RSC Advances

www.rsc.org/advances

COMMUNICATION

Lineariifolianoids A–D, rare unsymmetrical sesquiterpenoid dimers comprised of xanthane and guaiane framework units from *Inula lineariifolia*†

Jiang-Jiang Qin,[ac] **Ying Huang,**[a] **Dan Wang,**[b] **Xiang-Rong Cheng,**[a] **Qi Zeng,**[a] **Shou-De Zhang,**[a] **Zhen-Lin Hu,**[b] **Hui-Zi Jin*[a] and Wei-Dong Zhang*[ab]

Lineariifolianoids A–D (**1–4**), a rare class of unsymmetrical sesquiterpenoid dimers with xanthane and guaiane frameworks have been isolated for the first time from *Inula lineariifolia*. Notably, lineariifolianoid D (**4**) features the first *exo*-adduct of such dimers through a plausible Diels–Alderase catalyzed biogenetic pathway. Moreover, the absolute configuration of japonicone A (**5**) has been revised by the Flack method. The inhibitory effects of the new isolates on TNF-α-mediated L929 cytotoxicity have also been evaluated and only the *exo*-adduct lineariifolianoid D (**4**) displays the inhibitory property.

Sesquiterpenoid dimers have been reported to be a characteristic class of ingredients of the genus *Inula* (Asteraceae family), and exhibit more 'drug-like' and 'biologically friendly' molecular features compared with their monomers and they have recently received considerable interest.[1] In continuation of the study on the intriguing bioactive sesquiterpenoid dimers from this family, four new sesquiterpenoid dimers, lineariifolianoids A–D (**1–4**) (Fig. 1), have been isolated for the first time from *I. lineariifolia* Turcz., widely distributed in China and referred to as the traditional Chinese medicine "JinFeiCao".[2] Notably, lineariifolianoids A–D (**1–4**) represent a rare unsymmetrical sesquiterpenoid dimers class, comprising of xanthane and guaiane units with the linkage mode C–11–C–3′. So far, to our knowledge, only three cases of xanthane–guaiane dimers while with a linkage of C–11–C–4′ were reported two decades ago and their absolute configurations have yet to be determined.[3] Below are disclosed the details of the isolation, structure elucidation, and inhibitory effect evaluation on TNF-α-mediated L929 cytotoxicity of the new lineariifolianoids. Moreover, the revision of japonicone A's absolute structure has also been discussed.

[a] School of Pharmacy, Shanghai Jiao Tong University, Shanghai, 200240, P. R. China. E-mail: wdzhangy@hotmail.com; kimhz@sjtu.edu.cn; Fax: +86 (0)21 34205989; Tel: +86 (0)21 34205989
[b] Department of Phytochemistry, School of Pharmacy, Second Military Medical University, Shanghai, 200433, P. R. China
[c] Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX, 79106, USA

† Electronic supplementary information (ESI) available: Experimental procedures, plant material, crystallographic data (**1** and **5**), cytotoxicity assay (**1–4**), and 1D and 2D NMR spectra of **1–4**. CCDC reference numbers 806237 and 823557. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ra01014c

