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Chemical constituents from *Brucea javanica*

Shi-Hui Dong, Jia Liu, Ying-Zi Ge, Lei Dong, Cheng-Hui Xu, Jian Ding, Jian-Min Yue *

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, People's Republic of China

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ABSTRACT

Fourteen apotirucallane-type triterpenoids, named brujavanones A–N, were isolated from the twigs of *Brucea javanica*, along with four known quassinoids and seven known lignans from the seeds of *B. javanica*. Their structures were elucidated on the basis of extensive spectroscopic data analysis. The structure of a previously reported triterpenoid, bruceajavanin C, was revised as its C-21 epimer. The cytotoxic activities of triterpenoids and quassinoids against two human tumor cell lines, HL-60 and A-549, were evaluated, but all the compounds were inactive (IC_{50} > 10 μM).

1. Introduction

Brucea javanica (L.) Merr. (Simaroubaceae), an evergreen shrub, is widely distributed from southeast Asia to northern Australia. Its seeds have been used as an antimalarial in traditional Chinese medicine (Chen et al., 1997). Previous chemical investigations led mainly to the isolation of a number of apotirucallane-type triterpenoids and quassinoids, which showed a wide spectrum of biological effects, such as potential antibabesial, anti-HIV, antimalarial, antitubercular, and cytotoxic activities (Elkhateeb et al., 2008; Guo et al., 2005; Itokawa et al., 2000; Morre et al., 1998; Murakami et al., 2004; Okano et al., 1996; Pan et al., 2009; Subeki et al., 2007; Tamura et al., 2002). In the current study, 14 new apotirucallane-type triterpenoids, named brujavanones A–N, were isolated from the ethanolic extract of the twigs of *B. javanica*, and four known quassinoids together with seven known lignans from the ethanolic extract of seeds (Fig. 1). The structure of a previously reported triterpenoid, bruceajavanin C, was revised as its C-21 epimer, the epimer of brujavanone D, by comparing their spectroscopic data. The *O*-ethyl-bearing **14** is likely an artifact formed in the extraction process by involving EtOH as the solvent. The cytotoxic activities of 18 compounds against two tumor cell lines, HL-60 and A-549, were evaluated, and all compounds were inactive. Herein, the isolation, structural elucidation, and cytotoxic evaluation of these compounds are reported.

2. Results and discussion

Compound **1** was obtained as a white, amorphous powder. Its HRESIMS displayed a sodiated molecular ion peak at *m*/*z* 607.3236 $[M+Na]^+$, corresponding to the molecular formula $C_{34}H_{48}O_8$ with 11 degrees of unsaturation. A UV absorption band at 231 nm (log ε 4.09) implied the presence of an α,β-unsaturated carbonyl group, while the IR absorption bands indicated the presence of hydroxy (3429 cm^{-1}), ester carbonyl (1726 and 1709 cm^{-1}), and α,β-unsaturated carbonyl (1664 cm^{-1}) functional groups. The ^{13}C NMR spectroscopic data (Table 1) with DEPT experiments showed the presence of nine methyls, four methylenes, 12 methines (three olefinic and five oxygenated) and nine quaternary carbons (three carbonyls, one olefinic and one oxygenated). The α,β-unsaturated carbonyl group, two acetyl groups, and a trisubstituted double bond accounted for five out of the 11 degrees of unsaturation, and the remaining six degrees of unsaturation thus required **1** to be hexacyclic. In addition, a five-membered hemiacetal ring (δ_C 79.8 and 96.7) and a trisubstituted epoxide moiety (δ_H 2.64, d, 7.7; δ_C 57.2 and 66.6) were evident from its ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2). The above analysis suggested that **1** was an apotirucallane-type triterpenoid (Pan et al., 2009). Its structure was further elucidated by analysis of its 2D NMR spectra, especially the HMBC (Fig. 2A), in which the only keto group at C-3 (δ_C 204.3), conjugated with the Δ^1 double bond (δ_H 8.05, 5.77, each 1H, d, J = 10.7 Hz; δ_C 123.4 and 161.2), was assigned on the basis of the multiple HMBC correlations of Me-28 and Me-29/C-3, Me-19 and H-5/C-1, H-1/C-3 and C-10, and H-2/C-4 and C-10 (Fig. 2A). The trisubstituted Δ^{14} double bond (δ_H

* Corresponding author. Tel./fax: +86 21 50806718.
E-mail address: jmyue@mail.shcnc.ac.cn (J.-M. Yue).

