Chemical and Toxicological Investigations of a Previously Unknown Poisonous European Mushroom Tricholoma terreum

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Introduction

Cases of wild mushroom poisoning have been consistently linked to new syndromes worldwide. The established tradition of consuming and marketing wild mushrooms has focused attention on mycotoxicity, which has become a global issue. Guided by toxicity tests using mice, we recently isolated two new unusual toxic amino acids from the mushroom Trogia venenata. One of the toxic mushroom amino acids was detected directly from a blood sample from an individual who suffered a “sudden unexplained death”. Furthermore, we demonstrated that Trogia venenata was the likely cause of over 260 sudden unexplained deaths in Yunnan in the past 30 years.[1] In 2009, K. Hashimoto et al. isolated the small, highly strained carboxyl acid responsible for fatal rhabdomyolysis from the Asian toxic mushroom Russula subnigricans.[2] A recent report from Japan confirmed the existence of an unstable amino acid in the angel’s wing mushroom Pleurocybella porrigens, and seventeen people died of acute encephalopathy in Japan in 2004 after ingesting this mushroom.[3]

The wild mushroom Tricholoma equestre (or T. flavovirens) has resulted in poisoning in southwestern France. Twelve people were hospitalized for severe weakness and muscle loss after eating these mushrooms, and three died. The poisoning this mushroom causes is referred to as rhabdomyolysis. The patients reported fatigue, muscle weakness, myalgia, loss of appetite, mild nausea, profuse sweating, and darkening of the urine.[4] Despite the highly toxic nature of T. equestre, the poison in this mushroom has remained uncharacterized to date, which inspired us to investigate the toxic constituents of T. equestre and related species.

We collected T. equestre from sandy soils under pine trees on the coast of Arcachon in southwestern France. The Tricholoma terreum mushroom, another species of the same genus, was harvested from the same environment between late fall and midwinter. We postulated that T. equestre may have been mistaken for other members of the Tricholoma genus or that similar myotoxic effects may have been caused by another related fungus. Therefore, we tested the toxicities of the crude extracts of these two species. Both T. equestre and T. terreum were found to be toxic to mice when their extracts (CHCL/MeOH, 1:1) were administered orally. When the extracts were further partitioned between water and ethyl acetate, we found that the nonpolar fraction (ethyl acetate layer) of T. terreum and the polar fraction (water layer) of T. equestre were toxic, indicating that these mushrooms may contain different toxins.

Results and Discussion

Chemical investigation

To isolate the toxin responsible for Tricholoma poisoning, extracts of T. terreum were separated and examined using oral toxicity tests in mice. All of the animal experiments were performed with the approval of the Kunming Institute of Botany
Ethical Committee for the Experimental Use of Animals. We dried the fruiting bodies of *T. terreum* at 35 °C and cut them into pieces. The dried fruiting bodies (1.0 kg) were extracted with CHCl₃/MeOH (1:1), and the extract was separated by solvent partitioning between ethyl acetate and water. The ethyl acetate layer was concentrated under reduced pressure and purified using silica gel, Sephadex LH-20, RP-C₁₈ chromatography and HPLC, yielding 16 pure compounds (Figure 1).

Compound 1 was isolated as colorless crystals (CHCl₃/MeOH, 1:1), and determined to have a molecular formula of C₃₀H₄₄O₆ based on the HRIMS data, *m/z* 516.3090 [M]+ (calcd 516.3087). Many of the structural features of compound 1, including an 8,9-unsaturated-15-lactone moiety and a six-membered carbon ring cyclized by C₁ to C₆, were found to be the same as those of saponaceolide B.[⁵] Further analysis of the HMBC correlations established the presence of a substituted tetrahydrofuran ring consisting of carbons from C₃′ to C₆′. The ¹H NMR signals at δₗ = 1.38 (3H, s) and 1.39 ppm (3H, s) for the methyl groups at C₁₂′ and C₁₃′ displayed key HMBC correlations to the quaternary carbon at δₗ = 80.5 ppm (s, C₁) and the keto carbon at δₗ = 207.4 ppm (s, C₂′), and H₃′ also displayed HMBC correlations to C₁′ and C₂′. These data suggested that carbons C₁′ to C₅′ formed a pyran ring in which the ether bond was between C₁′ and C₅′, as deduced from the chemical shifts of C₁′ and C₅′ and the MS analysis. Therefore, compound 1 possessed a new 5/6/7 trioxaspiroketal system that may have resulted from a ketonaphthenic reaction between C₃′ and C₆′. Finally, a single-crystal X-ray diffraction (Figure 3) not only confirmed the new structure but also established the absolute configuration of the compound. Based on these findings, compound 1 was identified as terreolide A, as shown.

