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Toxic principles of a poisonous mushroom *Podostroma cornu-damae*

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Abstract—From the culture broth of a poisonous mushroom *Podostroma cornu-damae*, roridin E, verrucarin J, and satratoxin H were isolated. From the fruit bodies of the same fungus, satratoxin H, satratoxin H 12′,13′-diacetate, satratoxin H 12′-acetate, and satratoxin H 13′-acetate were isolated. All these macrocyclic trichothecenes except for verrucarin J had a lethal effect on mice by at least 0.5 mg per capita.

1. Introduction

In 1999, a lethal poisoning caused by the mushroom *Podostroma cornu-damae* happened in Niigata prefecture in Japan. Five people drank cups of sake containing some pieces of the fruit body and ate the soaked mushroom. The amount is estimated to be 1 g per capita. One of them died after two days of ingestion. In 2000, the poisoning happened again in Gunma prefecture in Japan. A couple ate the fried mushroom and one of them died the next day. Poisoning attributable to the fungus has been known to have occurred six times in Japan. This mushroom grows in Japan and Java, but it is a very rare fungus so these accidents have occurred very seldom.[1–4]

Some of the following symptoms are observed in these poisonings: gastrointestinal disorder, erroneous perception, decrease in the number of leukocytes and thrombocytes, deciduous skin of face, loss of hair, and atrophy of the cerebellum which brings about a speech impediment and voluntary movement problems.[1–4] These characteristic properties prompted us to study the toxic constituents of the mushroom.

2. Results and discussion

Due to the scarce amount of the mushroom, we initially attempted the cultivation to know whether the mycelium produces the toxic compounds or only the fruit body includes them. The fungus was isolated from the cultured tissue of *Podostroma cornu-damae*. The mycelium was grown in a stationary culture. The culture filtrate was evaporated and a portion (40 mg) of the residue was injected into the abdominal cavity of a mouse (weight: 25–30 g). The lethal effect was observed in the mouse one day after the injection. The culture filtrate was successively extracted with hexane and ether. The hexane extracts were chromatographed on silica gel with 1:1 hexane–ethyl acetate to give the major compound **1**, the minor compound **2**, and others. The ether extracts were also separated on silica-gel column chromatography with 97:3 chloroform–methanol to afford the major compound **3** and others.

The ^{1}H and ^{13}C NMR spectroscopic analyses revealed that these compounds **1**–**3** are members of the macrocyclic trichothecene group.[5,6] Comparison of the spectral data of **1**–**3** with those in the literature revealed that **1** is roridin E,[7–10] **2** is verrucarin J[7,11–14] (muconomycin B[15]), and **3** is satratoxin H.[16–18] (Fig. 1).

Using the above results as the guide, next, the fruit bodies of the mushrooms collected in Hokkaido and Niigata in Japan were used for isolation of the toxic principles. The methanol extracts of the mushroom were partitioned between ethyl acetate and water. The abdominal injection of these layers into mice (20 mg per capita) showed that both of the

Keywords: lethal toxins; mushroom; *Hypocreaceae*; *Podostroma cornu-damae*; trichothecenes; roridin E; verrucarin J; satratoxin H; satratoxin H 12′,13′-diacetate; satratoxin H 12′-acetate; satratoxin H 13′-acetate.

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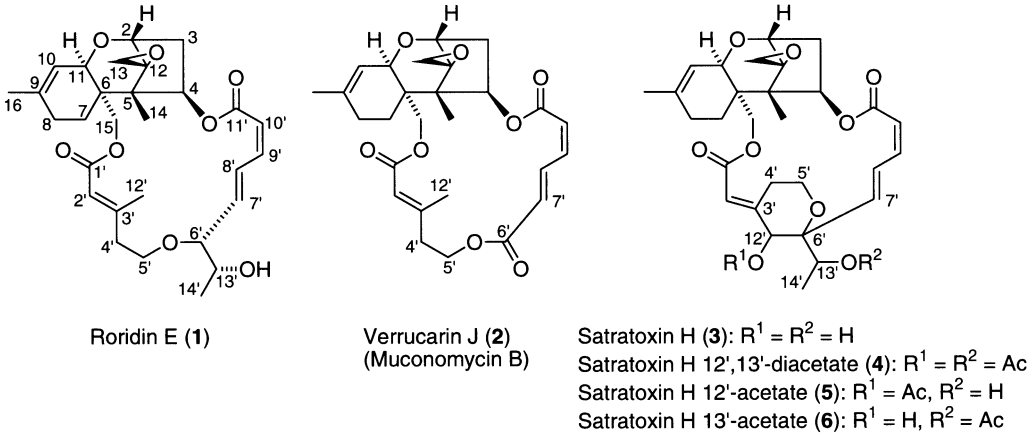

