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Natural Products

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

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Abstract: The established tradition of consuming and marketing wild mushrooms has focused attention on mycotoxicity, which has become a global issue. In the present study, we describe the toxins found in a previously unknown poisonous European mushroom *Tricholoma terreum*. Fifteen new triterpenoids terreolides A–F (**1**–**6**) and saponaceolides H–P (**8**–**16**) were isolated from the fruiting bodies of the toxic mushroom *T. terreum*. Terreolides A–C (**1**–**3**) possessed a unique 5/6/7 trioxaspiroketal system, whereas terreolides D–F (**4**–**6**) possessed an unprecedented carbon skeleton. Two abundant compounds in the mushroom, saponaceolide B (**7**) and saponaceolide M (**13**), displayed acute toxicity, with LD_{50} values of 88.3 and 63.7 mg kg^{-1} when administered orally in mice. Both compounds were found to increase serum creatine kinase levels in mice, indicating that *T. terreum* may be the cause of mushroom poisoning ultimately leading to rhabdomyolysis.

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 carboxylic 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] 脑病

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 ($CHCl_3$/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

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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 $CHCl_3$/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-C_{18} chromatography and HPLC, yielding 16 pure compounds (Figure 1).

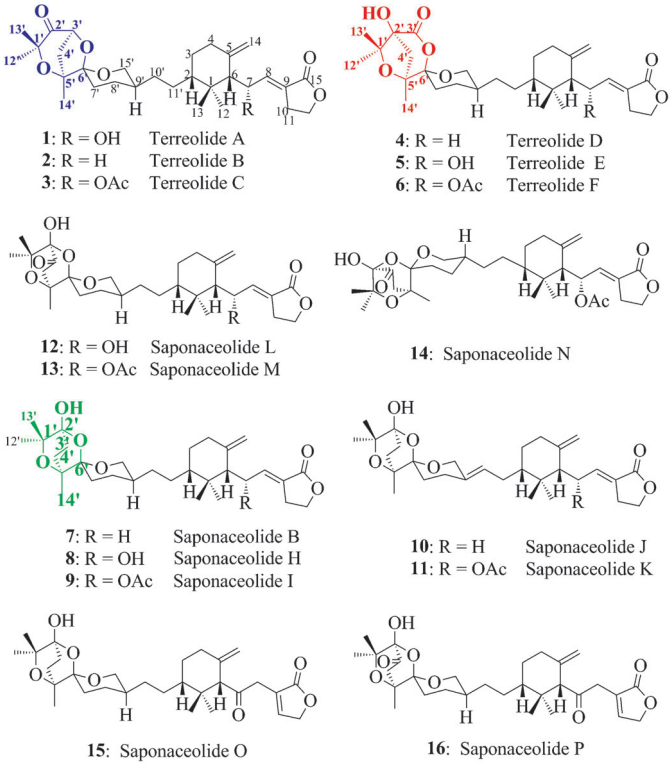

Figure 1. Structures of the triterpenoids from *Tricholoma terreum*.

Compound **1** was isolated as colorless crystals ($CHCl_3$/MeOH, 1:1), and determined to have a molecular formula of $C_{30}H_{44}O_7$ based on the HREIMS 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] on the basis of a preliminary analysis of the 1D- and 2D NMR spectroscopic analyses. However, instead of a methylene, an oxygenated methine was present (δ_H=4.84, δ_C=69.6 ppm) at C7 in **1**, as supported by the HMBC correlations (Figure 2) from H7 (δ_H=4.84 ppm, t, J=4.1 Hz) to C6 (δ_C=58.8 ppm) and C8 (δ_C=142.8 ppm). Moreover, the presence of signals for a keto carbonyl (δ_C=207.4 ppm) and an oxygenated methine (δ_H=4.19 ppm; δ_C=77.1 ppm) indicated that significant changes to the trioxaspiroketal system had occurred compared with saponaceolide B. In the ^{1}H-^{1}H COSY spectrum, the correlations of δ_H=4.19 ppm (1 H, br d, J=6.8 Hz, H3′) with the signals at 2.74 (1 H, dd, J=13.2, 6.8 Hz, H4′a) and 2.02 ppm (1 H, d, J=13.2 Hz, H4′b) revealed a connection between C3′ and C4′, whereas the key

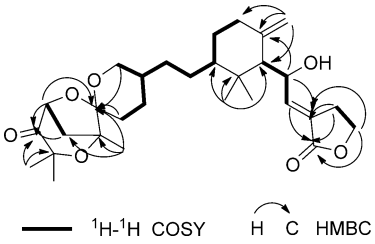

Figure 2. Key 2D NMR spectroscopic correlations of compound **1**.

HMBC correlation from H3′ to the ketal carbon at δ_C=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 ^{1}H NMR signals at δ_H=1.38 (3 H, s) and 1.39 ppm (3 H, s) for the methyl groups at C12′ and C13′ displayed key HMBC correlations to the quaternary carbon at δ_C=80.5 ppm (s, C1′) and the keto carbon at δ_C=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 ketal 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.

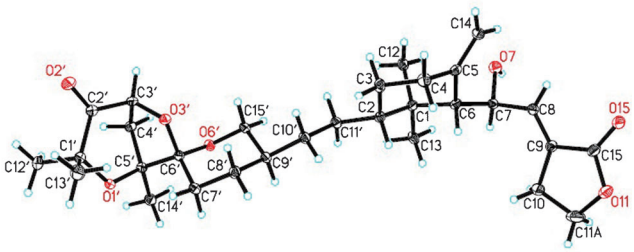

Figure 3. X-ray structures for terreolide A (**1**).

Compound **2** was isolated as colorless crystals ($CHCl_3$/MeOH, 1:1) and determined to have a molecular of $C_{30}H_{44}O_6$ based on the HREIMS 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 oxymethine at C7 in **1** was instead a methylene in **2**; this was confirmed by the HMBC correlations of δ_H=2.40 (1 H, m, H7a) and 2.27 ppm (1 H, m, H-7b) to δ_C=53.3 (d, C6) and 142.2 ppm (d, C8) and by the ^{1}H-^{1}H COSY correlation between H7 and δ_H=6.69 ppm (1 H, m, H8). A detailed analysis of the 2D NMR (^{1}H-^{1}H COSY, HMBC, and ROESY) spectroscopic data supported the structure of compound **2**, as shown, which was termed terreolide B.