Figure 1. Structures of the triterpenoids from *Tricholoma terreum*.

Figure 2. Key 2D NMR spectroscopic correlations of compound 1.

Figure 3. X-ray structures for terreolide A (1).

Compound 2 was isolated as colorless crystals (CHCl₃/MeOH, 1:1) and determined to have a molecular of C₃₀H₄₄O₆ based on the HRIMS data, *m/z* 500.3152 [M]+ (calcd 500.3138), which was 16 mass units lower than that of compound 1. A preliminary analysis of the ¹D NMR spectrum suggested that the oxygen at C₇ in 1 was instead a methylene in 2; this was confirmed by the HMBC correlations of δₗ = 2.40 (1H, m, H₇a) and 2.27 ppm (1H, m, H₇b) to δₗ = 53.3 (d, C₆′) and 142.2 ppm (d, C₈) and by the ¹H-¹H COSY correlation between H₇ and δₗ = 6.8 Hz, H₄′a) and 2.02 ppm (1H, d, J = 13.2 Hz, H₄′b) revealed a connection between C₃′ and C₄′, whereas the key HMBC correlation from H₃ to the ketal carbon at δₗ = 107.2 ppm (s, C₆′) indicated an ether bond between C₃′ and C₆′, which differed from the bond between C₂′ and C₆′ in saponaceolide B.[⁵] Further analysis of the HMBC correlations established the presence of a substituted tetrahydrofuran ring consisting of carbons from C₃′ to C₆′. The ¹H NMR signals at δₗ = 1.38 (3H, s) and 1.39 ppm (3H, s) for the methyl groups at C₁₂′ and C₁₃′ displayed key HMBC correlations to the quaternary carbon at δₗ = 80.5 ppm (s, C₁) and the keto carbon at δₗ = 207.4 ppm (s, C₂′), and H₃′ also displayed HMBC correlations to C₁′ and C₂′. These data suggested that carbons C₁′ to C₅′ formed a pyran ring in which the ether bond was between C₁′ and C₅′, as deduced from the chemical shifts of C₁′ and C₅′ and the MS analysis. Therefore, compound 1 possessed a new 5/6/7 trioxaspiroketal system that may have resulted from a ketonaphthenic reaction between C₃′ and C₆′. Finally, a single-crystal X-ray diffraction (Figure 3) not only confirmed the new structure but also established the absolute configuration of the compound. Based on these findings, compound 1 was identified as terreolide A, as shown.

Figure 1. Structures of the triterpenoids from *Tricholoma terreum*.

Figure 2. Key 2D NMR spectroscopic correlations of compound 1.

Figure 3. X-ray structures for terreolide A (1).
Compound 3 was obtained as colorless crystals (CHCl₃) and had a molecular formula of C₃₀H₄₆O₈ based on the HREIMS data, m/z: 518.3243 [M]+ (calcld 518.3244), which was 16 mass units higher than that of saponaceolide B (7). The IR spectrum displayed signals consistent with a hydroxy group (3424 cm⁻¹), a carbonyl group (1729 cm⁻¹) and a double bond (1642 cm⁻¹). The ¹H and ¹³C NMR data of 3 were quite similar to those of 7. The primary difference was the presence of a 7-hydroxy group in 8, which was supported by the HMBC correlation from δ₇H₁ = 4.85 ppm (1H, brt, J = 4.1 Hz, H7) to δC₇ = 142.6 ppm (d, C8) and the ¹H-¹³C COSY correlation between H7 and δC₇. The key ROEY correlations and the similarities of the chemical shifts suggested that 8 had the same absolute configuration as 7. In addition, the stereoconguration of C7 in 8 was determined to be S by comparison of the coupling constant of H7 and the chemical shifts of C7 with those of 1. Finally the structure of 5 was determined, and it was named terreolide E.

Compound 9, a white powder, was determined to have a molecular formula of C₃₀H₄₆O₈ based on the HREIMS data, m/z: 560.3350 [M]+ (calcld 560.3349). The IR absorption and ¹D NMR spectroscopic data were quite similar to those of 8. The obvious differences were the characteristic signals for an acetyl group (δC₇ = 170.3 ppm; δC₈ = 205 ppm (3H, s)) in 9. Further analysis of the HMBC spectrum suggested that the acetyl group was attached at C7 based on the correlations from δH₇ = 5.89 ppm (1H, dd, J = 8.0, 2.6 Hz, H7) to δC₇ = 170.2 ppm (β, CH₃COO–). Complete analysis of the NMR spectroscopic data ultimately established the structure of compound 6, which was named saponaceolide H.