Figure 1. Toxic principles isolated from *Podostroma cornu-damae*.

fractions contained lethal toxins. The ethyl acetate layers were chromatographed on silica gel with 1:1 hexane–ethyl acetate to give the major compound **4**, the minor compounds (**3**, **5** and **6**), and others. Compounds **4**–**6** were not detected in the cultured mixture. The ^{1}H NMR spectra of **3**–**6** are similar to each other. The significant peaks in the ^{1}H NMR spectrum of **4** in comparison with that of **3** are two acetyl peaks (δ 2.09 and 2.14 ppm) and two methine proton peaks shifted downfield (δ 5.26 and 5.41 ppm). These data showed that **4** is the 12′,13′-diacetate of satratoxin H (**3**). Although satratoxin H 12′,13′-diacetate (**4**) is a new compound that occurs in nature, it has previously been synthesized from satratoxin H (**3**) for the structure determination.[16] The ^{1}H NMR spectrum of **5** differs from that of **3** due to the presence of an acetyl group (δ 2.12 ppm) and the downfield shift of the 12′-methine proton (δ 5.29 ppm). Thus, **5** is the 12′-acetate of satratoxin H (**3**). The ^{1}H NMR spectrum of **6** differs from that of **3** due to the presence of an acetyl group (δ 2.10 ppm) and the downfield shift of the 13′-methine proton (δ 5.54 ppm). Thus, **6** is the 13′-acetate of satratoxin H (**3**). Satratoxin H 12′-acetate (**5**) and satratoxin H 13′-acetate (**6**) are new compounds that occur in nature. Moreover, we extracted the fruit bodies with water and the extracts were concentrated; the residue was chromatographed on octadecyl silica gel (ODS) with 1:1 methanol–water to afford satratoxin H (**3**).

The lethal effect on mice was again tested for the six isolated compounds; among them, **1**, **3**–**6** have a lethal effect on mice by at least 0.5 mg per capita one day after the injection. Verrucarin J (**2**) has not been tested for its lethal effect because of its solubility problem.

Some of the symptoms observed in the patients who ate *Podostroma cornu-damae* are similar to those observed in humans and animals that ate foods contaminated with the macrocyclic trichothecene toxins produced by several fungi.[5] Therefore, the mixture of the isolated compounds and the other minor components, of which some of the latter seem to belong to the macrocyclic trichothecene group based on the preliminary NMR studies, might be the main lethal toxins of *Podostroma cornu-damae*.

Structural studies on the other minor components are now in progress.

3. Experimental

The melting points were determined on a micro hot stage Yanaco MP-S3 and were uncorrected. ^{1}H- and ^{13}C NMR spectra including ^{1}H–^{1}H COSY, HMQC, and HMBC were measured at 300 and 75 MHz in $CDCl_3$ at 25°C on a JEOL LAMBDA 300 spectrometer. IR and UV spectra were recorded on JASCO FT IR-200 and HITACHI U-2001 spectrometers, respectively. Optical rotations were measured on a JASCO DIP-360 polarimeter. Low and high resolution mass spectra were recorded on a JEOL GCmate (FAB) and a PE SCOEX API-300 (ESI). Silica-gel TLC and preparative TLC (PTLC) were performed on a Merck TLC 60F-254 and column chromatography was performed on a Fuji–Davison BW-820MH. Cosmosil 75C_{18}-OPN (Nacalai tesque) was used for ODS.

3.1. Mushroom material

The mushroom was collected in September, 1999, in Hokkaido and in October, 1999, in Niigata, Japan.

3.2. Fermentation

The fungus was isolated from the cultured tissue of *Podostroma cornu-damae*. The mycelium was grown in a stationary culture in a 500 ml flask containing 100 ml of a medium consisting of 10 g of malt extract and 2 g of yeast extract per 1 liter of distilled water at 27°C for three weeks.

