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Diterpenoid constituents of *Euphorbia macrorrhiza*

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ABSTRACT

Ten diterpenoids, named macrorilone A–B, macroripremyrsinone A, macrorilathyrone A–B, macrorieuphorone A–B and macroricasbalone A–C, together with ten known diterpenoids, jatrophalone, sikkimenoids A–D, jatrophodione A, latilagascenes F, jolkinol B, 15β-*O*-benzoyl-5α-hydroxyisolathyrol and jatrophalactone were isolated from the whole plant of *Euphorbia macrorrhiza* C.A. Mey. These diterpenoids belong to six skeleton-types, including jatropholane, premyrsinane, lathyrane, euphoractin, casbene and rhamnofolane diterpenoids. Their structures were elucidated by extensive analysis of 1D, 2D NMR and HRESIMS spectroscopic data. The absolute configurations of macrorilone B, macroripremyrsinone A and macrorilathyrone A were established by comparing their experimental and calculated electronic circular dichroism (ECD) spectra. Several of the isolated compounds exhibited weak cytotoxicity against the KB and KBv200 cell lines with IC_{50} values ranging from 21.19 to 47.87 μM. Some also showed multidrug resistance (MDR) reversal activity, among which macrorilathyrone B exhibited a remarkable inhibitory effect on P-gp-mediated drug exclusion.

1. Introduction

Euphorbia is the largest genus in the Euphorbiaceae family, comprising more than 2000 species, which is well known for the structural and bioactive diversity of its diterpenoid constituents (Shi et al., 2008; Vasas et al., 2012). Diterpenoids with different skeletons, such as jatrophanes, lathyranes, casbenes, premyrsinanes, jatropholanes and euphoractins, exhibit a range of biological effects, such as modulability of multidrug resistance (Corea et al., 2003; Molnár et al., 2006), cytotoxicity (Fatope et al., 1996; Ravikanth et al., 2003), antiproliferative activity (Valente et al., 2004), anti-inflammatory (Ravikanth et al., 2002) and antimicrobial activities (Xu et al., 1998). These diterpenoids can be formed by several steps of intramolecular cyclization of a casbene precursor, many of which retain the *gem*-dimethylcyclopropane ring in their final structures, such as lathyranes, premyrsinanes and euphoractins (Durán-Peña et al., 2014; Thibodeaux et al., 2012). A casbene synthase has also been shown to cyclize geranylgeranyl pyrophosphate (GGPP) to casbene (Dueber et al., 1978; Kirby et al., 2010).

Euphorbia macrorrhiza C.A. Mey is mainly distributed in northwestern China, Russia and Kazakhstan (Editorial Committee of Flora of China, Chinese Academy of Sciences, 1997). A previous study showed that the essential oil of its roots exhibited antimicrobial activity and cytotoxicity (Lin et al., 2012). However, the chemical constituents, especially the diterpenoid constituents, of this plant have not been reported. As part of a program aimed at exploring novel, biologically active, diterpenoids from the genus *Euphorbia*, ten new (**1**–**10**) and ten known (**11**–**20**) diterpenoids were isolated from the whole plant of *E. macrorrhiza*, and their cytotoxicity and multidrug resistance (MDR) reversal activity were evaluated. In this study, the isolation and structural elucidation of the new diterpenoids are described, as well as their cytotoxicity against KB and KBv200 cell lines and the MDR reversal activity.

2. Results and discussion

The dried acetone extract of the whole plant of *E. macrorrhiza* was suspended in cyclohexane and partitioned with CH_3CN to yield the CH_3CN soluble extract, which was further subjected to silica gel and Sephadex LH-20 column chromatographic steps and semi-preparative HPLC to afford ten new (**1**–**10**) and ten known (**11**–**20**) diterpenoids (see Fig. 1).