Compound 7 was first isolated from T. saponaceum in 1991 and was designated saponaceolide B. It strongly inhibited the growth of P-388 mouse leukemia cells. The absolute configuration of this compound was corrected in 1993. Subsequently, some related synthetic efforts were reported. In the present study, saponaceolide B was isolated as one of the primary constituents of T. terreum, and X-ray crystallographic analyses further confirmed its structure to be that shown in Figure 5.
170.3 ppm (s). The structure of 9 was established as saponaceolide I.

Compound 10 was isolated as a white powder. The HREIMS data at m/z 500.3127 [M]+ (calcd 500.3138) indicated that its molecular formula was C29H39O8. The 13C NMR data were quite similar to those of 7 with the exception of signals that indicated the presence of an additional trisubstituted double bond at δc = 134.5 (s, C9) and 123.0 ppm (d, C10). The HMBC spectrum suggested that the double bond was located between C9′ and C10′ based on the correlations of δc = 44.4 (1H, d, J = 13.8, H15′a) and 4.26 ppm (1H, d, J = 13.8, H15′b) with C9′ and of δc = 2.11 (1H, m, H8′a) and 2.02 ppm (1H, m, H8′b) with C9′, which resulted in the upfield shift of C15′ (δc = 60.1 ppm, t) and a downfield shift of C8′ (δc = 27.9 ppm, t). Further analyses of the HMBC and ROESY correlations confirmed that the other constituents of 10 were the same as those of 7. Thus, compound 10 was named saponaceolide J.

Compound 11 was determined to have a molecular formula of C29H39O8 by the HREIMS data, m/z: 558.3201 [M]+ (calcd 558.3193). The 1D NMR spectroscopic data revealed that it was a C7 acetoxyl derivative of compound 10, which was further supported by the HMBC correlations from δc = 5.91 ppm (1H, brd, J = 7.8 Hz, H-7) to δc = 170.2 ppm (s, CH2COO−) and by the 1H–1H COSY correlation between H7 and δc = 6.85 ppm (1H, m, H8). Detailed analyses of the 2D NMR spectroscopic data suggested that the other constituents of 11 were the same as those of 10. Thus compound 11 was established as saponaceolide K.

Compound 12 displayed an [M]+ peak at m/z: 532.3033 in its HREIMS, suggesting a molecular formula C29H39O8 that is 14 mass units greater than that of compound 8. Comparison of the NMR data of 8 and 12 showed similarities: one exception was the presence of an additional keto group at C3′ (δc = 205.2 ppm) in 12, which caused a significant upfield chemical shift of C4′ (Δδ = 15.1 ppm) and an upfield chemical shift of C2′ (Δδ = 1.8 ppm) in the 13C NMR spectrum. These observations were further supported by the HMBC correlations from δc = 4.35 (1H, s, OH2′), 3.07 (1H, d, J = 18.9 Hz, H4′a), and 2.44 ppm (1H, d, J = 18.9 Hz, H-4b) to δc = 205.2 ppm (s, C3′). Therefore, compound 12 was established as saponaceolide L.

The molecular formula of compound 13 was determined to be C24H30O8 based on the HREIMS data, m/z: 574.3142 [M]+ (calcd 574.3142). By comparing the 1H and 13C NMR data with those of 12, the principal difference was the presence of an additional acetyl group (δc = 170.3, 21.3 ppm; δc = 207.0 ppm) at OH7 in 13. This conclusion was supported by the HMBC correlation from δc = 5.90 ppm (1H, dd, J = 7.9, 2.8 Hz, H7) to the quaternary carbon at δc = 170.3 ppm (s, CH2COO−). Therefore, compound 13 was identified as the 7-O-acetyl derivative of compound 12 and was named saponaceolide M.

Compound 14 possessed the same molecular formula as 13 (C24H30O8) from HREIMS data, m/z: 574.3135 [M]+ (calcd 574.3142). A preliminary analysis of the 1D- and 2D NMR spectroscopic data suggested that 13 and 14 possessed the same structure. However, a detailed comparison of the 13C NMR data revealed very small differences in the carbon shifts for C7′ (Δδ = 1.9 ppm), C8′ (Δδ = 1.9 ppm), C9′ (Δδ = 3.3 ppm), C10′ (Δδ = 3.0 ppm), and C15′ (Δδ = 3.1 ppm) between the two compounds that resulted from a different stereoconfiguration at C9′ in 14. Careful analyses of the 2D NMR data suggested that the other constituents of compound 14 were the same as those of 13. Thus, the structure of compound 14 was determined to be saponaceolide N.