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Compound **3** was obtained as colorless crystals ($CHCl_3$) and had a molecular formula of $C_{32}H_{46}O_8$ based on the HREIMS data, *m/z*: 558.3182 $[M]^+$ (calcd 558.3193). The IR spectrum showed absorption bands at 3432, 1735, 1726, and 1630 cm^{-1}, indicating the existence of hydroxy and carbonyl groups. The ^1H and ^{13}C NMR spectroscopic data were very similar to those of compound **1**, with the exception of an additional acetyl group in **3**. The strong HMBC correlations from $\delta_H = 2.06$ ppm (3H, s, CH_3COO–) to $\delta_C = 70.5$ ppm (d, C7) and from $\delta_H = 5.89$ ppm (1H, dd, $J = 7.7$, 2.3 Hz, H7) to $\delta_C = 170.2$ ppm (s, CH_3COO–) suggested that the acetyl group was attached at C7. Detailed analyses of the 2D NMR spectra ($^1H^1H$ COSY, HMBC, and ROESY) finally confirmed the structure of **3** to be that shown, and it was named terreolide C.

Compound **4** was isolated as colorless crystals (acetone) and had a molecular formula of $C_{30}H_{44}O_7$ (HREIMS, *m/z*: 516.3097 $[M]^+$, calcd 516.3087). According to analyses of the 1D- and 2D NMR spectroscopic data, many structural components of **4** were similar to those of **1**. However, a lactone carbon at $\delta_C = 174.5$ ppm (s, C3′) and an sp^3 quaternary carbon at $\delta_C = 80.2$ ppm (s, C2′) suggested significant differences in the trioxaspiroketal system. Due to the presence of multiple quaternary carbons, the 2D NMR data did not provide sufficient evidence to establish the structure. Fortunately, a single-crystal X-ray diffraction analysis elucidated both the planar structure and the absolute configuration of **4** (Figure 4). Thus, compound **4** was determined to be terreolide D and possessed a new carbon skeleton in which a bond between C2′ and C4′ was present as a result of cleavage of the bond between C3′ and C4′, and C3′ was oxygenated and was a lactone carbon cyclized with the spiroketal carbon at C6′.

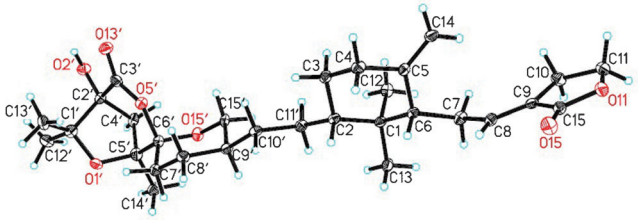

Figure 4. X-ray structures for terreolide D (**4**).

Compound **5**, a colorless oil, was determined to have a molecular formula of $C_{30}H_{44}O_8$ according to the HREIMS data, *m/z*: 532.3051 $[M]^+$ (calcd 532.3036). Compound **5** was readily identified as the 7-hydroxy derivative of **4**; this was supported by the HMBC correlations from $\delta_H = 4.86$ ppm (1H, brs, H7) to $\delta_C = 58.8$ ppm (d, C6) and 142.8 (d, C8) and by the ^1H-^1H COSY correlations from H6 to H8. Detailed analyses of the 2D NMR data suggested that the other constituents of **5** were the same as those of **4**. The stereoconfiguration of C7 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 **6**, a colorless oil, was determined to have a molecular formula of $C_{32}H_{46}O_9$ according to the HREIMS data, *m/z*: 574.3151 $[M]^+$ (calcd 574.3142), suggesting 10 degrees of unsaturation. By comparing the ^1H and ^{13}C NMR spectroscopic data, compound **6** was readily identified as the 7-*O*-acetyl derivative of **5**, as indicated by the HMBC correlation from $\delta_H = 5.90$ ppm (1H, dd, $J = 7.9$, 2.7 Hz, H7) to $\delta_C = 170.2$ ppm (s, CH_3COO–). Complete analysis of the NMR spectroscopic data ultimately established the structure of compound **6**, which was named terreolide F.

Compound **7** was first isolated from *T. saponaceum* in 1991 and was designated saponaceolide B.[5] It strongly inhibited the growth of P-388 mouse leukemia cells. The absolute configuration of this compound was corrected in 1993.[6] Subsequently, some related synthetic efforts were reported.[6,7] 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.

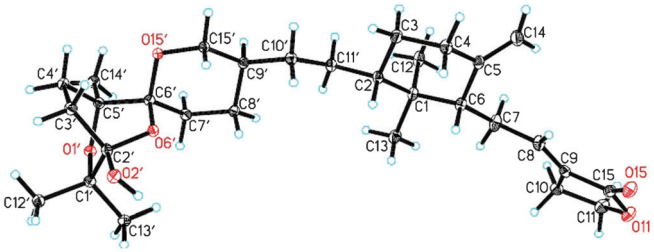

Figure 5. X-ray structures for saponaceolide B (**7**).

Compound **8** was obtained as a white powder with a molecular formula of $C_{30}H_{46}O_7$ based on the HREIMS data, *m/z*: 518.3243 $[M]^+$ (calcd 518.3244), which was 16 mass units higher than that of saponaceolide B (**7**).[5] 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 ^{13}C NMR data of **8** were quite similar to those of **7**. The primary difference was the presence of a 7-hydroxy group in **8**, this was supported by the HMBC correlations from $\delta_H = 4.85$ ppm (1H, brt, $J = 4.1$ Hz, H7) to $\delta_C = 58.9$ (d, C6) and 142.6 ppm (d, C8) and the ^1H-^1H COSY correlation between H7 and $\delta_H = 6.88$ ppm (1H, m, H8). The key ROESY correlations and the similarities of the chemical shifts suggested that **8** had the same absolute configuration as **7**.[5] In addition, the stereoconfiguration of C7 in **8** was determined to be *S* by comparison of the coupling constant of H7 and the chemical shifts of CH7 with those of **1**, as previously described. Compound **8** was ultimately established as saponaceolide H.

