

Sesquiterpenoids with New Carbon Skeletons from the Resin of *Toxicodendron vernicifluum* as New Types of Extracellular Matrix Inhibitors

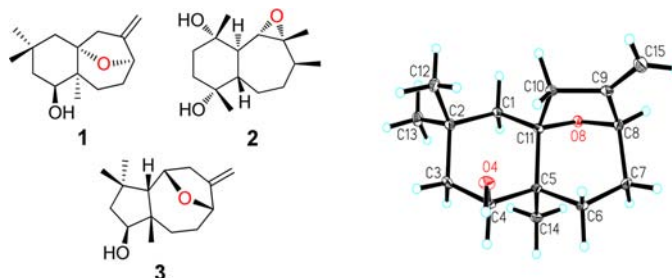
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ABSTRACT



Toxicodenanes A–C (1–3), representing sesquiterpenoids with three new carbon skeletons, were isolated from the dried resin of *Toxicodendron vernicifluum*. Their structures were identified by spectroscopic data and X-ray diffraction crystallography. Their plausible biosynthetic route was proposed via the isolated intermediate (4). Compounds 2 and 3 could significantly inhibit overproduction of fibronectin, collagen IV, and IL-6 in high-glucose-induced mesangial cells in a dose- and time-dependent manner, showing their potential in diabetic nephropathy.

Diabetic nephropathy (DN) is a major and serious complication of diabetes mellitus (DM), which affects 20% to 30% of patients with DM in their lifetime.¹ Due to the large prevalence of DM in the general population, the patients suffering from DN are increasing rapidly. DN as a consequence of DM in turn has become the most common cause of end-stage renal disease (ESRD), accounting for more than half of all cases of ESRD in the United States.²

For patients with ESRD, hemodialysis is an expedient measure but a heavy economic burden to patients. Renal

transplantation is an ultimate way out but meets with the big challenge of kidney supply and demand. As a consequence, the majority of patients with ESRD die during a waiting process. Evidence suggests that retarding the progression of DN will alleviate the conflicts between kidney supply and demand. Unfortunately, clinically available drugs for DN are scarce, and the data from Thomson Reuters Pharma showed that compounds launched in different research and development phases are no more than 100, of which only 2 are in phase III clinical trials. Therefore, an urgent need exists to identify new natural products with effects on DN which will provide new structure templates for drug optimization.

Resins are secreted by many plants, and their biological effects and defensive roles in the plants have been widely documented.³ Gambogic acid, for example, as a xanthone derived from the resin of *Garcinia hanburyi*, is well-known for its profound antitumor activity in clinical trials

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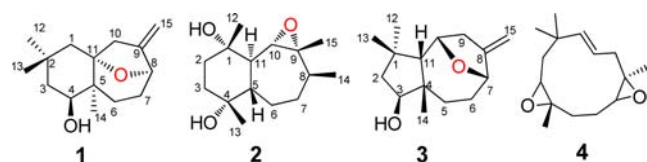
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and rodent experimental models.⁴ *Resina Toxicodendri* (ResT) is the dried resin produced by *T. vernicifluum* which is known as Chinese lacquer tree growing in East Asia and largely cultivated for its toxic sap. As ResT is used as a Chinese medicine for promoting blood circulation and removing blood stasis, it always occurs in prescriptions of Chinese medicine. We hypothesized that ResT might contain compounds which are beneficial for DN because DN is a microvascular-associated disease. With this motivation, we investigated the ethanol extract of ResT, from which three new sesquiterpenoids, toxicodenanes A–C (**1**–**3**), representing three new carbon skeletons, were isolated and structurally identified. Toxicodenanes A and B (**1** and **2**) possess a 6/7 ring system, and toxicodenane C (**3**) bears a 5/8 ring system. Both **1** and **3** have a tetrahedron furan ring moiety in the molecules which made them cage-like structures. Since oversecretion of extracellular matrix and proinflammatory factors are definitely implicated in DN, these isolates were purposely screened for their anti-diabetic nephropathy potential by using high-glucose-induced mesangial cells. Here, the isolation and structural elucidation of compounds **1**–**3**, representing the first example of three new classes of sesquiterpenoids, are reported as well as their antidiabetic nephropathy activity.