Compound 15, which was obtained as a white powder, displayed a [M]+ peak at m/z: 516.3087 in the HREIMS, corresponding to a molecular formula of C30H44O8. Comparison of the 1D NMR spectroscopic data with those of 8 showed that the oxymethylene at C7 in 8 was a keto carbonyl (δc = 205.7 ppm) in compound 15, resulting in a significant downfield chemical shift of CH6 (δc = 66.2 ppm, d; δc = 3.12 ppm, s). This was also supported by the HMBC correlations from H6 to δc = 205.7 ppm (s, C7). Furthermore, the double bond between C8 and C9 in 8 was instead located between C9 (δc = 126.7 ppm, s) and C10 (δc = 148.2 ppm, d) in compound 15, based on the 1H–1H COSY correlation between δc = 7.50 ppm (1H, br s, H10) and δc = 4.86 ppm (2H, m, H11). Further analysis of the 2D NMR data revealed that other structural features of 15 were the same as those of 8. Compound 15 was ultimately determined to be saponaceolide O.

Compound 16 was isolated as a white powder. Its molecular formula, C28H42O8, which was determined from its HREIMS data, m/z: 530.2891 [M]+ (calcd 530.2880), was 14 mass units higher than that of 15. The 1D NMR spectroscopic data revealed that 16 closely resembled 15; the primary difference was the presence of an additional keto group at δc = 205.2 ppm. The HMBC correlations from δc = 3.07 (1H, d, J = 18.8 Hz, H4′a) and 2.44 ppm (1H, d, J = 18.8 Hz, H4′a′) to δc = 205.2 ppm (s, C3′) supported the position of the keto group on C3′. The other constituents of 16 were to be the same as those of 15 through detailed analysis of the 2D NMR data. Thus, compound 16 was established as saponaceolide P.

Previously, only seven triterpenoids with this type of backbone were reported in the mushroom T. saponaceum.1,3,11 In this study, sixteen triterpenoids, including fifteen previously unidentified compounds, were isolated from T. terreum. Notably, two new triterpenoids backbone were discovered. A proposed biosynthetic pathway for these new triterpenoids backbone is provided in Scheme 1. These triterpenoids originated from two C15 sequences, which each followed the biogenetic isoprene rule. However, the two farnesyl units were not linked by tail-to-tail coupling as found in common triterpenes. The electrophilic attack of a formal carbocation at C1′ on the terminal double bond between C1 and C2 forms the C2′–C1′ linkage. The new 5/6/7 trioxaspiroketal system in terreolides A–C (1–3) results from a ketral reaction between C3′ and C6′ instead of between C2′ and C6′. In terreolides D–F (4–6), construction of the carbon system of the trioxaspiroketal moiety results from rearrangement of C4 to C2 under alkaline conditions.
Acute toxicity and the serum creatine kinase (CK) assay in mice

The crude *T. terreum* extract exhibited toxicity with an LD_{50} value of 1.51 g kg\(^{-1}\). The nonpolar fraction also displayed toxicity, with an LD_{50} value of 1.18 g kg\(^{-1}\), whereas the polar fraction did not exhibit toxicity, and the maximum tolerance dose was 10.0 g kg\(^{-1}\). Dose-dependent signs of intoxication, such as ataxia, reduced movement, and convulsions were observed and recurrent convulsions followed by death after 45–60 min. The LD_{50} values of 7 and 13 were determined to be 88.3 and 63.7 mg kg\(^{-1}\), respectively, following oral administration. Mice that were orally force-fed the two compounds exhibited dose-dependent signs of intoxication as the above.

The CK concentrations in sera from the treated animals were displayed a 1.52 difference in the mean value relative to the water control \(p < 0.01\) (t-test). The CK concentrations in sera from the treated animals were displayed a 1.52 difference in the mean value relative to the 1 %-Tween-80 control, \(p < 0.05\) (t-test).

As discussed previously, we found that *T. equestre* extract was also toxic but that the toxins differed from those found in *T. terreum*. The *T. equestre* toxins were present only in the polar fraction. We also examined the *T. equestre* species for the presence of saponaceolide B and the terreolides present in *T. terreum*, but none of them were detected. It is likely that *T. equestre* contains other, as yet unidentified toxins. We are continuing our attempts to determine which of the toxins found in *T. equestre* is responsible for its observed toxicity.