Lineariifolianoid A (**1**) was obtained as colorless monoclinic crystal, giving an [M + Na]⁺ ion in the HRESIMS at *m/z* 617.2746, appropriate for a molecular formula of $C_{34}H_{42}O_9$ with 14 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3437 cm⁻¹), carbonyl (1751 cm⁻¹), and olefinic (1637 cm⁻¹) functionalities. The ¹³C NMR spectrum of **1** exhibited 34 carbon signals in agreement with the HRESIMS data (Table S1, ESI†). The ¹H and ¹³C NMR spectra also suggested the presence of two acetoxy groups (δ_H 2.01, δ_C 169.9 and 21.0; δ_H 2.01, δ_C 170.0 and 21.1). Their positions were determined by HMBC correlations (Fig. 2). A further analysis of 1D and 2D NMR data of the remaining 30 carbon signals revealed that they belonged to two different sesquiterpenoid moieties, A_1 and B_1 (Fig. 2). Compared with the NMR data of related japonicone A (**5**) isolated from *I. japonica*, an identical guaianolide framework A_1 with a characteristic α-methylene lactone functionality can be authenticated by olefinic carbons C–11′ (δ_C 139.4), C–13′ (δ_C 119.5), and exocyclic olefinic protons H–13′a (δ_H 6.25) and H–13′b (δ_H 5.52).[1c] ¹H–¹H COSY and HMBC correlations (Fig. 2) enabled the determination of the structure of the B_1 fragment, by comparison with inuchinenolide A,[4] previously isolated from the same plant.[5] The NMR data of moiety B_1 mainly differed in the chemical shifts of C–11 and C–13 from inuchinenolide A, suggesting that the two
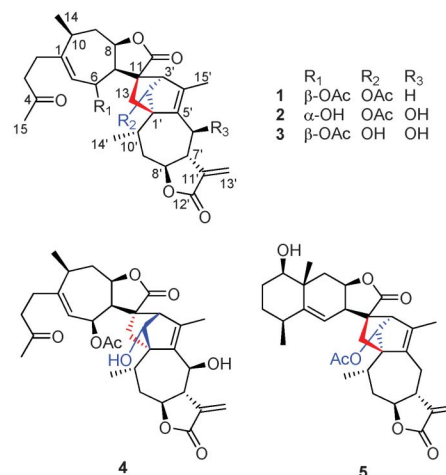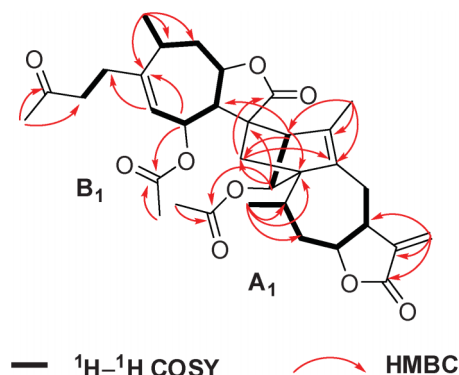

Fig. 1 The structures of compounds **1–5**.

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

moieties were connected through these units. The key HMBC correlations from H–2′ to C–11 and C–13, H–3′ to C–7 and C–13, and H$_2$–13 to C–2′ and C–5′ indicated the presence of a bridged ring norbornene system.[1]

The relative configuration of **1** was determined by NOESY NMR experiment (Fig. S1, ESI†) and further confirmed by single X-ray crystallography study (Fig. 3). These studies suggested that **1** was an *endo*-cylcoadduct of xanthane and guaiane from a Diels–Alder reaction.[6] The strong NOESY correlations of H–7 with H–6, H–8, and H–10; and of H–8 with H–10 were observed in moiety B$_1$, indicating that H–6, H–7, H–8, and H–10 adopted the same orientation, arbitrarily designated as α-orientation. In addition, the correlations of H–2′ with H–3′ and H$_3$–14′; of H–3′ with H–7 and H–8; and of H–8′ with H–6′α and H$_3$–14′ concluded that H–2′, H–3′, H–8′ and H$_3$–14′ were also α-orientated. On the other hand,
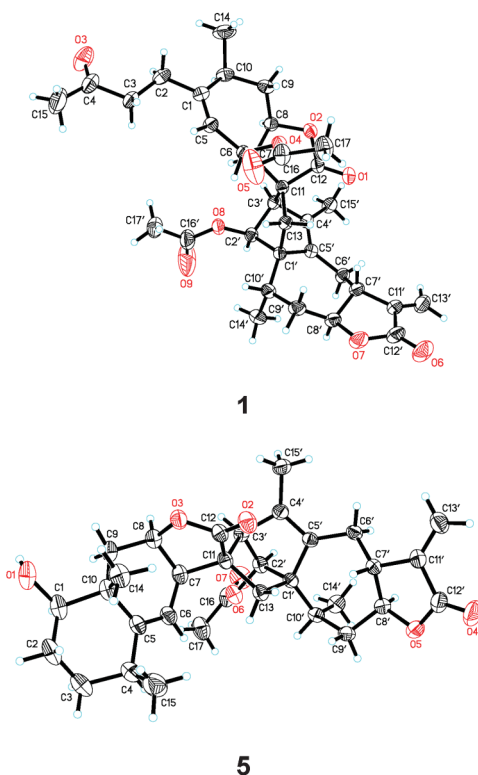