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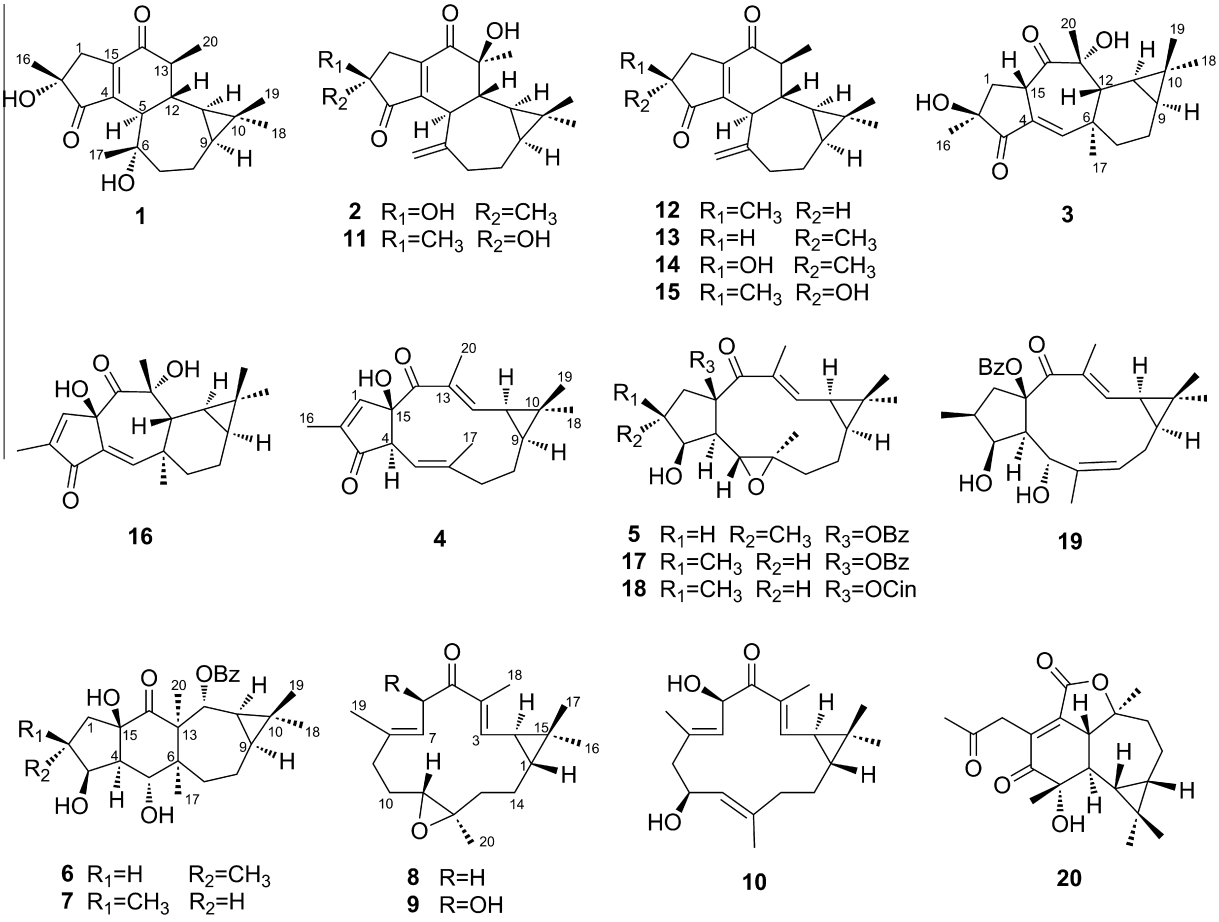

Fig. 1. Structures of the diterpenoids **1**–**20** isolated from *Euphorbia macrorrhiza*.

Macrorilone A (**1**), molecular formula $C_{20}H_{28}O_4$ by HRESI(+)MS (*m*/*z* 355.1892 $[M+Na]^+$, calcd for $C_{20}H_{28}O_4Na$ 355.1885), was obtained as an amorphous colorless powder. Analysis of its 1D and 2D NMR data indicated that **1** was an analogue of the known jatropholane diterpenoid, sikkimenoid D (**15**) (Yang et al., 2013). The only difference lay in the replacement of the 6(17)-exomethylene group by a methyl and a hydroxyl group substituted at C-6, which was supported by the chemical shifts of Me-17 (δ_c 21.6) and C-6 (δ_c 76.5) and the HMBC correlations (Fig. S1 in Supplementary Information). The NOESY spectrum confirmed that **1** had the same relative configurations as **15** (Fig. S2 in Supplementary Information), and the configuration of Me-17 was shown to be β by the NOESY correlation of H_3-17/H-12.