### Table 1. Serum creatine kinase (CK) level in mice after five consecutive daily oral exposures to the nonpolar fraction (NF), compounds 7, 13, and \(p\)-phenylenediamine.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg kg(^{-1}))</th>
<th>n(^{[a]})</th>
<th>24 h Mean CK</th>
<th>Rel. Mean CK</th>
<th>48 h Mean CK</th>
<th>Rel. Mean CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^{[b]})</td>
<td>–</td>
<td>12</td>
<td>867.3</td>
<td>inc.(^{[c]})</td>
<td>724.3</td>
<td>inc.(^{[c]})</td>
</tr>
<tr>
<td>1 %-Tween-80(^{[b]})</td>
<td>–</td>
<td>12</td>
<td>949.6</td>
<td>inc.(^{[c]})</td>
<td>679.3</td>
<td>inc.(^{[c]})</td>
</tr>
<tr>
<td>Pos. cont.</td>
<td>50</td>
<td>12</td>
<td>1616.4</td>
<td>1.86</td>
<td>1286.6(^{[f]})</td>
<td>1.78</td>
</tr>
<tr>
<td>NF</td>
<td>300</td>
<td>12</td>
<td>1075.8</td>
<td>1.13</td>
<td>1709.9(^{[e]})</td>
<td>2.52</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>12</td>
<td>1056.7</td>
<td>1.11</td>
<td>1120.1(^{[f]})</td>
<td>1.65</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>12</td>
<td>1082.1</td>
<td>1.14</td>
<td>1034.2(^{[e]})</td>
<td>1.52</td>
</tr>
</tbody>
</table>

\(^{[a]}\) The number (n) of mice tested. \(^{[b]}\) Control mice were treated with water or with 1 %-Tween-80. \(^{[c]}\) Rel. inc. is the relative increase compared to control mice treated with water or with 1 %-Tween-80. \(^{[d]}\) Difference in the mean value relative to the water control \(p < 0.01\) (t-test). \(^{[e]}\) Difference in the mean value relative to the 1 %-Tween-80 control, \(p < 0.01\) (t-test). \(^{[f]}\) Difference in the mean value relative to the 1 %-Tween-80 control, \(p < 0.05\) (t-test).

### Conclusion

We have identified a hitherto unknown poisonous European mushroom, *Tricholoma terreum*, and have isolated the main toxins, saponaceolide B (7) and saponaceolide M (13), both of which are abundantly expressed in *T. terreum*. The two toxic compounds (7 and 13) showed LD_{50} values of 88.3 and 63.7 mg kg\(^{-1}\) and were lipophilic, thus, they differed from the hydrophilic constituents recently reported in the literature\(^{[1–3]}\). Additionally, these compounds increased serum CK levels in mice. The new triterpenoids terreolides A–C (1–3) possessed a unique 5/6/7 trioxaspiroketal system, and terreolides D–F (4–6) possessed an unprecedented carbon skeleton. The absolute configurations of the structures of these triterpenoids were confirmed by single-crystal X-ray crystallography. The results of the present study indicate that the previously reported mycotoxic effects of *T. equestre* may not be specific to one mushroom species, and the observed toxicity may be caused by a similar or related fungus. The harmful effects likely result from combined effects because all reported cases of intoxication have involved at least three consecutive meals of *T. equestre*. This mushroom may have been confused with *T. terreum* given that the two mushrooms grow in similar environments and that they belong to the same genus. Mushrooms may be more complex and opportunistic than previously realized.

### Experimental Section

#### General

Melting points were determined by using a Yuhua X-4 digital micro-display melting point apparatus. Optical rotations (OR) were recorded on a JASCO P-1020 digital polarimeter. UV spectra were obtained using a Shimadzu UV2401PC spectrometer, and IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. Nuclear magnetic resonance (NMR) spectra were obtained at room temperature using a Bruker AM-400, a Bruker DRX-500 and a Bruker Avance III 600 MHz spectrometer. Tetramethylsilane (TMS) was used as an internal standard. HREIMS were record-
ed on a Waters AutoSpec Premier P776 instrument. X-ray diffraction was performed on an APEX DUO spectrophotometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., P.R. China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for open column chromatography (CC). Preparative HPLC was performed on an Agilent 1100 liquid chromatography system equipped with a Zorbax SB-C18 column (21.2 mm × 150 mm). Fractions were monitored using TLC. Spots were visualized by heating silica gel plates immersed in vanillin/H2SO4 in ethanol.

**Fungi materials**

The *Tricholoma terreum* mushroom was collected from Arcachon in southwestern France in December 2011 and was identified by Prof. Zhu-Liang Yang of Kunming Institute of Botany, Chinese Academy of Sciences. A specimen (No. KIB201112) was deposited at the Kunming Institute of Botany, Chinese Academy of Sciences. Pileus: 5–8 cm in diameter, convex-conical to umbo-nate, blackish grey, fibrilllose to innately streaky. Lamellae: not very crowded, pale greyish. Stipe: 6–10.0×0.8–1.2 cm, subylindrical or attenuate towards the base, white, glabrous to fibrillose. Context: whitish to dirty white. Smell: indistinct. Taste: mild. Spores: 5.5–7.8–4.5 μm, ellipsoid to oblong, thin-walled, smooth, non-amyloid, colorless and hyaline. Clamp connections absent. The mushrooms were growing on calcareous soils under coniferous trees.