Compound **1**⁵ was isolated as colorless crystals (CH₃OH). Its molecular formula was established as C₁₅H₂₄O₂ by HRESIMS (m/z 235.1696 [M – H][–], calcd for 235.1698), requiring four degrees of unsaturation. The IR spectrum displayed the existence of hydroxyl (3409 cm^{–1}) and double bond (1669 cm^{–1}) groups. The ¹H NMR spectroscopic data of **1** (Table 1 in the Supporting Information (SI)) revealed the presence of three single methyl [δ_H 0.96 (3H, s, H-12), 1.16 (3H, s, H-13), 1.08 (3H, s, H-14)] and two terminal olefinic protons [δ_H 4.84 and δ_H 4.79 (each 1H, s, H-15a and H-15b)]. The ¹³C NMR and DEPT spectroscopic data (Table 1 in the SI) of **1** indicated 15 carbon resonances, including three methyl, six methylene (including one terminal double bond), two oxygenated methine, and four quaternary carbons (including one oxygenated sp³ quaternary carbon, one sp² quaternary carbon). Inspection of the ¹H and ¹³C NMR spectra of **1** indicated a sesquiterpenoid. The total NMR spectroscopic

data of **1** were assigned by a combined analyses of 1D and 2D NMR spectra including HSQC, HMBC, and ¹H–¹H COSY spectra. The ¹H–¹H COSY correlations (Figure 1) of H-3/H-4 and H-6/H-7/H-8 displayed the key spin systems. In the HMBC spectrum (Figure 1), the correlations from H-12 and H-13 to C-1, C-2, and C-3 indicated that the 12-CH₃ and 13-CH₃ were both connected to C-2; the correlations from H-14 to C-4, C-5, C-6, and C-11 suggested that the 14-CH₃ was linked to C-5; the correlations from H-15a and H-15b to C-8, C-9, and C-10 confirmed the presence of the terminal double bond in the molecule, which was established to be located at C-9. The two oxymethine carbons and one oxygenated quaternary carbon were assigned to C-4, C-8, and C-11 from its downfield chemical shifts, along with the HMBC and ¹H–¹H COSY correlations. Apart from one double bond and two carbon ring systems, the remaining unsaturation in **1** was assumed to be representative of an oxygen bridge between C-11 and C-4 or C-11 and C-8, forming another ring system. The oxygen bridge was deduced to be between C-11 and C-8 from the HMBC correlation from H-8 to C-11, forming a cage-like structure.

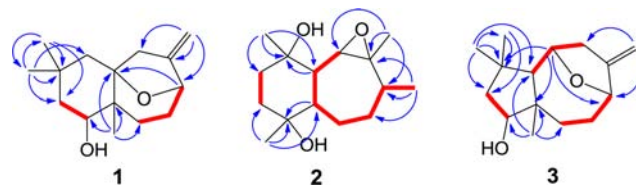


Figure 1. Key HMBC (blue arrows) and ¹H–¹H COSY (red lines) correlations of **1**–**3**.

The relative configuration of **1** was assigned by ROESY experiments and X-ray crystallographic diffraction. In the ROESY spectrum (Figure 2), the correlations of H-14/H-4 and H-14/H-12 indicated that 14-CH₃, 12-CH₃, and H-4 were on the same side of the ring, possessing α -orientation. The strong correlation of H-8 with H-10a (δ_H 3.62) indicated that the oxygen bridge was on the same side. H-8 was determined to be β -oriented based on the correlations of H-10a/H-13 and H-10a/H-8 as shown in the molecular model. The structure and relative configuration of **1** were finally confirmed by a single-crystal X-ray diffraction (Figure 3)⁶ using anomalous scattering of Cu K α radiation, which indicated that compound **1** is a racemic mixture, comprised of equal amounts of left- and right-handed enantiomers (4*S*, 5*S*, 8*R*, 11*R* and 4*R*, 5*R*, 8*S*, 11*S*). Therefore, the structure of **1** was established and named (\pm)-toxicodenane A.

Compound **2**,⁷ obtained as a colorless gum, was determined to have the molecular formula C₁₅H₂₆O₃ by HRESIMS (m/z 253.1802 [M – H][–], calcd for 253.1803), with three degrees of unsaturation. The IR spectrum of **2**

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(5) (\pm)-Toxicodenane A (**1**): colorless crystals; [α]_D²⁵ – 11.9 (this may result from the residual impurity) (c 0.1, CHCl₃); IR (KBr) ν_{\max} 3409, 2955, 2924, 2857, 1669, 1463, 1377, 1173, 1124, 1050, 1033, 1008, 763 cm^{–1}; ¹H and ¹³C NMR spectroscopic data, see SI; ESIMS: (negative) m/z 235 [M – H][–]; HRESIMS: m/z 235.1696 [M – H][–] (calcd for C₁₅H₂₄O₂, 235.1698).