3.3. Extraction and isolation

A culture broth (1010 ml) of *Podostroma cornu-damae* was extracted with hexane (300 ml×3), and the hexane layers were evaporated. The obtained mixture (28.0 mg) was chromatographed on silica-gel PTLC (1:1 hexane–ethyl acetate) to give roridin E (**1**) (22.1 mg) and verrucarin J (**2**) (3.2 mg). The culture broth was then extracted with ether (300 ml×3), and the ether layers were evaporated. The obtained mixture (24.5 mg) was chromatographed on ODS (20 g) with 1:1 methanol–water, and then the main component was purified by silica-gel PTLC (97:3 chloroform–methanol) to give satratoxin H (**3**) (1.5 mg). The fruit bodies (100 g, collected in Hokkaido) of *Podostroma cornu-damae* were cut and half (50 g) was extracted with

water (100 ml×2). Part (90.1 mg) of the extracts (3.27 g) was chromatographed on ODS (8.0 g) with 1:1 methanol–water to give satratoxin H (**3**) (2.5 mg). The rest of the fruit bodies (50 g) was extracted with methanol (100 ml×2). To part (700 mg) of the extracts (1.29 g) was added water (5 ml) and this was extracted with ethyl acetate (5 ml×3). The ethyl acetate layers were evaporated and the residue (48.8 mg) was chromatographed on silica gel (40 g) with 1:1 hexane–ethyl acetate to give satratoxin H (**3**) (5.5 mg) and satratoxin H 12′,13′-diacetate (**4**) (13.4 mg). The other fractions were concentrated and the residue was subjected to silica-gel PTLC with 2:1 hexane–ethyl acetate to give satratoxin H 12′-acetate (**5**) (3.0 mg) and satratoxin H 13′-acetate (**6**) (4.9 mg). Consequently, the rough amount (results are obtained from the repeated extraction experiments) of these toxins contained in the fruit bodies was the followings; **3** (0.13–0.15% of fresh weight), **4** (0.05–0.08%/f.w.), **5** (0.01–0.03%/f.w.), and **6** (0.02–0.03%/f.w.). CAUTION! These macrocyclic trichothecens are highly corrosive toxins. Therefore, avoid any contact with skin.

3.3.1. Roridin E (1). A colorless amorphous powder; R_f=0.47 (2:3 hexane–ethyl acetate); $C_{29}H_{38}O_8$; FABMS *m/z*: 515 $[M+H]^+$; $[\alpha]_D^{22.5}=-22$ (*c* 0.38, $CHCl_3$) [lit.[7] $[\alpha]_D^{25}=-24\pm2$ (*c* 0.798, $CHCl_3$). Lit.[8] $[\alpha]_D^{23}=-16\pm1$ (*c* 0.815, $CHCl_3$)]; UV (EtOH) λ_{max} nm (log ϵ): 223 (4.26), 263 (4.18) [lit.[7] 195 (4.2), 223 (4.40), 263 (4.30). Lit.[8] 198.5 (4.26), 218 (4.25), 262 (3.98)]; IR (CH_2Cl_2) ν_{max} cm^{-1}: 3578, 2976, 2930, 1712, 1650, 1600, 1438, 1420, 1398, 1362, 1221, 1180, 1150, 1121, 1082, 1038, 1010, 998, 967, 923, 865 [lit.[7] 3570, 3050, 2975, 1712, 1647, 1603, 1365, 1220, 1180, 1148, 1142, 1096, 1090, 1080, 966, 814. Lit.[8] 3500, 1705, 1640, 1595]; ^{1}H NMR ($CDCl_3$, $CHCl_3$=7.26): δ 0.78 (3H, s, H-14), 1.18 (3H, d, *J*=6.1 Hz, H-14′), ~1.66 (1H, m, H-7), 1.70 (3H, br s, H-16), ~2.00 (1H, m, H-7), ~2.02 (2H, m, H-8), 2.05 (1H, m, H-3), 2.26 (3H, d, *J*=1.4 Hz, H-12′), ~2.50 (2H, m, H-4′), 2.53 (1H, m, H-3), 2.81 (1H, d, *J*=4.1 Hz, H-13), 3.13 (1H, d, *J*=4.1 Hz, H-13), ~3.56 (2H, m, H-5′), 3.63 (1H, m, H-13′), 3.69 (1H, m, H-6′), 3.84 (1H, d, *J*=4.9 Hz, H-2), 3.89 (1H, d, *J*=4.3 Hz, H-11), 3.93 (1H, d, *J*=12.0 Hz, H-15), 4.30 (1H, d, *J*=12.0 Hz, H-15), 5.47 (1H, br d, *J*=4.3 Hz, H-10), 5.74 (1H, d, *J*=11.4 Hz, H-10′), 5.90 (1H, dd, *J*=15.4, 3.2 Hz, H-7′) 5.95 (1H, br, H-2′), 6.20 (1H, dd, *J*=8.2, 4.0 Hz, H-4), 6.56 (1H, dd, *J*=11.4, 11.4 Hz, H-9′), 7.51 (1H, dd, *J*=15.4, 11.4 Hz, H-8′); ^{13}C NMR ($CDCl_3$, $CDCl_3$=77.00): δ 6.64 (C-14), 18.23 (C-14′), 20.30 (C-12′), 21.55 (C-7), 23.23 (C-16), 27.63 (C-8), 35.78 (C-3), 41.21 (C-4′), 42.67 (C-6), 48.11 (C-13), 48.36 (C-5), 63.69 (C-15), 65.57 (C-12), 67.18 (C-11), 69.83 (C-5′), 70.63 (C-13′), 74.14 (C-4), 79.19 (C-2), 83.89 (C-6′), 117.12 (C-2′),† 117.76 (C-10′), 118.80 (C-10),† 126.50 (C-8′), 138.00 (C-7′), 140.18 (C-9), 143.62 (C-9′), 159.20 (C-3′), 165.78 (C-11′), 166.42 (C-1′).