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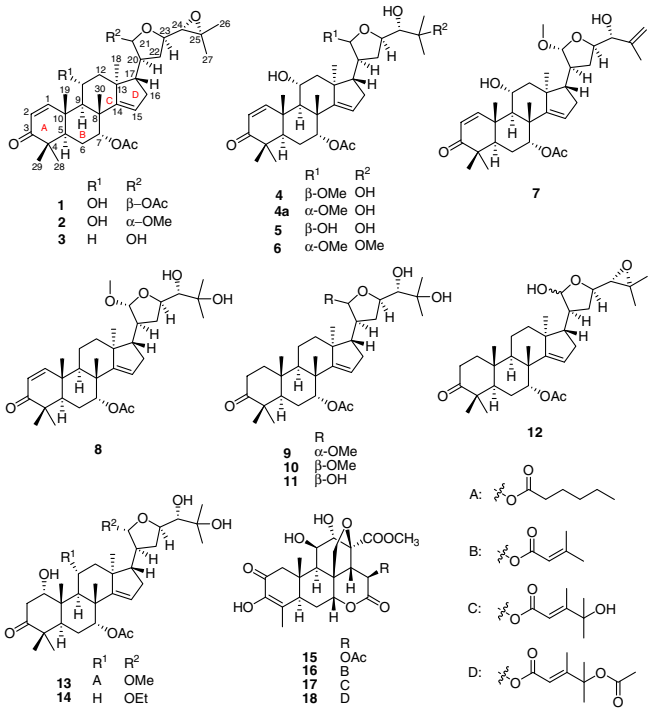

Fig. 1. Structures of compounds **1**–**18**.

5.27; δ_C 118.0 and 159.3) (Tables 1 and 2) was located by the HMBC correlation networks of Me-18, Me-30, H-9, and H_2-16/C-14, H-15/C-8, C-13, C-14, C-16, and C-17, and H_2-16/C-15 (Fig. 2A). The presence of the five-membered hemiacetal ring was confirmed by the HMBC correlations of H-21/C-20, C-22, and C-23 (δ_C 79.8). Two acetoxyl groups were placed at C-7 (δ_C 73.8) and C-21 (δ_C 96.7) by the HMBC correlations from H-7 and H-21 to each of the corresponding carbonyl of the acetyls, respectively (Fig. 2A). The chemical shifts of C-24 at δ_C 66.6 and C-25 at δ_C 57.2 featured a typical trisubsituted 24,25-epoxide, which was confirmed by the HMBC correlations of Me-26 (or Me-27)/C-24 and C-25 (Fig. 2A). The last oxygenated methine was assigned to C-11 (δ_C 66.4) bearing a hydroxyl group based on the HMBC correlations of H-11/C-10, C-12, and C-13, and H-9/C-11 (Fig. 2A). Thus, the skeletal structure of **1** was determined.

The relative configuration of **1** was defined by analysis of its ROESY spectrum (Fig. 2B). The ROESY cross-peaks of Me-29/Me-19, Me-19/Me-30, and Me-30/H-17 indicated that Me-19, Me-29, Me-30, and H-17 were cofacial and assigned in a β-orientation according to previously reported analogues (Pan et al., 2009). In consequence, the ROESY correlations of Me-28/H-5, H-5/H-9, and H-9/Me-18 suggested that they were α-oriented. H-7 and H-11 were assigned a β-orientation by ROESY correlations of H-11/Me-19, H-11/Me-30, and H-7/Me-30. The ROESY correlation between H-20 and Me-18 suggested that H-20 was α-oriented. By the same argument, H-21 and H-23 were also assigned an α-orientation from the ROESY correlations of H-21/H_2-12, H-21/H-20, H-21/Me-18, and H-23/H-20, while H-24 was β-oriented based on the correlation of H-24/H-22β (Fig. 2B). Thus, the structure of brujavanone A (**1**) was elucidated as shown, 7α,21β-diacetoxy-21*R**, 23*R**:24α,25-diepoxy-11α-hydroxy-4,4,8-trimethyl-cholesta-1,14-dien-3-one.