**Extraction and isolation**

The dried fruiting bodies of *T. terreum* (1.0 kg) were extracted with CHCl3/MeOH (1:1). The extract was separated by partitioning between EtOAc and water. The EtOAc layer was concentrated under reduced pressure to generate a crude extract (23 g), and this residue was subjected to silica gel column chromatography (CC) eluting with a gradient of CHCl3/MeOH (from 1:0 to 0:1) to obtain seven fractions (A–G). A mixture was crystallized from fraction B (1.2 g) and purified using semi-preparative HPLC (MeCN/H2O, from 3:7 to 3:2, 20 mL/min−1, 30 min) to yield compounds 4 (2 mg), 7 (414 mg) and 1 (208 mg). Fraction C (2.6 g) was subjected to CC over RP-18 (MeOH/H2O from 1:1 to 1:0) to obtain fractions C1–C14.

Fraction C7 (0.8 g) was separated by repeated CC over silica gel with petroleum ether/EtOAc (from 6:1 to 2:1) to yield 19 (152 mg), 11 (11 mg), 13 (184 mg), 14 (4 mg), 16 (8 mg), and 6 (68 mg). Fraction C8 (0.5 g) was separated using Sephadex LH-20 (CHCl3/MeOH, 1:1) followed by preparative HPLC (MeCN/H2O, from 7:3 to 1:0, 20 mL/min−1, 40 min) to yield 8 (145 mg), 10 (4 mg), 12 (10 mg), 15 (7 mg), and 16 (15 mg). Fraction C11 (700 mg) was separated using silica gel CC eluting with CHCl3/acetonitrile (100:1) and purified using preparative HPLC (MeCN/H2O, from 1:1 to 9:1, 20 mL/min−1, 30 min) to yield 2 (32 mg) and 3 (28 mg).

**Terreolide A (1):** Colorless crystals (aq. CHCl3/MeOH, 1:1). M.p. 209−210 °C; [α]20D = +27.4° (c = 0.15, CHCl3/MeOH, 1:1); [α]20D (MeOH) = +224.3 (log ε: 224.3292, 0.202 nm (3.28 dm3 mol−1 cm−1)); HREIMS: m/z: calc’d for C55H60O4: 856.3087; found: 856.3090.

**Terreolide B (2):** Colorless crystals (aq. CHCl3), M.p. 158−160 °C; [α]20D = +21.1° (c = 0.23, MeOH); [α]20D (MeOH) = +390.8 (log ε: 224.329, 0.202 nm (3.28 dm3 mol−1 cm−1)); HREIMS: m/z: calc’d for C55H60O4: 856.3087; found: 856.3090.

**Terreolide C (3):** Colorless crystals (aq. CHCl3), M.p. 98−100 °C; [α]20D = +54.1° (c = 0.19, MeOH); [α]20D (MeOH) = +390.8 (log ε: 224.329, 0.202 nm (3.28 dm3 mol−1 cm−1)); HREIMS: m/z: calc’d for C55H60O4: 856.3188; found: 856.3190.

**Terreolide D (4):** Colorless crystals (aq. acetone). M.p. 195−196 °C; [α]20D = +14.7° (c = 0.23, MeOH); [α]20D (MeOH) = +390.8 (log ε: 224.329, 0.202 nm (3.28 dm3 mol−1 cm−1)); HREIMS: m/z: calc’d for C55H60O4: 856.3192; found: 856.3193.

**Terreolide E (5):** White powder. [α]20D = −7.4° (c = 0.33, MeOH); [α]20D (MeOH) = +390.8 (log ε: 224.329, 0.202 nm (3.28 dm3 mol−1 cm−1)); HREIMS: m/z: calc’d for C55H60O4: 856.3087; found: 856.3097.
Saponaceolide J (11): White, amorphous powder. [α]D20 = −48.5 (c = 0.22, MeOH); 1H NMR (400 MHz, CDCl3, 25 °C, TMS): δ = 6.85 (m, 1 H), 1.62 (m, 1 H), 1.05 (m, 1 H), 0.59 (s, 3 H), 1.98 (br s, 1 H), 4.98 (brs, 1 H), 5.48 (d, J = 14.6 Hz, 1 H); 4.40 (m, 2 H); 1.94 (d, J = 14.6 Hz, 1 H), 1.31 (s, 3 H), 1.27 (s, 3 H), 1.22 (s, 3 H), 0.75 ppm (s, 3 H); 13C NMR (100 MHz, CDCl3, 25 °C, TMS): δ = 171.1, 170.2, 144.2, 137.7, 134.0, 123.6, 122.8, 110.7, 101.7, 97.7, 77.3, 72.4, 70.4, 65.5, 61.6, 57.0, 48.9, 39.7, 37.8, 30.8, 29.7, 28.8, 28.0, 27.9, 26.4, 25.3, 24.9, 23.0, 21.3, 18.7, 16.6 ppm; IR (KBr): υmax (log ε) = 3431, 2943, 1743, 1643, 1373, 1322, 1195, 1071, 1028, 992 cm−1; UV (MeOH): λmax (log ε) = 202 nm (3.33 dm3 mol−1 cm−1); HREIMS: m/z: calcld for C30H44O8: 558.3193; found: 558.3201.