Compound **9**, a white powder, was determined to have a molecular formula of $C_{32}H_{48}O_8$ based on the HREIMS data, *m/z*: 560.3350 $[M]^+$ (calcd 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 ($\delta_C = 170.3$ (s), 21.3 ppm (q); $\delta_H = 2.05$ 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 $\delta_H = 5.89$ ppm (1H, dd, $J = 8.0$, 2.6 Hz, H7) to $\delta_C =$

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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 $C_{30}H_{44}O_6$. The ^{13}C 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 $\delta_C = 134.5$ (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 $\delta_H = 4.44$ (1 H, d, $J = 13.8$, H15′a) and 4.26 ppm (1 H, d, $J = 13.8$, H15′b) with C9′ and of $\delta_H = 2.11$ (1 H, m, H8′a) and 2.02 ppm (1 H, m, H8′b) with C9′, which resulted in the upfield shift of C15′ ($\delta_C = 60.1$ ppm, t) and a downfield shift of C8′ ($\delta_C = 27.9$ 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 $C_{32}H_{46}O_8$ 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 $\delta_H = 5.91$ ppm (1 H, br d, $J = 7.8$ Hz, H-7) to $\delta_C = 170.2$ ppm (s, CH_3COO–) and by the ^1H-^1H COSY correlation between H7 and $\delta_H = 6.85$ ppm (1 H, 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 $C_{30}H_{44}O_8$ 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′ ($\delta_C =$ 205.2 ppm) in **12**, which caused a significant downfield chemical shift of C4′ ($\Delta\delta = 15.1$ ppm) and an upfield chemical shift of C2′ ($\Delta\delta = 1.8$ ppm) in the ^{13}C NMR spectrum. These observations were further supported by the HMBC correlations from $\delta_H = 4.35$ (1 H, s, OH2′), 3.07 (1 H, d, $J = 18.9$ Hz, H4′a), and 2.44 ppm (1 H, d, $J = 18.9$ Hz, H-4′b) to $\delta_C = 205.2$ ppm (s, C3′). Therefore, compound **12** was established as saponaceolide L.

The molecular formula of compound **13** was determined to be $C_{32}H_{46}O_9$ based on the HREIMS data, *m/z*: 574.3142 $[M]^+$ (calcd 574.3142). By comparing the ^1H and ^{13}C NMR data with those of **12**, the principal difference was the presence of an additional acetyl group ($\delta_C = 170.3$, 21.3 ppm; $\delta_H = 2.07$ ppm) at OH7 in **13**. This conclusion was supported by the HMBC correlation from $\delta_H = 5.90$ ppm (1 H, dd, $J = 7.9$, 2.8 Hz, H7) to the quaternary carbon at $\delta_C = 170.3$ ppm (s, CH_3COO–). 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** ($C_{32}H_{46}O_9$) 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 ^{13}C NMR data revealed very small differences in the carbon shifts for C7′ ($\Delta\delta = 1.9$ ppm), C8′ ($\Delta\delta = 1.9$ ppm), C9′ ($\Delta\delta = 3.3$ ppm), C10′ ($\Delta\delta = 3.0$ ppm), and C15′ ($\Delta\delta = 3.1$ 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 $C_{30}H_{44}O_7$. Comparison of the 1D NMR spectroscopic data with those of **8** showed that the oxymethine at C7 in **8** was a keto carbonyl ($\delta_C =$ 205.7 ppm) in compound **15**, resulting in a significant downfield chemical shift of CH6 ($\delta_C = 66.2$ ppm, d; $\delta_H = 3.12$ ppm, s). This was also supported by the HMBC correlations from H6 to $\delta_C = 205.7$ ppm (s, C7). Furthermore, the double bond between C8 and C9 in **8** was instead located between C9 ($\delta_C =$ 126.7 ppm, s) and C10 ($\delta_C = 148.2$ ppm, d) in compound **15**, based on the ^1H-^1H COSY correlation between $\delta_H = 7.50$ ppm (1 H, br s, H10) and $\delta_H = 4.86$ ppm (2 H, 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, $C_{30}H_{42}O_8$, 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 $\delta_C = 205.2$ ppm. The HMBC correlations from $\delta_H = 3.07$ (1 H, d, $J = 18.8$ Hz, H4′a) and 2.44 ppm (1 H, d, $J = 18.8$ Hz, H4′b) to $\delta_C = 205.2$ 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*.[5,8] 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 C11′ on the terminal double bond between C1 and C2 forms the C2–C11′ linkage. The new 5/6/7 trioxaspiroketal system in terreolides A–C (**1**–**3**) results from a ketal 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.

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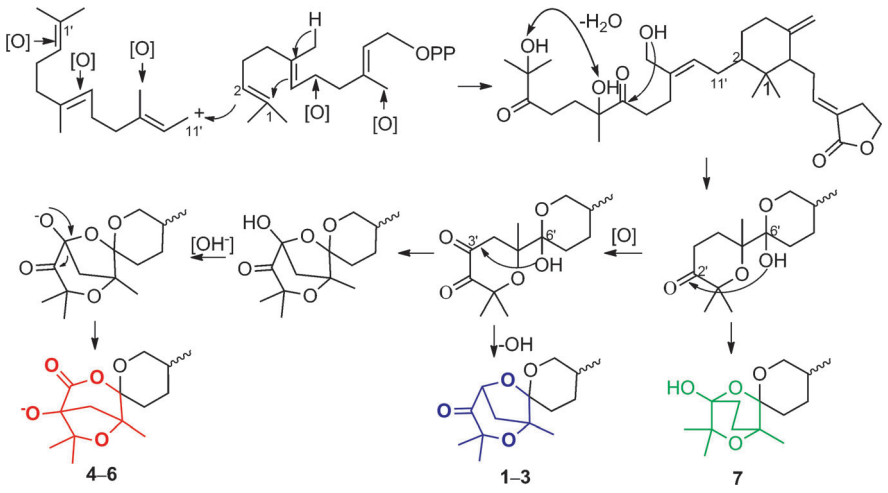

Scheme 1. A proposed biosynthetic pathway for three kinds of backbones.

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 determined by using a Roche Automated Biochemical Analyzer (Combas Integra 400 plus). Mice treated with compounds **7** and **13** displayed a 1.52- to 1.65-fold increase in serum CK levels relative to mice that received water or 1%-Tween-80 (Table 1).

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.

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

[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).

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.