3.3.2. Verrucarin J (2). A colorless amorphous powder; $C_{27}H_{32}O_8$; R_f=0.66 (2:3 hexane–ethyl acetate); ESIMS *m/z*: 485.5 $[M+H]^+$, 507.4 $[M+Na]^+$; $[\alpha]_D^{21}=+8.1$ (*c* 0.20, $CHCl_3$) [lit.[7] $[\alpha]_D^{22}=+20\pm2$ (*c* 1.011, $CHCl_3$)]; UV (EtOH) λ_{max} nm (log ϵ): 220 (4.00), 262 (3.90) [lit.[7] 196 (4.19), 219 (4.30), 262 (4.16). Lit.[15] 220.5 (4.33), 261 (4.34)]; IR (CH_2Cl_2) ν_{max} cm^{-1}: 3601, 2958, 2932, 2877, 1712, 1662, 1590, 1462, 1413, 1380, 1358, 1225, 1182, 1151, 1085, 1043, 1000, 968, 880, 822 [lit.[7] 2810, 1710, 1650, 1630, 1588, 1352, 1221, 1180, 1147, 1070–1088, 1042, 995, 968, 877, 820. Lit.[13] 3022, 2962, 1712, 1650, 1224, 1182. Lit.[15] (KBr) 1705, 1650, 1595]; ^{1}H NMR ($CDCl_3$, $CHCl_3$=7.26): δ 0.83 (3H, s, H-14), 1.7–2.1 (4H, m, H-7, 8), 1.72 (3H, br s, H-16), 2.16 (1H, ddd, *J*=15.2, 5.2, 5.2 Hz, H-3), 2.28 (1H, d, *J*=1.5 Hz, H-12′), 2.45–2.60 (3H, m, H-3, 4′), 2.83 (1H, d, *J*=4.1 Hz, H-13), 3.14 (1H, d, *J*=4.1 Hz, H-13), 3.75 (1H, d, *J*=5.1 Hz, H-11), 3.85 (1H, d, *J*=5.2 Hz, H-2), 3.97 (1H, d, *J*=12.2 Hz, H-15), 4.15 (1H, ddd, *J*=11.7, 8.0, 5.3 Hz, H-5′), 4.42 (1H, d, *J*=12.2 Hz, H-15), ~4.45 (1H, m, H-5′), 5.46 (1H, br d, *J*=5.1 Hz, H-10), 5.83 (1H, br s, H-2′), 5.99 (1H, dd, *J*=8.2, 4.3 Hz, H-4) 6.00 (1H, d, *J*=15.2 Hz, H-7′), 6.10 (1H, d, *J*=11.5 Hz, H-10′), 6.62 (1H, dd, *J*=11.5, 11.5 Hz, H-9′), 8.06 (1H, dd, *J*=15.2, 11.5 Hz, H-8′); ^{13}C NMR ($CDCl_3$, $CDCl_3$=77.00): δ 6.95 (C-14), 17.19 (C-12′), 20.74 (C-7), 23.26 (C-16), 27.65 (C-8), 35.10 (C-3), 40.20 (C-4′), 43.03 (C-6), 48.05 (C-13), 48.84 (C-5), 60.42 (C-5′), 63.31 (C-15), 65.45 (C-12), 67.28 (C-11), 75.32 (C-4), 79.04 (C-2), 118.12 (C-10), 118.62 (C-2′), 125.51 (C-10′), 127.37 (C-7′), 139.11 (C-8′), 139.46 (C-9′), 140.44 (C-9), 156.58 (C-3′), 165.53 (C-11′), 165.79 (C-1′), 166.09 (C-6′).