Saponaceolide L (12): White, amorphous powder. [α]D20 = −20.1 (c = 0.28, MeOH); 1H NMR (400 MHz, CDCl3, 25 °C, TMS): δ = 6.67 (m, 1 H), 1.97 (m, 1 H), 4.98 (brs, 1 H), 4.39 (m, 2 H), 2.90 (m, 1 H), 1.41 (m, 1 H), 1.16 (m, 1 H), 1.02 (s, 3 H), 0.98 ppm (s, 3 H); 13C NMR (100 MHz, CDCl3, 25 °C, TMS): δ = 128.3, 124.7, 124.6, 110.6, 101.2, 96.7, 77.5, 72.9, 69.6, 65.9, 65.6, 58.9, 48.0, 39.5, 36.4, 35.7, 31.6, 29.2, 29.1, 28.5, 27.8, 27.3, 25.8, 25.3, 24.8, 22.4, 18.8, 18.4 ppm; IR (KBr): υmax (log ε) = 3424, 2923, 1729, 1642, 1379, 1206, 1138, 1074, 996 cm−1; UV (MeOH): λmax (log ε) = 223 (3.30), 203 nm (3.29 dm3 mol−1 cm−1); HREIMS: m/z: calcld for C30H44O8: 514.324; found: 514.323.

Saponaceolide M (13): White, amorphous powder. [α]D20 = −29.4 (c = 0.15, MeOH); 1H NMR (400 MHz, CDCl3, 25 °C, TMS): δ = 6.85 (m, 1 H), 1.97 (m, 1 H), 4.98 (brs, 1 H), 4.40 (m, 2 H), 2.90 (m, 1 H), 1.41 (m, 1 H), 1.16 (m, 1 H), 1.02 (s, 3 H), 0.98 ppm (s, 3 H); 13C NMR (100 MHz, CDCl3, 25 °C, TMS): δ = 171.2, 170.3, 144.2, 137.8, 126.3, 106.1, 101.6, 96.6, 77.5, 72.7, 70.5, 65.8, 65.5, 57.1, 48.8, 39.9, 37.8, 35.6, 31.5, 29.7, 29.2, 28.5, 27.9, 27.2, 26.3, 25.8, 24.8, 24.7, 22.4, 21.3, 20.8, 16.7 ppm; IR (KBr): υmax (log ε) = 3426, 2924, 1740, 1630, 1460, 1377, 1218, 1076, 995 cm−1; UV (MeOH): λmax (log ε) = 256 (2.96), 203 nm (4.53 dm3 mol−1 cm−1); HREIMS: m/z: calcld for C30H44O8: 560.3349; found: 560.3350.
26.7, 26.4, 24.9, 22.1, 21.1, 20.0, 16.6 ppm; IR (KBr): \( \nu \text{max} = 3439, 2935, 1753, 1722, 1368, 1241, 1201, 1076, 1037, 995 \text{ cm}^-1 \); UV (MeOH): \( \lambda_{\text{max}} \) (log \( e \)) = 219 (3.36), 202 nm (3.37 \text{ dm}^3 \text{ mol}^-1 \text{ cm}^-1 ); HREIMS: \( m/z \) 752.3412; found: 752.3135.