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-

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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/H_2SO_4 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. KIB20111212) was deposited at the Kunming Institute of Botany, Chinese Academy of Sciences. Pileus: 5–8 cm in diameter, convex-conical to umbonate, blackish grey, fibrillose to innately streaky. Lamellae: not very crowded, pale greyish. Stipe: 6–10×0.8–1.2 cm, subcylindrical or attenuate towards the base, white, glabrous to fibrillose. Context: whitish to dirty white. Smell: indistinct. Taste: mild. Spores: 5.5–7×4–5 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 $CHCl_3$/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 $CHCl_3$/MeOH (from 1:0 to 0:1) to obtained seven fractions (A–G). A mixture was crystallized from fraction B (1.2 g) and purified using semi-preparative HPLC (MeCN/H_2O, 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/H_2O from 1:1 to 1:0) to obtain fractions C1–C14. Fraction C7 (0.8 g) was separated by repeated CC over silica gel eluting with petroleum ether/EtOAc (from 6:1 to 2:1) to yield **9** (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 ($CHCl_3$/MeOH, 1:1) followed by preparative HPLC (MeCN/H_2O, 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 **5** (16 mg). Fraction C11 (700 mg) was separated using silica gel CC eluting with $CHCl_3$/acetone (100:1) and purified using preparative HPLC (MeCN/H_2O, 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. $CHCl_3$/MeOH, 1:1). M.p. 209–210 °C; $[a]_D^{27}=+27.4°$ ($c=0.15$, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=6.86$ (m, 1H), 4.97 (br s, 1H), 4.96 (br s, 1H), 4.84 (m, 1H), 4.36 (t, $J=7.5$ Hz, 2H), 4.19 (d, $J=6.8$ Hz, 1H), 3.58 (m, 1H), 3.45 (t, $J=11.1$ Hz, 1H), 3.04 (m, 1H), 2.99 (m, 1H), 2.74 (dd, $J=13.2$, 6.8 Hz, 1H), 2.26 (dt, $J=13.1$, 4.0 Hz, 1H), 2.02 (d, $J=13.2$ Hz, 1H), 1.98 (m, 1H), 1.97 (m, 1H), 1.95 (m, 1H), 1.76 (m, 1H), 1.75 (m, 1H), 1.57 (td, $J=13.4$, 4.5 Hz, 1H), 1.55 (m, 1H), 1.49 (m, 1H), 1.41 (m, 1H), 1.39 (s, 3H), 1.38 (s, 3H), 1.30 (s, 3H), 1.24 (m, 1H), 1.21 (m, 1H), 1.09 (m, 1H), 1.08 (s, 3H), 1.00 (m, 1H), 0.87 (m, 1H), 0.87 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=207.4$, 171.7, 145.6, 142.8, 124.5, 110.5, 107.2, 82.3, 80.5, 77.1, 69.6, 66.3, 65.6, 58.8, 48.1, 39.6, 36.6, 35.4, 35.2, 32.0, 31.5, 29.2, 28.1, 27.2, 27.0, 26.7, 26.2, 25.3, 18.9, 18.0 ppm; IR (KBr): $\tilde{\nu}_{max}=3432$, 2932, 1735, 1631, 1028, 1191, 1075, 1018 cm^{-1}; UV (MeOH): λ_{max} (log ε):=224 (3.29), 202 nm (3.28 dm^3 M^{-1} cm^{-1}); HREIMS: *m/z*: calcd for $C_{30}H_{44}O_7^+$: 516.3087; found: 516.3090.

Terreolide B (**2**): Colorless crystals (aq. $CHCl_3$); M.p. 158–160 °C; $[a]_D^{24}=+21.1$ ($c=0.23$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=6.69$ (m, 1H), 4.82 (br s, 1H), 4.39 (br s, 1H), 4.36 (t, $J=7.4$ Hz, 2H), 4.19 (d, $J=6.8$ Hz, 1H), 3.58 (m, 1H), 3.45 (t, $J=11.0$ Hz, 1H), 2.86 (m, 2H), 2.74 (dd, $J=13.3$, 6.9 Hz, 1H), 2.40 (m, 1H), 2.29 (dt, $J=12.8$, 3.7 Hz), 2.27 (m, 1H), 2.01 (d, $J=13.3$, 1H), 1.98 (m, 1H), 1.93 (m, 1H), 1.90 (m, 1H), 1.78 (m, 1H), 1.76 (m, 1H), 1.59 (m, 1H), 1.55 (m, 1H), 1.50 (m, 1H), 1.40 (m, 1H), 1.39 (s, 3H), 1.38 (s, 3H), 1.31 (s, 3H), 1.24 (m, 1H), 1.10 (m, 2H), 1.01 (m, 1H), 1.00 (s, 3H), 0.81 (m, 1H), 0.56 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=207.4$, 171.3, 148.0, 142.2, 124.5, 107.5, 107.2, 82.3, 80.5, 77.1, 66.3, 65.2, 53.3, 48.0, 39.5, 37.1, 35.4, 35.2, 32.0, 31.5, 30.0, 28.1, 27.6, 26.8, 26.7, 26.4, 26.2, 25.1, 18.9, 14.7 ppm; IR (KBr): $\tilde{\nu}_{max}=3432$, 2932, 1630, 1225, 1180, 1120, 1074, 1020 cm^{-1}; UV (MeOH): λ_{max} (log ε):=218 (3.24), 202 nm (3.22 dm^3 M^{-1} cm^{-1}); HREIMS: *m/z*: calcd for $C_{30}H_{44}O_6^+$: 500.3138; found: 500.3152.

Terreolide C (**3**): Colorless crystals (aq. $CHCl_3$). M.p. 98–100 °C; $[a]_D^{24}=+54.1$ ($c=0.19$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=6.83$ (m, 1H), 5.89 (dd, $J=7.7$, 2.3 Hz, 1H), 5.03 (br s, 1H), 4.96 (br s, 1H), 4.38 (m, 2H), 4.19 (d, $J=6.8$ Hz, 1H), 3.58 (m, 1H), 3.45 (t, $J=11.0$ Hz, 1H), 3.14 (m, 1H), 2.86 (m, 1H), 2.74 (dd, $J=13.2$, 6.9 Hz, 1H), 2.27 (dt, $J=12.9$, 3.5 Hz, 2H), 2.06 (s, 3H), 2.02 (d, $J=13.2$ Hz, 1H), 1.98 (m, 1H), 1.95 (m, 1H), 1.94 (m, 1H), 1.76 (m, 1H), 1.75 (m, 1H), 1.59 (m, 1H), 1.52 (m, 1H), 1.48 (m, 1H), 1.40 (m, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.30 (s, 3H), 1.23 (m, 1H), 1.15 (m, 1H), 1.06 (m, 1H), 1.05 (s, 3H), 0.99 (m, 1H), 0.83 (m, 1H); 0.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=207.4$, 171.1, 170.2, 144.2, 137.8, 126.3, 110.6, 107.5, 82.3, 80.5, 77.1, 70.5, 66.3, 65.5, 57.1, 48.9, 39.9, 37.9, 35.4, 35.2, 32.0, 31.5, 29.8, 28.1, 27.2, 26.7, 26.3, 26.2, 24.9, 21.3, 18.9, 16.6 ppm; IR (KBr): $\tilde{\nu}_{max}=3431$, 2932, 1738, 1630, 1384, 1222, 1116, 1086 cm^{-1}; UV (MeOH): λ_{max} (log ε): 224 (3.29), 202 nm (3.20 dm^3 M^{-1} cm^{-1}); HREIMS: *m/z*: calcd for $C_{32}H_{46}O_8^+$: 558.3182; found: 558.3193).