Fig. 3 Single X-ray crystallographic structures of **1** and **5** showing their absolute configurations.

the correlations of H–7′ with H–6′β and H–9′β revealed the *trans*-fused lactone ring of moiety A$_1$. The relative configuration of **1** established by NOESY NMR experiments was in good agreement with that determined by X-ray diffraction study with copper radiation (CCDC 806236) (Fig. 3). The absolute configuration of **1** was determined to be 6*S*, 7*R*, 8*R*, 10*S*, 11*R*, 1′*S*, 2′*S*, 3′*R*, 7′*R*, 8′*S*, and 10′*S*, respectively, which were deduced from the Flack parameter, 0.04 (14), refined using 2359 Friedel pairs.[7]

Surprisingly, the obtained experimental data was inconsistent with the structurally related sesquiterpenoid dimer, japonicone A (**5**), whose absolute configuration was previously established by the modified Mosher method.[1b] In order to solve the contradiction between **1** and **5**, an X-ray crystallographic analysis of **5** (CCDC 823557) (Fig. 3) as an anomalous dispersion with copper radiation was performed [Flack parameter, 0.10 (14)]. The careful analysis led to the exactly opposite of the reported result and accordingly the revised absolute configuration of **5**, 1*R*, 4*S*, 7*R*, 8*R*, 10*R*, 11*S*, 1′*S*, 2′*S*, 3′*R*, 7′*R*, 8′*S*, and 10′*S* was proposed.

Lineariifolianoids B–D (**2**, **3** and **4**) have the same molecular formula $C_{32}H_{40}O_9$, established from their HRESIMS at *m/z* 591.2566, 591.2587, and 591.2576 [M + Na]$^+$, respectively, possessing 13 degrees of unsaturation. Comparison of their 1D and 2D NMR data with those of **1** led to establishing their structures (Table S1 and Fig. S3–S5, ESI†). The NMR spectra of the three compounds suggested the presence of only one acetoxy group, and their positions were determined by the HMBC experiments (Fig. S3–S5, ESI†). In addition, the presence of oxygenated methines resonating at δ_C 69.7, 69.9, and 68.4 (C–6′) in the respective guaianolide substructures A$_2$, A$_3$, and A$_4$ (see Fig. S3–S5, ESI†) of **2**, **3**, and **4**, along with the absence of a methylene at δ_C 26.0 (C–6′) of **1**, indicated that they all had an additional hydroxyl substituent at C–6′. The conclusions were further drawn by analyses of their 2D NMR data. Compared with **1**, similar NOESY patterns allowed us to determine the relative configurations of **2** and **3** (Fig. S2 and 4, respectively, ESI†). They were also the dimers of the *endo*-adducts of xanthane and guaiane. The important NOESY correlations of H–8′ with H–6′ of the two compounds suggested the hydroxyl at C–6′ was β-orientation, while in the ^{1}H NMR spectrum of **2** (Table S1, ESI†), the coupling constant between H–6 and H–7 changed to 9.6 Hz (but 0 Hz in **1**), indicating that the hydroxyl at C–6 was α-orientation. Detailed comparison analyses of 2D NMR data of **3** and **4** (Fig. S4 and S5, respectively, ESI†) also suggested that **4** had the same planar structure as **3**. However, the significant differences were also observed in the NMR resonances of their corresponding norbornene structures (Table S1, ESI†), mainly from C–11 to C–13 and from C–1′ to C–4′. These observations implied that the xanthane and guaiane units of **4** were cycloadded in an *exo* fashion. The absence of the important correlations of H–3′ with H–7 and H–8 in the NOESY spectrum of **4** (Fig. 4) further confirmed the above supposition. Thus, compound **4** was identified as the first *exo*-adduct with xanthane and guaiane units.