**Saponacide P (15):** White, amorphous powder. \( a^2_{0.0} = 16.7 \) (c = 0.34, MeOH); \( \mu \) (MeOH) = 0.45 (40 MHz, CDCl3, 25 °C, TMS). \( \delta \) = 7.50 (brs, 1H), 4.86 (brs, 1H), 4.85 (brs, 1H), 4.43 (brs, 1H), 3.56 (d, \( J = 19.0 \) Hz, 1H), 3.55 (m, 2H), 3.39 (d, \( J = 19.0 \) Hz, 1H), 3.13 (s, 1H), 3.07 (d, \( J = 18.8 \) Hz, 1H), 2.44 (d, \( J = 18.8 \) Hz, 1H), 2.36 (m, 1H), 2.13 (m, 1H), 2.06 (m, 1H), 1.75 (m, 1H), 1.73 (m, 1H), 1.62 (m, 1H), 1.51 (m, 1H), 1.48 (m, 1H), 1.36 (s, 3H), 1.25 (m, 1H), 1.16 (m, 1H), 1.11 (s, 3H), 1.09 (m, 1H), 1.01 (m, 1H), 0.98 (s, 3H), 0.89 (s, 3H), 0.79 ppm (m, 1H); \( \mu \) (CD3OD, 25 °C, TMS). \( \delta \) = 205.7, 173.9, 148.2, 144.2, 126.7, 109.0, 101.2, 96.9, 77.5, 72.7, 60.6, 66.2, 48.3, 40.9, 39.6, 36.4, 35.6, 31.2, 29.2, 28.9, 28.5, 27.8, 26.3, 25.8, 24.8, 22.4, 20.8, 14.9 ppm; IR (KBr): 3439, 2937, 1759, 1722, 1451, 1365, 1209, 1075, 994 \text{ cm}^-1 ; UV (MeOH): \( \lambda_{\text{max}} \) (log \( e \)) = 204 nm (3.37 \text{ dm}^3 \text{ mol}^-1 \text{ cm}^-1 ); positive ion HREIMS: \( m/z \) 752.3087; found: 752.3087.

**Acute toxicity experiments in mice:**

Healthy male and female KM mice, weighing 18–22 g, were purchased from the Experimental Centre of Kunming Medical College (Certificate No. SCXK2005-0008). The animals were housed on a constant 12-h-light/12-h-dark cycle in a temperature-controlled central animal facility (18–22 °C) and allowed free access to solid food and tap water. All of the procedures conformed to the guidelines of the Ethical Committee for the Experimental Use of Animals.

**Toxicities of the extracts following oral administration:** We dried the fruiting bodies of *T. terreum* at 35 °C and cut the fruiting bodies into pieces. These were extracted with CHCl3/MeOH (1:1) to generate a crude extract. The crude extract was separated by partitioning between ethyl acetate and water. The crude extract, together with the polar fraction and the nonpolar fractions, was dissolved in 1 %-Twee-80 and orally force-fed to mice to determine the toxicities of the fractions. Pilot tests were conducted to determine the dose range of the three fractions to be administered. The maximum dose that produced 0 % death and the minimum dose that produced 100 % death were determined. Then, for each of the three fractions, 5 graded single doses were administered orally to 5 groups of 10 mice each. The crude extract displayed toxicity with an LD50 value of 1.51 g kg^-1_. The nonpolar fraction displayed toxicity with an LD50 value of 1.18 g kg^-1_. Whereas the polar fraction did not exhibit toxicity, showing a maximum tolerated dose of 10.0 g kg^-1_. Dose-dependent signs of intoxication such as ataxia, reduced movement, and convulsions were observed, and recurrent convulsions followed by death after 45–60 min.

**Toxicities of compounds 7 and 13 following oral administration:** Guided by the methods outlined above, the LD50 values of 7 and 13 were determined to be 88.3 and 63.7 mg kg^-1_, respectively, following oral administration. Mice that were orally force-fed one of the two compounds exhibited dose-dependent signs of intoxication as outlined above.
Serum creatine kinase (CK) assay in mice

Healthy male ICR mice, weighting 18–22 g, were purchased from Chengdu Dossy Biological Technology Co. (Certificate No. SCXK-(CHUAN)2013-24). The animals were housed on a constant 12 h-light/12 h-dark cycle in a temperature-controlled central animal facility (18–22 °C) and allowed free access to solid food and tap water. All of the procedures were in accordance with the Ethical Committee for the Experimental Use of Animals.

Pilot tests were conducted to determine the appropriate dose ranges of the nonpolar fraction and of compounds 7 and 13. The maximum tolerated doses of nonpolar fraction, compound 7, and compound 13 were 390, 31.1, and 28.1 mg kg$^{-1}$, respectively. The ICR mice were randomly divided into six groups of 12 mice each: Group 1 was the negative control group (water-treated); group 2 was treated with solvent (1 %-Tween-80); group 3 was the positive control group and received 50 mg kg$^{-1}$ p-phenylenediamine dissolved in water; group 4 received 300 mg kg$^{-1}$ of the nonpolar fraction (dissolved in 1 %-Tween-80); groups 5 and 6 were treated with 20 mg kg$^{-1}$ of compounds 7 and 13 (dissolved in 1 %-Tween-80), respectively. Each sample was orally force-fed to mice once daily for 5 days. Blood samples were collected from the tail vein of the mice 24 and 48 h after the final dose and centrifuged at 3000 rpm for 10 min in 4 °C to obtain serum. The creatine kinase concentration of the serum was determined by Roche Automated Biochemical Analyzer (Combas Integra 400 plus).

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[9] Tables containing the proton and the carbon shift values are available in the Supporting Information.

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