3.3.3. Satratoxin H (3). A colorless amorphous powder; R_f=0.23 (2:3 hexane–ethyl acetate); $C_{29}H_{36}O_9$; ESIMS ($CH_3CO_2NH_4$ was added) *m/z*: positive: 529.5 $[M+H]^+$, 551.3 $[M+Na]^+$; negative: 483.2 $[M-H-CO_2]^-$, 527.2 $[M-H]^-$, 587.2 $[M+CH_3CO_2]^-$; $[\alpha]_D^{22.5}=+73$ (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ): 205 (4.21), 229 (4.29), 255 (4.04) [lit.[18] 225 (4.17), 255 (4.02)]; IR (CH_2Cl_2) ν_{max} cm^{-1}: 3580, 2979, 1718, 1652, 1595, 1437, 1410, 1361, 1306, 1259, 1239, 1218, 1192, 1153, 1081, 1043, 1002, 964, 882 [lit.[17] 1720, 1650, 1595]; ^{1}H NMR ($CDCl_3$, TMS=0.00): δ 0.82 (3H, s, H-14), 1.16 (3H, d, *J*=6.6 Hz, H-14′), 1.73 (3H, br s, H-16), 1.88–1.96 and 2.00–2.09 (each 2H, each m, H-7 and 8), 2.20 (1H, ddd, *J*=15.2, 4.9, 4.9 Hz, H-3), 2.36 (1H, d, *J*=6.5 Hz, 12′-OH), 2.41 (1H, s, 13′-OH), 2.45 (1H, dd, *J*=15.2, 8.2 Hz, H-3), 2.66 (1H, m, H-4′), 2.83 (1H, d, *J*=4.1 Hz, H-13), 3.14 (1H, d, *J*=4.1 Hz, H-13), 3.60 (1H, d, *J*=5.1 Hz, H-11), 3.74 (1H, ddd, *J*=11.7, 11.7, 2.9 Hz, H-5′), ~3.85 (2H, m, H-4′, 5′), 3.85 (1H, d, *J*=4.9 Hz, H-2), 3.86 (1H, d, *J*=12.5 Hz, H-15), 3.99 (1H, d, *J*=6.5 Hz, H-12′), 4.36 (1H, q, *J*=6.6 Hz, H-13′), 4.56 (1H, d, *J*=12.5 Hz, H-15), 5.45 (1H, br d, *J*=5.1 Hz, H-10), 5.84 (1H, d, *J*=2.2 Hz, H-2′), 5.90 (1H, d, *J*=10.0 Hz, H-10′), 5.90 (1H, m, H-4), 6.08 (1H, d, *J*=16.7 Hz, H-7′), 6.61 (1H, dd, *J*=10.0, 10.0 Hz, H-9′), 7.35 (1H, dd, *J*=16.7, 10.0 Hz, H-8′); ^{13}C NMR ($CDCl_3$, TMS=0.00): δ 7.58 (C-14), 15.73 (C-14′), 20.30 (C-7), 23.38 (C-16), 25.25 (C-4′), 27.59 (C-8), 34.35 (C-3), 43.33 (C-6), 48.10 (C-13), 48.89 (C-5), 60.42 (C-5′), 64.17 (C-15), 65.47 (C-12), 68.12 (C-11), 69.65 (C-13′), 73.55 (C-12′), 74.09 (C-4), 79.11 (C-2), 81.26 (C-6′), 118.83 (C-10),† 119.03 (C-2′),† 120.36 (C-10′), 132.01 (C-7′), 134.23 (C-8′), 140.38 (C-9), 142.93 (C-9′), 154.96 (C-3′), 166.26 (C-1′), 167.11 (C-11′).