Terreolide D (**4**): Colorless crystals (aq. acetone). M.p. 195–196 °C; $[a]_D^{27}=-14.7$ ($c=0.23$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=6.72$ (m, 1H), 4.84 (br s, 1H), 4.40 (br s, 1H), 4.39 (t, $J=7.5$ Hz, 2H), 3.72 (m, 1H), 3.63 (t, $J=11.0$ Hz, 1H), 2.88 (t, $J=7.5$ Hz, 2H), 2.56 (d, $J=12.0$ Hz, 1H), 2.43 (m, 1H), 2.31 (dt, $J=13.4$, 3.5 Hz, 1H), 2.28 (m, 1H), 2.27 (d, $J=12.0$ Hz, 1H), 2.10 (dt, $J=13.4$, 4.1 Hz, 1H), 1.94 (m, 1H), 1.93 (m, 1H), 1.78 (m, 1H), 1.76 (m, 1H), 1.65 (td, $J=13.4$, 4.1 Hz, 1H), 1.60 (m, 1H), 1.57 (m, 1H), 1.53 (m, 1H), 1.34 (s, 3H), 1.30 (m, 1H), 1.27 (s, 3H), 1.16 (s, 3H), 1.12 (m, 1H), 1.10 (m, 1H), 1.07 (m, 1H), 1.02 (s, 3H), 0.82 (m, 1H), 0.57 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): $\delta=174.5$, 171.3, 147.8, 142.2, 124.5, 109.9, 107.8, 82.2, 80.5, 80.2, 67.8, 65.3, 53.3, 48.0, 39.5, 38.1, 37.1, 34.9, 31.3, 29.9, 27.5, 27.0, 26.7, 26.4, 25.4, 25.2, 24.5, 23.1, 21.5, 14.7 ppm; IR (KBr): $\tilde{\nu}_{max}=3432$, 1631, 1178, 1085, 1032 cm^{-1}; UV (MeOH): λ_{max} (log ε): 224 (3.27), 202 nm (3.19 dm^3 M^{-1} cm^{-1}); positive ion HREIMS: *m/z*: calcd for $C_{30}H_{44}O_7^+$: 516.3087; found: 516.3097.

Terreolide E (**5**): White powder. $[a]_D^{27}=-7.4$ ($c=0.33$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=6.88$ (m, 1H), 4.97 (br s, 2H), 4.86 (br s, 1H), 4.38 (t, $J=7.3$ Hz, 2H), 3.70 (m, 1H), 3.62 (t, $J=10.6$ Hz, 1H), 3.06 (m, 1H), 3.00 (m, 1H), 2.55 (d, $J=12.2$ Hz, 1H), 2.27 (m, 1H), 2.26 (d, $J=12.2$ Hz, 1H), 2.10 (m, 1H), 2.00 (td, $J=13.2$, 3.6 Hz, 1H), 1.97 (m, 1H), 1.76 (m, 1H), 1.75 (m, 1H), 1.63 (td, $J=12.7$, 4.0 Hz, 1H), 1.61 (m, 1H), 1.57 (m, 1H), 1.53 (m, 1H), 1.34 (s, 3H), 1.29 (m, 1H), 1.28 (s, 3H), 1.24 (m, 1H), 1.15 (s, 3H), 1.09 (m, 1H), 1.09 (s, 3H), 1.04 (m, 1H), 0.89 (m, 1H), 0.88 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=174.5$, 171.6, 145.5, 142.8, 124.6, 110.5, 109.9, 82.2, 80.5, 80.2, 69.6, 67.7, 65.6, 58.8,

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48.1, 39.5, 38.1, 36.6, 34.9, 31.4, 29.2, 27.2, 27.0, 26.9, 25.3, 25.2, 24.5, 22.8, 21.5, 18.0 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3439, 2932, 1735, 1387, 1254, 1204, 1180, 1114, 1083, 962 cm^{-1}; UV (MeOH): λ_{max} (log ε): 224 (3.35), 202 nm (3.33 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{30}H_{44}O_8^+$: 532.3036; found: 532.3051.

Terreolide F (**6**): White powder. $[a]_D^{24} = -23.2$ (c = 0.32, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.84 (m, 1H), 5.90 (dd, J = 7.9, 2.7 Hz, 1H), 5.04 (brs, 1H), 4.98 (brs, 1H), 4.39 (m, 2H), 3.69 (m, 1H), 3.61 (t, J = 11.2 Hz, 1H), 3.15 (m, 1H), 2.85 (m, 1H), 2.54 (d, J = 12.4 Hz, 1H), 2.28 (dt, J = 13.2, 3.6 Hz, 1H), 2.25 (d, J = 12.4 Hz, 1H), 2.10 (m, 1H), 1.96 (m2H), 1.76 (m, 1H), 1.75 (m, 1H), 1.63 (td, J = 12.6, 3.8 Hz, 1H), 1.57 (m, 1H), 1.54 (m, 1H), 1.53 (m, 1H), 1.33 (s, 3H), 1.27 (m, 1H), 1.26 (s, 3H), 1.17 (m, 1H), 1.15 (s, 3H), 1.05 (m, 1H), 1.05 (s, 3H), 1.03 (m, 1H), 0.83 (m, 1H), 0.72 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 174.5, 171.1, 170.2, 144.1, 137.7, 126.4, 110.7, 109.8, 82.2, 80.5, 80.2, 70.4, 67.6, 65.5, 57.1, 48.9, 39.9, 38.1, 37.9, 34.9, 31.3, 29.8, 27.2, 27.0, 26.3, 25.2, 24.9, 24.5, 22.8, 21.5, 21.3, 16.6 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3448, 2939, 1753, 1453, 1374, 1236, 1202, 1079, 1032, 991, 960, 910 cm^{-1}; UV (MeOH): λ_{max} (log ε): 219 (3.37), 203 nm (3.31 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{32}H_{46}O_9^+$: 574.3142; found: 574.3151.