(6) Crystallographic data of (\pm)-toxicodenane A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (Deposition No. CCDC 926067). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

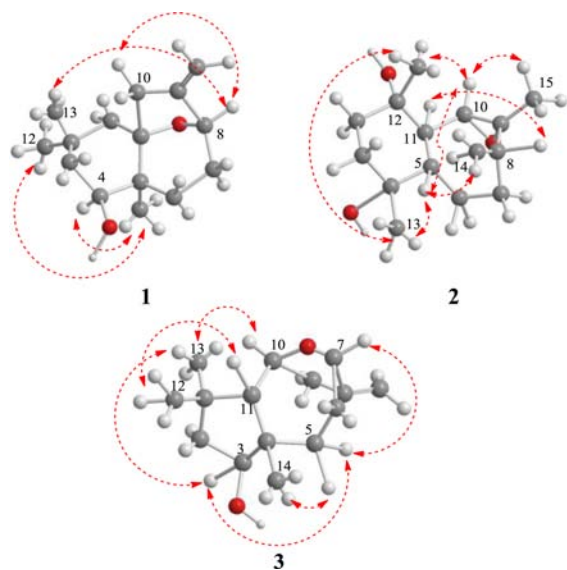


Figure 2. ROESY (red arrows) correlations of **1**–**3**.

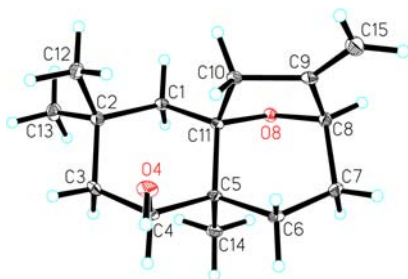


Figure 3. X-ray crystallographic structure of **1**.

revealed the presence of a hydroxyl group (3423 cm^{-1}). The ^1H NMR showed four methyl [δ_{H} 1.33 (3H, s, H-12), 1.13 (3H, s, H-13), 1.00 (3H, d, $J = 7.2\text{ Hz}$, H-14), 1.21 (3H, s, H-15)] signals. The ^{13}C and DEPT NMR data of **2** (see SI) also showed 15 carbon resonances, indicating a sesquiterpenoid skeleton. The ^1H – ^1H COSY (Figure 1) correlations of H-2/H-3 and H-10/H-11/H-5/H-6/H-7/H-8/H-14 revealed two spin systems. In the HMBC spectrum (Figure 1), the correlations from H-12 to C-1, C-2, and C-11 and from H-13 to C-3, C-4, and C-5 indicated that 12- CH_3 and 13- CH_3 were connected to C-1 and C-4, respectively. The HMBC correlations from H-14 to C-7, C-8, and C-9 and correlations from H-15 to C-8, C-9, and C-10 suggested that 14- CH_3 and 15- CH_3 were located at C-8 and C-9, respectively. C-9 and C-10 were oxygen-bearing carbons based on their downfield chemical shifts in the ^{13}C NMR spectrum. Analysis of the unsaturation of **2** revealed

the presence of another epoxy group in this molecule, as there were only two carbon rings deduced by the HMBC and ^1H – ^1H COSY correlations. Thus, the planar structure of **2** was determined as shown, with a 6/7 ring sesquiterpenoid.

The relative configuration of **2** was established by ROESY experiments. In the ROESY spectrum (Figure 2), the correlations of H-5/H-10, H-13, H-12/H-10, H-13 indicated the β -orientation of H-5, H-10, 12- CH_3 , and 13- CH_3 . 14- CH_3 and 15- CH_3 were deduced to be β -oriented from the correlations of H-15/H-10, H-14, which were further confirmed by the correlation of H-14/H-12, H-13. The α -orientation of H-11 was assigned by the correlation of H-11/H-8. Therefore, the structure of **2** was assigned as shown, named toxicodenane B.