Compound **2**, a white, amorphous powder, gave a molecular formula of $C_{33}H_{48}O_7$ as determined by HRESIMS ion peak at *m*/*z* 579.3281 $[M+Na]^+$. A UV absorption band at 228 nm (log ε 4.07) implied the presence of an α,β-unsaturated carbonyl group. IR absorption bands indicated the presence of hydroxyl (3477 cm^{-1}), ester carbonyl (1732 cm^{-1}), and α,β-unsaturated carbonyl (1666 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) showed the presence of eight methyls, an *O*-methyl group, an acetyl group, an α,β-unsaturated keto group, a trisubstituted double bond, five oxygenated methines, and an oxygenated quaternary carbon. These data established that **2** was also an apotirucallane-type triterpenoid and shared a similar structure with **1** based on the analysis of its HMBC spectrum (Supplementary data). The only difference was the presence of an *O*-methyl group at C-21 in **2** instead of the AcO-21 in **1**, which was confirmed by the HMBC correlation from OCH_3 to C-21 (δ_C 109.2) (Supplementary data). As the result, proton resonance of H-21 in **2** shifted upfield at δ_H 4.85 as compared with that of **1** at δ_H 6.28. The C-21 chemical shift of **2** at δ_C 109.2 suggested that OCH_3-21 was in an α-orientation (Xie et al., 2007), which was confirmed by ROESY correlations of H-21/H-17 and H-21/H_2-12 (Supplementary data). The other stereocenters in **2** were established to be identical to those in **1** by the analysis of its ROESY spectrum (Supplementary data), as well as their similar NMR spectroscopic patterns. Thus, structure **2** (brujavanone B) was determined as 7α-acetoxy-21*R**,23*R**:24α,25-diepoxy-11α-hydroxy-21α-methoxy-4,4,8-tri- methyl-cholesta-1,14-dien-3-one.

Compound **3**, a white, amorphous powder, was obtained as a mixture of C-21 epimers, with a ratio of OH-21α and OH-21β being about 3:5, as determined by peak area on HPLC. Its molecular formula was determined to be $C_{32}H_{46}O_6$ on the basis of its HRESIMS. The ^1H and ^{13}C NMR spectroscopic data of **3** (Tables 1 and 2) showed close similarities to those of bruceajavanin B (Kitagawa et al., 1994), with the only difference being due to the presence of a hydroxyl group at C-21 in **3** replacing an *O*-methyl at C-21 in bruceajavanin B. This deduction was further confirmed by its HMBC spectrum (Supplementary data). The relative configuration of **3** was established to be identical with that of bruceajavanin B (Kitagawa et al., 1994) by analysis of its ROESY spectrum (Supplementary data) and comparing their NMR spectroscopic data. Therefore, the structure of **3** (brujavanone C) was assigned as 7α-acetoxy-21,23*R**:24α,25-diepoxy-21-hydroxy-4,4,8-trimethyl-cholesta-1,14-dien-3-one.

Compound **4a** gave a molecular formula of $C_{33}H_{50}O_8$ as established by its HRESIMS. Analysis of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated that **4a** was also an apotirucallane-type triterpenoid, and shared the same tetracyclic core and five-membered hemiacetal ring with **2**, with the difference being due to the absence of the typical 24,25-epoxide moiety, with concomitant presence of two downfield-shifted carbon signals at δ_C 75.3 and 73.1 ppm, indicating that **4a** was the hydrolytic product of the 24,25-epoxide in **2**. This deduction was supported by its molecular weight showing 18 mass units more than that of **2** and confirmed by the HMBC spectrum (Supplementary data). The non-detectable coupling constant of H-24 (brs) and the ROESY correlations of H-24/H_2-22 resulted from the fairly fixed C-23/C-24 rotation, which might be induced by the hydrogen bond between OH-24 and the oxygen of the furan ring (Xie et al., 2007). The stereochemistry of C-24 of eight apotirucallane-type triterpenoids representing four substituted patterns was assigned all in a *S*-configuration by chemical correlation (Mitsui et al., 2005), which accordingly showed four patterns for the chemical shifts of relevant protons H-23 and H-24, and carbons C-23, C-24, C-25 and C-26 as summarized in Table 3. The chemical shifts of relevant protons and carbons in compound **4a** well matched those of pattern 1 (Table 3), indicating that the C-24 of **4a** is also in a *S**-configuration. The other stereocenters of **4a** were established to be identical with those of **2** by the analysis of its ROESY spectrum (Supplementary data) and comparing their NMR spectroscopic data. Thus, the structure of **4a** was elucidated as 7α-acetoxy-21*R**,23*R**-epoxy-11α,24*S**,25-trihydroxy-21β-methoxy-4,4,8-trimethyl-cholesta-1,