It has been documented that the sesquiterpenoid dimers, a group of naturally occurring metabolites with C$_{30}$ cores, almost always originate biosynthetically from two identical or different sesquiterpenoid units. The Diels–Alder reaction, as a powerful biological tool, has exhibited its versatility during the formation of such natural products.[6b] In the previous investigation, all the sesquiterpenoid dimers from the *Inula* genus have been reported to be formed by *endo*-selective Diels–Alder reactions, which are attributed to the most
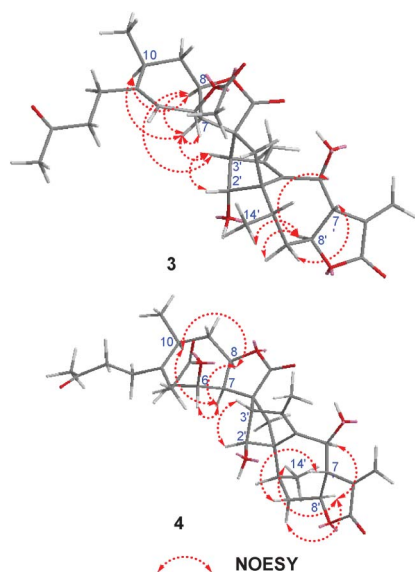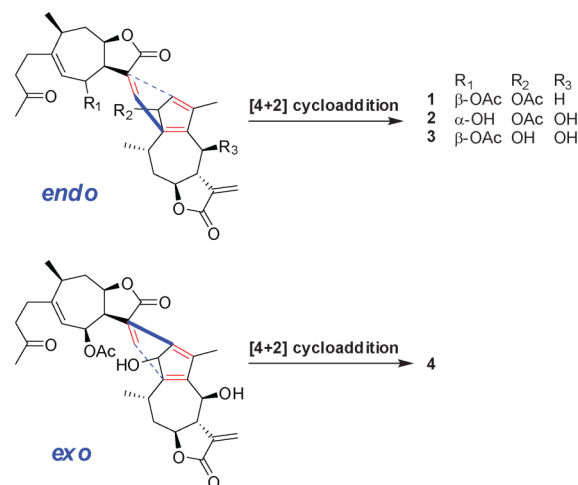
Fig. 4 Key NOESY correlations of **3** and **4**.

Scheme 1 Plausible biogenetic pathway of Lineariifolianoids A–D (**1–4**).

stable transition states.[1,8] In contrast, the unusual natural *exo* cycloaddition catalyzed by Diels–Alderase in the *Inula* genus has not been reported despite the fact that they have been occasionally observed from other plants.[9]

As a rare unsymmetrical sesquiterpenoid dimers class, comprised of xanthane and guaiane units with the linkage mode C–11–C–3′, lineariifolianoids A–D (**1–4**) are presumably formed by [4 + 2] cycloaddition of xanthane and guaiane catalyzed by Diels–Alderase as proposed in Scheme 1. Lineariifolianoids A–C (**1–3**) are constructed by a commonly observed *endo*-selective Diels–Alder reaction; nevertheless, lineariifolianoid D (**4**) is biosynthesized in an unusual *exo* fashion and serves as the first *exo*-adduct of such xanthane–guaiane dimers from a natural source.

In the previous study, japonicone A was identified as a novel TNF-α antagonist by directly binding to TNF-α and therefore blocking TNF-α-mediated activities in cell-based assays. To determine whether lineariifolianoids have similar activities, in preliminary investigations, we accordingly tested the inhibitory effects on the TNF-α-sensitive L929 cells.[10] The obtained results showed that the only *exo*-adduct, lineariifolianoid D (**4**), inhibited TNF-α-mediated cytotoxicity in a dose-dependent manner from 2.5 to 10 μM, but the three *endo*-adducts **1–3** didn't exhibit such property (Fig. S6, ESI†).

