19, H_3-19/H-6β, H_3-19/H-8, H-8/H-15β, H-15β/H-16, and H-16/H_3-18 indicated that they were on the same side with a β-orientation. In addition, the double bond between C-24 and C-25 of **2** was deduced as a *Z*-configuration by the clear presence of the ROESY correlation of H-24 with H_3-27 (Figure 1). Considering the free rotation of the single bond between C-17 and C-20, the relative stereochemistry of C-20 of **1** could not be determined by the ROESY data. Thereby, the relative configuration of **1** was elucidated as shown with C-20 remaining to be determined.

Kadcoccitone B (**2**) had the same molecular formula as that of kadcoccitone A (**1**). Side-by-side comparison of their NMR data indicated that **1** and **2** were a pair of C-20 epimers.

The absolute configuration of **1** and **2** was elucidated by quantum chemial calculation. The theoretical calculation method of the electronic circular dichroism (ECD) spectra was adopted as a complementary approach using the time dependent DFT (TDDFT) method with the B3LYP-SCRF/6-31+G(d,p)//B3LYP/6-31G(d) level. The overall predicted ECD spectra of **1** and **2** were subsequently compared with the experimental ones (Figure 2).

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Table 1. ^{13}C NMR Assignments of Kadcoccitones A–C (**1**–**3**) (δ in ppm, J in Hz)

position	**1**[a]	**1**[b]	**2**[a]	**3**[a]
1	35.2 t	35.1 t	35.2 t	30.3 t
2	34.5 t	34.3 t	34.5 t	33.9 t
3	216.3 s	215.1 s	216.3 s	216.1 s
4	47.8 s	47.2 s	47.8 s	47.8 s
5	53.1 d	53.2 d	53.2 d	50.7 d
6	22.7 t	22.3 t	22.7 t	22.5 t
7	31.3 t	30.8 t	31.3 t	24.4 t
8	55.9 d	54.9 d	55.9 d	38.3 d
9	161.6 s	159.5 s	161.6 s	74.1 s
10	37.5 s	37.0 s	37.5 s	35.7 s
11	118.0 d	118.9 d	118.0 d	65.3 d
12	94.7 s	94.0 s	94.6 s	81.0 s
13	81.1 s	81.0 s	81.1 s	125.9 s
14	49.2 s	47.5 s	49.1 s	41.0 s
15	46.3 t	43.6 t	46.5 t	30.2 t
16	53.1 d	56.3 d	53.6 d	19.9 t
17	219.8 s	214.2 s	219.8 s	140.1 s
18	22.2 q	22.9 q	22.2 q	13.8 q
19	19.7 q	19.1 q	19.6 q	18.8 q
20	46.9 d	44.3 d	46.7 d	35.4 d
21	16.4 q	15.6 q	15.9 q	18.6 q
22	31.4 t	32.9 t	32.3 t	34.0 t
23	27.6 t	26.7 t	27.4 t	28.5 t
24	145.0 d	140.6 d	144.7 d	146.4 d
25	127.0 s	128.0 s	127.2 s	126.2 s
26	172.7 s	168.9 s	172.6 s	173.0 s
27	20.5 q	20.6 q	20.5 q	20.6 q
28	19.4 q	19.7 q	19.4 q	22.5 q
29	21.7 q	21.3 q	21.7 q	21.6 q
30	25.9 q	25.7 q	25.8 q	25.2 q

[a] Recorded in $CDCl_3$ at 100 MHz. [b] Recorded in DMSO 125 MHz, and the assignments were based on DEPT, HSQC, COSY, HMBC, and ROESY experiments.

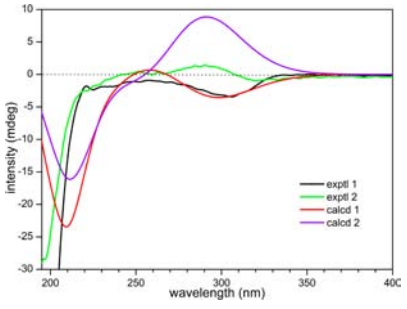


Figure 2. Calculated and experimental ECD spectra of **1** and **2**.

This comparsion revealed good agreement between the calculated and the experimental ECD curves. Furthermore, the calculated spectra for the 20*R*- and 20*S*-diastereomers were nearly identical with the experimental ones of **1** and **2** over the whole range of wavelengths under investigation, respectively. Thus, the absolute configurations of C-20 in **1** and **2** were assigned as *R* and *S*, respectively.

Molecular orbital (MO) analysis used conformer **1t** as an example to provide comprehension of the experimental ECD curve (Figures 3 and S40). The weak negative Cotton effect (CE) at 292 nm could be assigned to the experimental negative CE at 307 nm, which was caused by the electronic transitions from MO134 to MO138 involving an n→π* transition in the carbonyl group of the side chain. In addtion, the next negative CE at 207 nm could be ascribed to the experimental negative CE at 197 nm, which resulted from the electronic transitions from MO136 to MO141 involving a π→π* transition in the double bond of ring C.

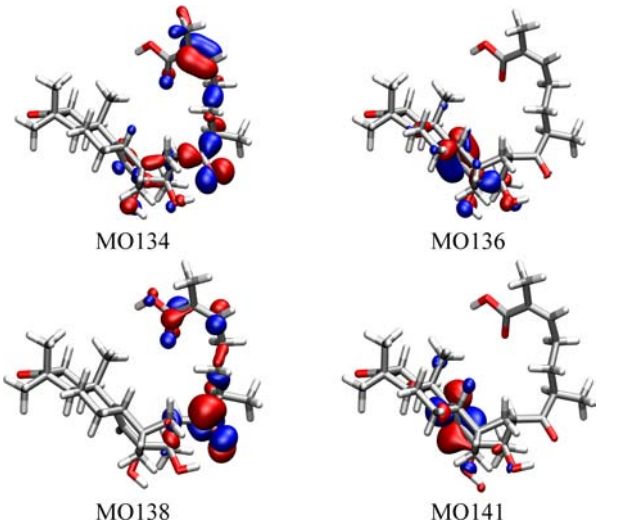


Figure 3. The most important orbitals involved in the key transitions of the conformer **1t** at the B3LYP/6-31+G(d,p) level in MeOH with the PCM model.