Saponaceolide H (**8**): White, amorphous powder. $[a]_D^{27}$ = <-M> 22.6 (c = 0.39, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.88 (m, 1H); 4.97 (s, 2H); 4.85 (brt, J = 4.1 Hz, 1H); 4.38 (t, J = 7.5 Hz, 2H); 3.68 (t, J = 11.0 Hz, 1H), 3.58 (m, 1H), 3.06 (m, 1H); 2.99 (m, 1H); 2.28 (dt, J = 13.4, 4.0 Hz, 1H); 2.15 (m, 1H), 2.00 (m, 2H); 1.98 (m, 1H); 1.97 (m, 1H), 1.86 (m, 1H), 1.78 (m, 1H); 1.67 (m, 1H), 1.66 (m, 2H), 1.56 (m, 1H), 1.50 (m, 1H), 1.49 (m, 1H), 1.29 (s, 3H), 1.25 (m, 1H), 1.24 (m, 1H); 1.21 (s, 3H), 1.11 (m, 1H), 1.09 (s, 3H), 1.08 (s, 3H), 1.02 (m, 1H), 0.91 (m, 1H), 0.88 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.8, 145.7, 142.6, 124.7, 110.6, 101.2, 96.7, 77.5, 72.7, 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, 27.0, 25.8, 25.3, 24.8, 22.4, 20.8, 18.4 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3424, 2933, 1729, 1642, 1379, 1206, 1138, 1074, 996 cm^{-1}; UV (MeOH): λ_{max} (log ε): 223 (3.33), 203 nm (3.34 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{30}H_{46}O_7^+$: 518.3244; found: 518.3243.

Saponaceolide I (**9**): White, amorphous powder. $[a]_D^{26} = -10.1$ (c = 0.18, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.83 (m, 1H); 5.89 (dd, J = 8.0, 2.6 Hz, 1H), 5.02 (brs, 1H), 4.96 (brs, 1H); 4.37 (m, 2H); 3.67 (t, J = 11.2 Hz, 1H), 3.56 (m, 1H), 3.13 (m, 1H); 2.85 (m, 1H); 2.27 (dt, J = 12.8, 3.4 Hz, 1H); 2.14 (m, 1H), 2.05 (s, 3H), 1.98 (m, 1H), 1.96 (m, 1H), 1.95 (m, 1H); 1.94 (m, 1H); 1.85 (m, 1H), 1.76 (m, 1H); 1.65 (m, 1H), 1.63 (m, 1H), 1.52 (m, 2H), 1.47 (m, 1H), 1.46 (m, 1H), 1.27 (s, 3H), 1.23 (m, 1H), 1.19 (s, 3H), 1.16 (m, 1H); 1.06 (s, 3H), 1.05 (m, 1H), 1.04 (s, 3H), 1.00 (m, 1H), 0.82 (m, 1H), 0.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.2, 170.3, 144.2, 137.8, 126.3, 110.6, 101.1, 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): $\tilde{\nu}_{max}$ = 3426, 2924, 1740, 1630, 1460, 1377, 1218, 1076, 995 cm^{-1}; UV (MeOH): λ_{max} (log ε): 256 (2.96), 203 nm (4.53 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{32}H_{48}O_8^+$: 560.3349; found: 560.3350.

Saponaceolide J (**10**): White, amorphous powder. $[a]_D^{27} = +43.6$ (c = 0.24, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.71 (m, 1H); 5.15 (t, J = 7.4 Hz, 1H), 4.84 (brs, 1H), 4.44 (d, J = 13.8 Hz, 1H), 4.40 (brs, 1H); 4.37 (t, J = 7.6 Hz, 2H); 4.26 (d, J = 13.8 Hz, 1H), 2.87 (m, 2H); 2.59 (m, 1H), 2.43 (m, 1H), 2.28 (m, 2H), 2.19 (m, 1H), 2.11 (m, 1H), 2.02 (m, 1H), 1.97 (m, 1H), 1.93 (m, 1H); 1.91 (m, 1H); 1.90 (m, 1H), 1.72 (m, 1H), 1.68 (m, 1H); 1.66 (m, 2H), 1.29 (s, 3H), 1.25 (m, 1H), 1.22 (s, 3H), 1.07 (s, 3H), 1.05 (s, 3H), 1.04 (m, 1H), 0.59 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.4, 148.0, 142.0, 134.5, 124.5, 123.0, 107.6, 101.9, 96.8, 77.6, 72.7, 65.2, 60.1, 53.4, 48.3, 39.4, 37.1, 30.9, 29.8, 28.5, 28.4, 27.9, 27.8, 26.7, 26.5, 25.8, 25.2, 22.4, 20.8, 14.8 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3440, 2976, 1740, 1640, 1377, 1248, 1086, 1019, 997 cm^{-1}; UV (MeOH): λ_{max} (log ε): 224 (3.48), 202 nm (3.57 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{30}H_{44}O_6^+$; 500.3138; found: 500.3127.

Saponaceolide K (**11**): White, amorphous powder. $[a]_D^{27} = -48.5$ (c = 0.22, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.85 (m, 1H); 5.91 (brd, J = 7.8 Hz, 1H), 5.12 (t, J = 7.7 Hz, 1H), 5.05 (brs, 1H), 4.98 (brs, 1H), 4.58 (d, J = 14.6 Hz, 1H); 4.40 (m, 2H); 4.19 (d, J = 14.6 Hz, 1H), 3.16 (m, 1H), 2.87 (m, H); 2.60 (m, 1H), 2.22 (m, 1H), 2.17 (m, 3H), 2.08 (s, 3H), 2.00 (m, 1H), 1.97 (m, 2H), 1.94 (m, 1H); 1.90 (m, 1H), 1.70 (m, 1H), 1.69 (m, 1H); 1.67 (m, 1H), 1.31 (s, 3H), 1.28 (s, 3H), 1.22 (m, 1H), 1.10 (s, 3H), 1.10 (m, 1H), 1.06 (s, 3H), 0.75 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.1, 170.2, 144.2, 137.7, 134.0, 126.3, 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, 27.9, 26.4, 25.3, 24.9, 23.0, 21.3, 18.7, 16.6 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3431, 2934, 1743, 1643, 1373, 1232, 1195, 1071, 1028, 992 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 202 nm (3.53 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{32}H_{46}O_8^+$: 558.3193; found: 558.3201.