The ^{13}C NMR spectroscopic data of macrorilone B (**2**), molecular formula $C_{20}H_{26}O_4$ by HRESI(+)MS (*m*/*z* 353.1738 $[M+Na]^+$, calcd for $C_{20}H_{26}O_4Na$ 353.1729), were nearly identical to those of the known jatropholane diterpenoid, jatrophalone (**11**) (Liu et al., 2012), with only differences in the chemical shifts of C-2 (δ 75.6 in **2** and 76.9 in **11**), C-3 (δ 208.5 in **2** and 210.6 in **11**) and C-16 (δ 24.8 in **2** and 26.3 in **11**). Interpretation of their 2D NMR data established the same overall structure. The NOESY correlation of H-1α/H_3-16 suggested, however, an α-orientation of the Me-16 in **2**, while the Me-16 in **11** was β-oriented, indicating that **2** and **11** were C-2 epimers. The absolute configurations of **2** and **11** were determined as 2*S*, 5*S*, 9*S*, 11*S*, 12*R*, 13*S* for compound **2**, and 2*R*, 5*S*, 9*S*, 11*S*, 12*R*, 13*S* for compound **11**, respectively, by comparing their experimental and quantum chemical calculated electronic circular dichroism (ECD) spectra (Fig. S3 in Supplementary Information).

Macroripremyrsinone A (**3**), molecular formula $C_{20}H_{28}O_4$ by HRESI (+) MS (*m*/*z* 355.1887 $[M+Na]^+$, calcd for $C_{20}H_{28}O_4Na$ 355.1885), showed NMR data similar to those of jatrophodione A (**16**) (Xu et al., 2011), a premyrsinane diterpenoid possessing a 5/7/6/3 fused-ring skeleton. The main differences were that the 1,2-double bond in **16** was replaced by a methylene at C-1 (δ 34.2) and an oxygenated quaternary carbon at C-2 (δ 76.1) in **3**. Meanwhile, the –OH group at C-15 in **16** was absent in **3**, this being supported by the chemical shifts of C-15 (δ 42.7). Analysis of the NOESY spectrum of compound **3** established the same relative configurations as those of **16**. The NOESY correlations of H_3-16/H-1α and H-12/H-15/H-1β indicated an α-orientation of Me-16 and a β-orientation of H-15, respectively. The calculated ECD spectrum of **3** showed a negative Cotton effect at 219 nm and a positive Cotton effect at 280 nm, which were in good agreement with its experimental ECD spectrum (Fig. S3 in Supplementary Information). Thus, the absolute configurations of C-2, C-6, C-9, C-11, C-12, C-13 and C-15 were determined as *S*, *S*, *S*, *S*, *R*, *R*, *S*, respectively.

Macrorilathyrone A (**4**) was assigned as $C_{20}H_{26}O_3$ on the basis of its ^{13}C NMR data and HRESI(+)MS results (*m*/*z* 315.1965 $[M+H]^+$, calcd for $C_{20}H_{27}O_3$ 315.1960), indicative of eight indices of hydrogen deficiency. Analysis of its 1D (Table 2) and 2D NMR spectra established a 5/11/3 fused-ring skeleton of a lathyrane diterpenoid, with two ketone carbonyl groups at C-3 and C-14, as well as three olefinic double bonds at $\Delta^{1,2}$, $\Delta^{5,6}$ and $\Delta^{12,13}$. The chemical shifts of C-15 (δ 85.1), together with the HRESI(+)MS data, demonstrated the existence of an –OH group located at C-15. The normal H-4 α and 15-OH β configurations of lathyranes were assumed (Appendino et al., 2003). The NOESY correlations of H-4/H_3-17,