Compound **3**⁸ possessed the same molecular formula as that of **1**, deduced from the HREIMS (m/z 236.1778 [M]⁺, calcd for $\text{C}_{15}\text{H}_{24}\text{O}_2$, 236.1776), indicating 4 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3426 cm^{-1}) and double bond (1640 cm^{-1}) groups. The ^1H NMR spectrum of **3** was similar to that of **1**, suggesting a sesquiterpenoid, with three single methyl [δ_{H} 1.09 (3H, s, H-12), 1.14 (3H, s, H-13), 1.02 (3H, s, H-14)] and two terminal olefinic protons [δ_{H} 4.95 (1H, d, $J = 2.0\text{ Hz}$, H-15a) and δ_{H} 4.90 (1H, d, $J = 2.0\text{ Hz}$, H-15b)]. The ^{13}C NMR and DEPT spectroscopic data of **3** (see SI) were also highly similar to those of **1**, except that the oxygenated quaternary carbon at C-11 in **1** was replaced by an oxymethine at C-10 (δ_{C} 78.2) in **3**, which was established by the HMBC correlations (Figure 1) from H-10 to C-1 and C-4, along with the ^1H – ^1H COSY correlations (Figure 1) of H-9/H-10/H-11. Furthermore, another major difference between these two compounds was that the methylene signal at C-1 in **1** was replaced by a methine at C-11 (δ_{C} 64.1) in **3**, which was determined on the basis of the HMBC correlations from H-12 and H-13 to C-1, C-2, and C-11 and from H-14 to C-3, C-4, C-5, and C-11. The HMBC correlations from H-15a and H-15b to C-7, C-8, and C-9 confirmed that the terminal double bond was located at C-8. The hydroxyl group was deduced to be located at C-3 based on the downfield chemical shift and the HMBC correlation from H-14 to C-3, together with the ^1H – ^1H COSY correlation of H-2/H-3. The key HMBC correlation from H-10 to C-7 confirmed that the oxygen bridge was between C-10 and C-7, in accordance with the molecular formula revealed by the HREIMS and the degrees of unsaturation in the molecule. The formation of the oxygen bridge made the structure cage-like.

The relative configuration of **3** was assigned by ROESY experiment. In the ROESY spectrum (Figure 2), the correlations of H-13/H-3, H-10, and H-12/H-11 indicated the α -orientation of H-10, 13- CH_3 , and H-3 and the β -orientation of 12- CH_3 and H-11. The β -orientation of 14- CH_3 was assigned by the correlations of H-3/H-5a (δ_{H} 2.09) and H-14/H-5b (δ_{H} 1.56). Therefore, the structure of **3** was assigned as shown, named toxicodenane C.

(7) Toxicodenane B (**2**): colorless gums; $[\alpha]_{\text{D}}^{15} + 7.69$ (c 0.24, CHCl_3); IR (KBr) ν_{max} 3423, 2958, 2924, 2853, 1727, 1462, 1377, 1159, 1065, 1033, 968 cm^{-1} ; ^1H and ^{13}C NMR spectroscopic data, see SI; ESIMS (negative): m/z 507 [$2\text{M} - \text{H}$][−]; HRESIMS (negative): m/z 253.1802 [$\text{M} - \text{H}$][−] (calcd for $\text{C}_{15}\text{H}_{25}\text{O}_3$, 253.1803).

(8) Toxicodenane C (**3**): colorless gums; $[\alpha]_{\text{D}}^{15} - 4.9$ (c 0.35, CHCl_3); IR (KBr) ν_{max} 3426, 2954, 2925, 2854, 1640, 1463, 1378, 1168, 1093, 1042, 1033, 881 cm^{-1} ; ^1H and ^{13}C NMR spectroscopic data, see SI; EIMS: m/z 236 [M]⁺; HREIMS: m/z 236.1778 [M]⁺ (calcd for $\text{C}_{15}\text{H}_{24}\text{O}_2$, 236.1776).

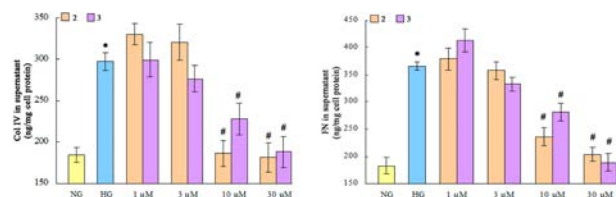


Figure 4. Compounds inhibited collagen IV and fibronectin production in a dose-dependent manner; * $p < 0.05$ vs NG; # $p < 0.05$ vs HG.

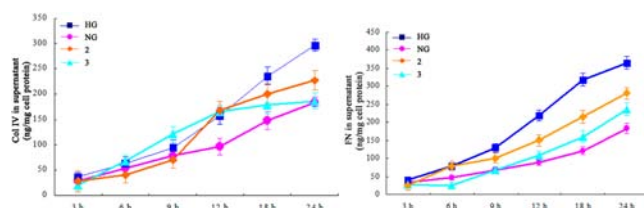


Figure 5. Compounds inhibited collagen IV and fibronectin production in a time-dependent manner at 10 μM .