The molecular formula of kadcoccitone C (**3**) was determined to be $C_{30}H_{44}O_5$ by its positive HREIMS m/z 484.3189 ($[M]^+$, calcd 484.3189), requiring 8 degrees of unsaturation. Extensive analysis of the 1D and 2D NMR spectra (Supporting Information (SI)), particularly HMBC, revealed that **3** had a 14(13→12)-*abeo*-Lanostane triterpenoid skeleton with a 6/6/5/6-fused tetracyclic ring system, in which the D ring remained as a six-membered ring, while the side chain still had eight cabons. But the 2D NMR spectra in $CDCl_3$ (SI) could not provide sufficient information to elucidate the assignment of the epoxide and hydroxyl group, since the key hydroxyl signals in the ^1H NMR spectrum did not exist. Fortunately, an X-ray diffraction experiment with suitable crystals was conducted by Cu Kα radiation with a Flack parameter of 0.24 (18) and a Hooft parameter of 0.10 (7) for 1661 Bijvoet pairs (CCDC 910835). Thus, the structure and absolute stereochemistry of **3** were established (Figure 4).

Generally, most of the discovered *Schisandra* triterpenoids were supposed to derive from cycloartane[1,2] and only a few were deduced from lanostane.[1] Besides, a large part of the novel *Schisandra* triterpenoids, especially

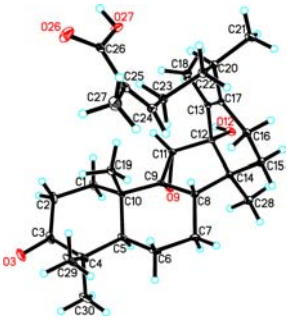


Figure 4. Single-crystal X-ray structure of **3**.

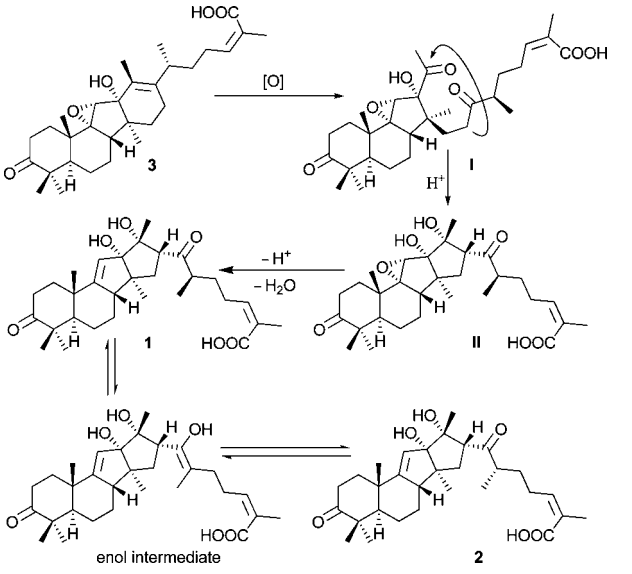
schinortriterpenoids (*Schisandra* nortriterpenoids), were highly oxygenated and endowed different complicated polycyclic rings. To the best of our knowledge, kadcoccitones A (**1**) and B (**2**), representing the first examples of triterpenoids with a unique 6/6/5/5-fused tetracyclic ring system with a C_9 side chain, were intriguingly possibly derived from 14(13→12)-*abeo*-lanostane.

The previous studies on *K. coccinea* have discovered several structurally interesting triterpenoids.[5] But they are quite distinct from those described here for this plant collected from Ziyuan prefecture of Guangxi province, China. This might be caused by either environmental or genetic differences leading to the differences in cyclization and producing these metabolites.

The biogenetic routes of **1** and **2** (Scheme 1) could be plausibly traced back to kadcoccitone C (**3**), since **3** may be an intermediate initially transformed from lanostane[6] and the structure of **1** and **2** are very similar to that of **3**, i.e. rings A, B, and C and the side chain, which was also isolated from the same plant in a large amount. The key biochemical reactions we deduced would be a C-13/C-17 C-bond opening and then a reconstruction of a five-membered D ring intermediate (**II**) through an aldo-reation. Finally, **2** was produced from **1** through an enol intermediate.[7]

Compounds **1** and **3** were tested for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW- 480 human cancer cell lines by using the MTT method.[8] However, none of them showed obvious inhibitory activity against those cells with $IC_{50} > 40\,\mu g/mL$. Additionally, the anti-HIV-1 activities of **1** and **3** were tested by microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[9] Compound **1** demonstrated anti-HIV-1 activity with an EC_{50} value of 47.91 $\mu g/mL$ (AZT: EC_{50} = 0.00534 $\mu g/mL$). Compound **3** showed weak anti-HIV-1 activity with EC_{50} values of 32.66 $\mu g/mL$.

Scheme 1. Hypothetical Biogenetic Pathway of **1** and **2**



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Supporting Information Available. Detailed experimental procedures; physicochemical properties; 1D and 2D NMR, MS, UV, and IR spectra of compounds **1**–**3**; X-ray data of **3**; and related original ECD calculation data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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