Saponaceolide L (**12**): White, amorphous powder. $[a]_D^{27} = -20.1$ (c = 0.28, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.87 (m, 1H); 4.98 (brs, 1H), 4.97 (brs, 1H), 4.84 (brs, 1H), 4.39 (t, J = 7.6 Hz, 2H); 3.55 (m, 2H), 3.07 (d, J = 18.9 Hz, 1H), 3.06 (m, 1H), 3.00 (m, 1H), 2.44 (d, J = 18.9 Hz, 1H), 2.27 (m, 1H), 2.13 (m, 1H), 1.99 (m, 1H), 1.97 (m, 1H), 1.76 (m, 1H), 1.72 (m, 1H), 1.62 (m, 2H), 1.54 (m, 1H), 1.49 (m, 1H), 1.35 (s, 3H), 1.25 (s, 3H), 1.24 (m, 1H), 1.20 (m, 1H), 1.10 (s, 3H), 1.09 (m, 1H), 1.08 (s, 3H), 1.02 (m, 1H), 0.88 (m, 1H), 0.87 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 205.2, 171.5, 145.6, 142.6, 124.7, 110.5, 101.5, 94.9, 76.3, 75.4, 69.6, 66.3, 65.6, 58.9, 48.0, 43.6, 39.5, 36.5, 35.4, 31.4, 29.1, 28.6, 27.2, 26.9, 26.2, 25.3, 24.6, 21.5, 20.6, 18.1 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3425, 2930, 1756, 1732, 1382, 1201, 1151, 1071, 992 cm^{-1}; UV (MeOH): λ_{max} (log ε): 223 (3.30), 203 nm (3.29 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{30}H_{44}O_8^+$: 532.3036; found: 532.3033.

Saponaceolide M (**13**): White, amorphous powder. $[a]_D^{26} = -29.4$ (c = 0.15, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.85 (m, 1H); 5.90 (dd, J = 7.9, 2.8 Hz, 1H), 5.04 (brs, 1H), 4.97 (brs, 1H), 4.40 (m, 2H); 3.54 (m, 2H), 3.16 (m, 1H), 3.07 (d, J = 18.8 Hz, 1H), 2.87 (m, 1H), 2.44 (d, J = 18.8 Hz, 1H), 2.27 (m, 1H), 2.13 (m, 1H), 2.07 (s, 3H), 1.95 (m, 1H), 1.94 (m, 1H), 1.75 (m, 1H), 1.72 (m, 1H), 1.62 (m, 1H), 1.62 (m, 1H), 1.52 (m, 1H), 1.48 (m, 1H), 1.35 (s, 3H), 1.25 (s, 3H), 1.23 (m, 1H), 1.15 (m, 1H), 1.11 (s, 3H), 1.05 (m, 1H), 1.05 (s, 3H), 1.00 (m, 1H), 0.80 (m, 1H), 0.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 205.2, 171.1, 170.3, 144.2, 137.8, 126.3, 110.7, 101.4, 94.9, 76.3, 75.4, 70.4, 66.3, 65.5, 57.1, 48.9, 43.6, 39.9, 37.9, 35.5, 31.3, 29.8, 28.6, 27.2, 26.3, 26.2, 24.9, 24.6, 21.5, 21.3, 20.8, 16.6 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3441, 2936, 1754, 1377, 1236, 1151, 1073, 1032, 991 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 220 (3.38), 203 nm (3.32 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{32}H_{46}O_9^+$: 574.3142; found: 574.3142.

Saponaceolide N (**14**): White, amorphous powder; $[a]_D^{26} = -72.9$ (c = 0.28, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.80 (m, 1H); 5.91 (dd, J = 7.9, 2.7 Hz, 1H), 5.04 (brs, 1H), 4.98 (brs, 1H), 4.39 (m, 2H); 4.25 (t, J = 11.7 Hz, 1H), 3.66 (m, 1H), 3.15 (m, 1H), 2.86 (m, 1H), 2.68 (d, J = 19.3 Hz, 1H), 2.61 (d, J = 19.3 Hz, 1H), 2.29 (dt, J = 13.2, 3.3 Hz, 1H), 2.14 (m, 1H), 2.07 (s, 3H), 1.97 (m, 1H), 1.96 (m, 1H), 1.80 (m, 1H), 1.75 (m, 1H), 1.69 (m, 1H), 1.62 (m, 1H), 1.50 (m, 2H), 1.49 (m, 1H), 1.46 (s, 3H), 1.31 (s, 3H), 1.20 (m, 1H), 1.14 (m, 1H), 1.10 (s, 3H), 1.10 (m, 1H), 1.06 (s, 3H), 0.97 (m, 1H), 0.74 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 202.9, 171.3, 170.3, 144.2, 137.8, 126.3, 110.7, 101.7, 95.6, 76.4, 75.7, 70.5, 65.5, 63.2, 57.2, 48.8, 43.7, 39.9, 37.9, 32.2, 29.8, 28.4, 28.3, 26.8,

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26.7, 26.4, 24.9, 22.7, 21.3, 21.1, 20.0, 16.6 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3439, 2935, 1753, 1722, 1368, 1241, 1201, 1076, 1037, 995 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 219 (3.36), 202 nm (3.37 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{32}H_{46}O_9^+$: 574.3142; found: 574.3135.

Saponaceolide O (**15**): White, amorphous powder. $[a]_D^{27} = +16.7$ (c = 0.34, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.50 (br s, 1 H), 4.86 (br s, 3 H), 4.43 (br s, 1 H), 3.68 (t, J = 11.0 Hz, 1 H), 3.58 (m, 1 H), 3.56 (d, J = 19.0 Hz, 1 H), 3.38 (d, J = 19.0 Hz, 1 H), 3.12 (s, 1 H), 2.35 (m, 1 H), 2.16 (m, 1 H), 2.06 (m, 1 H), 1.98 (m, 1 H), 1.97 (m, 1 H), 1.86 (m, 1 H), 1.77 (m, 1 H), 1.66 (m, 1 H), 1.65 (m, 2 H), 1.52 (m, 1 H), 1.50 (m, 1 H), 1.48 (m, 1 H), 1.29 (s, 3 H), 1.25 (m, 1 H), 1.20 (s, 3 H), 1.17 (m, 1 H), 1.11 (m, 1 H), 1.08 (s, 3 H), 1.01 (m, 1 H), 0.99 (s, 3 H), 0.89 (s, 3 H), 0.82 ppm (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 205.7, 173.9, 148.2, 144.2, 126.7, 109.0, 101.2, 96.9, 77.5, 72.7, 70.6, 66.2, 65.8, 48.3, 40.9, 39.6, 36.4, 35.6, 31.2, 29.2, 28.9, 28.5, 27.8, 26.3, 26.2, 25.8, 24.8, 22.4, 20.8, 14.9 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3434, 2937, 1759, 1721, 1451, 1365, 1209, 1075, 994 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 204 nm (3.37 $dm^3 M^{-1} cm^{-1}$); positive ion HREIMS: *m/z*: calcd for $C_{30}H_{44}O_7^+$: 516.3087; found: 516.3087.