In conclusion, a rare unsymmetrical sesquiterpenoid dimer class, comprising of xanthane and guaiane units with the linkage mode C–11–C–3′, have been isolated and elucidated for the first time. Notably, an unusual *exo*-Diels–Alder adduct, lineariifolianoid D (**4**) comprising of xanthane–guaiane dimers from the Asteraceae family is discovered for the first time. Such a configuration is also critical for the observed TNF-α-mediated cytotoxicity. Moreover, we have revised the structure of japonicone A. Further structural and biological investigations of these intriguing compounds are being carried out in our laboratory and the results will be reported in due course.

References

1 (a) J. J. Qin, L. Y. Wang, J. X. Zhu, H. Z. Jin, J. J. Fu, X. F. Liu, H. L. Li and W. D. Zhang, *Chem. Commun.*, 2011, **47**, 1222–1224; (b) J. J. Qin, H. Z. Jin, J. X. Zhu, J. J. Fu, X. J. Hu, X. H. Liu, Y. Zhu, S. K. Yan and W. D. Zhang, *Planta Med.*, 2010, **76**, 278–283; (c) J. J. Qin, H. Z. Jin, J. J. Fu, X. J. Hu, Y. Wang, S. K. Yan and W. D. Zhang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 710–713; (d) H. Z. Jin, D. Lee, J. H. Lee, K. Lee, Y. S. Hong, D. H. Choung, Y. H. Kim and J. J. Lee, *Planta Med.*, 2006, **72**, 40–45.

2 Y. Ling, in *Flora of China*, ed. Y. Ling, Science Press, Beijing, 1979, **vol. 75**, pp. 265–266.

3 (a) J. Jakupovic, C. Zdero, M. Grenz, F. Tsichritzis, L. Lehmann, S. M. Hashemi-Nejad and F. Bohlmann, *Phytochemistry*, 1989, **28**, 1119–1131; (b) C. Zdero and F. Bohlmann, *Phytochemistry*, 1989, **28**, 3105–3120.

4 K. Ito and T. Iida, *Phytochemistry*, 1981, **20**, 271–273.

5 L. Y. Nie, J. J. Qin, Y. Huang, L. Yan, Y. B. Liu, Y. X. Pan, H. Z. Jin and W. D. Zhang, *J. Nat. Prod.*, 2010, **73**, 1117–1120.

6 (a) F. Fringuelli and A. Taticchi, in *The Diels–Alder Reaction: Selected Practical Methods*, ed. F. Fringuelli and A. Taticchi, John Wiley & Sons, West Sussex, 2002, **chp. 1**, 14–15; (b) H. Oikawa and T. Tokiwano, *Nat. Prod. Rep.*, 2004, **21**, 321–352.

7 H. D. Flack, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 1983, **A39**, 876–881.

8 (a) B. N. Su, Y. Takaishi, M. Tori, S. Takaoka, G. Honda, M. Itoh, Y. Takeda, O. K. Kodzhimatov and O. Ashurmetov, *Tetrahedron Lett.*, 2000, **41**, 1475–1479; (b) B. Fu, B. N. Su, Y. Takaishi, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov and O. Ashurmetov, *Phytochemistry*, 2001, **58**, 1121–1128.

9 (a) C. Zdero, F. Bohlmann and H. M. Niemeyer, *Phytochemistry*, 1991, **30**, 1597–1601; (b) M. Li, J. S. Zhang and M. Q. Chen, *J. Nat. Prod.*, 2001, **64**, 971–972; (c) S. B. Singh, D. L. Zink, B. Heimbach, O. Genilloud, A. Teran, K. C. Silverman, R. B. Lingham, P. Felock and D. J. Hazuda, *Org. Lett.*, 2002, **4**, 1123–1126.

10 F. Denizot and R. J. Lang, *Immunol. Methods*, 1986, **89**, 271–277.