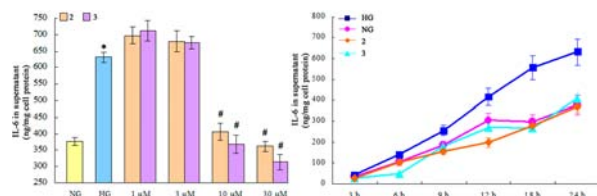
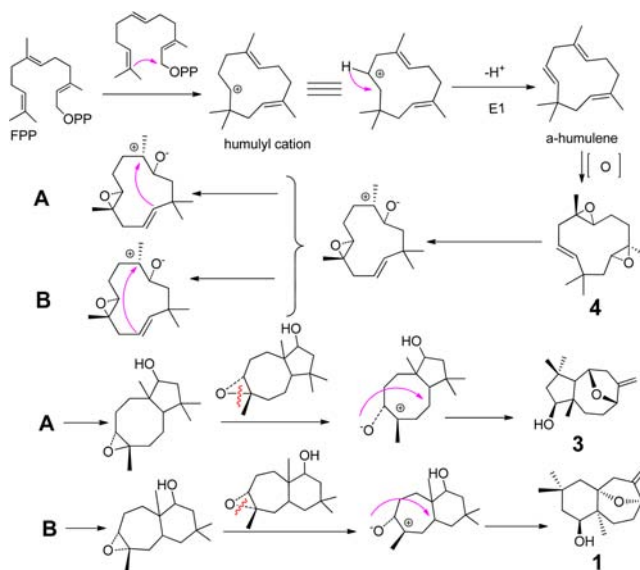


Figure 6. Compounds inhibited IL-6 production in a dose- (left) and time- (right) dependent manner; * $p < 0.05$ vs NG; # $p < 0.05$ vs HG.

Excessive accumulation of the extracellular matrix (fibronectin, collagen IV, etc.) in the glomerular mesangium is the major pathologic feature of DN and a cause of renal fibrosis or glomerulosclerosis. Therefore it has been considered as a crucial therapeutic target of DN.⁹ Chronic inflammation is definitely implicated in DN.¹⁰ Therefore, inhibition of proinflammatory factors (such as cytokin IL-6) will be beneficial for arresting the progression of DN. In this study, compounds **2** and **3** were examined for their inhibitory effects in high-glucose-induced mesangial cells.

As shown in Figures 4 and 5, high glucose levels induced overproduction of fibronectin (FN) and collagen IV (col IV) but this overproduction was significantly inhibited by pretreating mesangial cells with **2** and **3**. In addition, the inhibitory effects of **2** and **3** were both dose- and time-dependent. As shown in Figure 6, the high-glucose-induced overproduction of IL-6 was also alleviated significantly by

Scheme 1. Plausible Biogenetic Route of **1** and **3**



pretreating mesangial cells with **2** and **3**. TGF- β /Smads pathway is a critical pathway, but we found that the inhibitory effects of **2** and **3** are not via this pathway. As many signal pathways are involved in DN, the mode of action requires further investigation. In addition, to exclude the effects of **2** and **3** as being caused by their cellular toxicity, a cytotoxicity assay was performed in every experiment. The results showed that there was no significant difference among the groups (data not shown).

The isolate **4** was derived from α -humulene, which is formed from a common farnesyl pyrophosphate by an enzymatic cyclization reaction. We suggested that compounds **1** and **3** started from **4**. Oxidation of **4** followed by carbon bond formation led to A (5/8 ring system) and B (6/7 ring system). Starting from A and B, the formation of the oxygen bridge led to **3** and **1**, respectively.

A plausible biogenetic pathway of **1** and **3** was proposed as shown in Scheme 1.

To our knowledge, natural products which regulated the extracellular matrix of mesangial cells are relatively rare. Compounds **2** and **3** might provide new structure templates for antidiabetic nephropathy drug optimization.

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Supporting Information Available. Detailed experimental procedures, bioassay methods, 1D and 2D NMR, MS, and IR spectra of compounds **1–3**, X-ray crystal data of **1**, NMR data of **1–3**, and partial biological data. This material is available free of charge via Internet at <http://pubs.acs.org>.

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The authors declare no competing financial interest.