Saponaceolide P (**16**): White, amorphous powder. $[a]_D^{24} = +0.33$ (c = 0.16, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.50 (br s, 1 H), 4.86 (br s, 2 H), 4.85 (br s, 1 H), 4.43 (br s, 1 H), 3.56 (d, J = 19.0 Hz, 1 H), 3.55 (m, 2 H), 3.39 (d, J = 19.0 Hz, 1 H), 3.13 (s, 1 H), 3.07 (d, J = 18.8 Hz, 1 H), 2.44 (d, J = 18.8 Hz, 1 H), 2.36 (m, 1 H), 2.13 (m, 1 H), 2.06 (m, 1 H), 1.75 (m, 1 H), 1.73 (m, 1 H), 1.62 (m, 2 H), 1.51 (m, 1 H), 1.48 (m, 1 H), 1.36 (s, 3 H), 1.25 (m, 1 H), 1.25 (s, 3 H), 1.16 (m, 1 H), 1.11 (s, 3 H), 1.09 (m, 1 H), 1.01 (m, 1 H), 0.98 (s, 3 H), 0.89 (s, 3 H), 0.79 ppm (m, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 205.7, 205.2, 174.0, 148.2, 144.1, 126.7, 109.6, 101.4, 94.9, 76.4, 75.5, 70.7, 66.3, 66.2, 48.2, 43.6, 40.9, 39.6, 36.4, 35.4, 31.0, 28.9, 28.6, 26.3, 26.2, 26.2, 24.6, 21.5, 20.6, 14.9 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3442, 2921, 1737, 1725, 1463, 1377, 1156, 1079, 1017, 993 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 203 nm (3.26 $dm^3 M^{-1} cm^{-1}$); HREIMS: *m/z*: calcd for $C_{30}H_{42}O_8^+$: 530.2880[9]; found: 530.2891.

X-ray crystal data

Compound 1: $C_{30}H_{44}O_7$, M = 516.65, orthorhombic, a = 13.3565(4), b = 34.5376(11), c = 6.1473(2) Å, α = 90.00, β = 90.00, γ = 90.00 °, V = 2835.76(15) Å^3, T = 100(2) K, space group $P2_12_12$, Z = 4, $\mu(Cu_{K\alpha})$ = 0.685 mm^{-1}, 12 150 reflections measured, 4688 independent reflections (R_{int} = 0.0311). The final R1 values were 0.0636 ($I > 2\sigma(I)$). The final *wR*(*F*2) values were 0.2120 ($I > 2\sigma(I)$). The final *R*1 values were 0.0639 (all data). The final *wR*(*F*2) values were 0.2127 (all data). The goodness of fit on *F*2 was 1.088. Flack parameter = 0.2(3). The Hooft parameter is 0.06(6) for 1889 Bijvoet pairs. The crystal structure of compound **1** was solved by using the direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **1** have been deposited in the Cambridge Crystallographic Data Centre. CCDC-901676 (**1**) contains the supplementary crystallographic data. This, and the following ccdc data, can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Compound 4: $C_{30}H_{44}O_7$, M = 516.65, orthorhombic, a = 6.30190(10), b = 7.91690(10), c = 54.6887(10) Å, α = 90.00, β = 90.00, γ = 90.00°, V = 2728.50(7) Å^3, T = 100(2) K, space group $P2_12_12_1$, Z = 4, $\mu(Cu_{K\alpha})$ = 0.712 mm^{-1}, 13 534 reflections measured, 4748 independent reflections (R_{int} = 0.0251). The final *R*1 values were 0.0660 ($I > 2\sigma(I)$). The final *wR*(*F*2) values were 0.1551 ($I > 2\sigma(I)$). The final *R*1 values were 0.0664 (all data). The final *wR*(*F*2) values were 0.1553 (all data). The goodness of fit on *F*2 was 1.225. Flack parameter = 0.2(3). The Hooft parameter is 0.07(6) for 1914 Bijvoet pairs. The crystal structure of compound **4** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **4** have been deposited in the Cambridge Crystallographic Data Centre as CCDC-927519.

Compound 7: $C_{30}H_{46}O_6{\cdot}CH_4O$, M_w = 534.71, monoclinic, a = 7.22180(10), b = 11.7698(2), c = 17.4760(3) Å, α = 90.00, β = 101.5110(10), γ = 90.00°, V = 1455.57(4) Å^3, T = 100(2) K, space group *P*21, Z = 2, $\mu(Cu_{K\alpha})$ = 0.680 mm^{-1}, 12 299 reflections measured, 4675 independent reflections (R_{int} = 0.0260). The final R_1 values were 0.0290 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0755 ($I > 2\sigma(I)$). The final R_1 values were 0.0290 (all data). The final $wR(F^2)$ values were 0.0755 (all data). The goodness of fit on F^2 was 1.073. Flack parameter = 0.15(10). The Hooft parameter is 0.09(4) for 1918 Bijvoet pairs. The crystal structure of compound **7** was solved by using the direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **7** have been deposited in the Cambridge Crystallographic Data Centre as CCDC-927518.

Acute toxicity experiments in mice

Healthy male and female KM mice, weighting 18–22 g, were purchased from the Experimental Animal 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 $CHCl_3$/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 %-Tween-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 LD_{50} value of 1.51 g kg^{-1}. The nonpolar fraction displayed toxicity with an LD_{50} 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 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 one of the two compounds exhibited dose-dependent signs of intoxication as outlined above.

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Serum creatine kinase (CK) assay in mice

Healthy male ICR mice, weighting 18–22 g, were purchased from Chengdu Dossy Bioligical 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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Keywords: enzymes · natural products · NMR spectroscopy · structure elucidation · X-ray diffraction

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

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