3.3.4. Satratoxin H 12′,13′-diacetate (4). A colorless amorphous powder; R_f=0.63 (2:3 hexane–ethyl acetate);

† Interchangeable.

mp 167–172°C (not recrystallized); HRFABMS *m/z*: positive: 635.2448 $[M+Na]^+$, calcd for $C_{33}H_{40}O_{11}Na$, 635.2469; $[\alpha]_D^{25.0}$=+28 (*c* 0.87, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ): 230 (3.98); IR (CH_2Cl_2) ν_{max} cm^{-1}: 2976, 1738, 1655, 1595, 1376, 1242, 1226, 1190, 1157, 1094, 1043, 1020, 963; ^1H NMR ($CDCl_3$, $CHCl_3$=7.26): δ 0.83 (3H, s, H-14), 1.08 (3H, d, *J*=6.6 Hz, H-14′), 1.72 (3H, br s, H-16), 1.8–2.1 (4H, m, H-7, 8), 2.09 (3H, s, OAc), 2.14 (3H, s, OAc), 2.19 (1H, ddd, *J*=15.3, 5.1, 5.1 Hz, H-3), 2.44 (1H, dd, *J*=15.3, 8.4 Hz, H-3), 2.56 (1H, m, H-4′), 2.84 (1H, d, *J*=4.1 Hz, H-13), 3.14 (1H, d, *J*=4.1 Hz, H-13), 3.58 (1H, d, *J*=5.1 Hz, H-11), 3.70 (1H, ddd, *J*=12.0, 12.0, 3.1 Hz, H-5′), 3.84 (1H, d, *J*=5.1 Hz, H-2), ~3.85 (2H, m, H-4′, H-5′), 3.85 (1H, d, *J*=12.2 Hz, H-15), 4.52 (1H, d, *J*=12.2 Hz, H-15), 5.26 (1H, s, H-12′), 5.41 (1H, q, *J*=6.6 Hz, H-13′), 5.43 (1H, br d, *J*=5.1 Hz, H-10), 5.89 (1H, dd, *J*=8.4, 4.9 Hz, H-4), 5.93 (1H, d, *J*=10.0 Hz, H-10′), 6.02 (1H, d, *J*=2.2 Hz, H-2′), 6.03 (1H, d, *J*= 16.8 Hz, H-7′), 6.63 (1H, dd, *J*=10.0, 10.0 Hz, H-9′), 7.38 (1H, dd, *J*=16.8, 10.0 Hz, H-8′); ^{13}C NMR ($CDCl_3$, $CDCl_3$=77.00): δ 7.65 (C-14), 14.89 (C-14′), 20.25 (C-7), 21.20 (OCO*Me*), 21.33 (OCO*Me*), 23.36 (C-16), 25.84 (C-4′), 27.45 (C-8), 34.30 (C-3), 43.26 (C-6), 48.08 (C-13), 48.89 (C-5), 60.30 (C-5′), 64.15 (C-15), 65.45 (C-12), 68.10 (C-11), 71.32 (C-13′), 73.23 (C-12′), 74.17 (C-4), 79.06 (C-2), 79.39 (C-6′), 118.77 (C-10), 120.90 (C-10′), 122.34 (C-2′), 131.42 (C-7′), 134.33 (C-8′), 140.40 (C-9), 142.40 (C-9′), 149.84 (C-3′), 166.04 (C-1′), 166.98 (C-11′), 169.88 (O*C*OMe), 170.20 (O*C*OMe).

3.3.5. Satratoxin H 12′-acetate (5). A colorless amorphous powder; R_f=0.46 (2:3 hexane–ethyl acetate); mp 116–120°C (not recrystallized); HREIMS *m/z*: 570.2454 $[M]^+$, calcd for $C_{31}H_{38}O_{10}$, 570.2465; $[\alpha]_D^{31}$=+25 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ): 208 (4.08), 230 (4.19), 255 (4.03); IR (CH_2Cl_2) ν_{max} cm^{-1}: 3581, 2978, 2937, 1721, 1657, 1595, 1413, 1372, 1230, 1193, 1157, 1082, 1043, 1018, 964; ^1H NMR ($CDCl_3$, $CHCl_3$=7.26): δ 0.82 (3H, s, H-14), 1.02 (3H, d, *J*=6.7 Hz, H-14′), 1.72 (3H, br s, H-16), 1.86–2.14 (4H, m, H-7, 8), 2.12 (3H, s, 12′-OAc), 2.20 (1H, ddd, *J*=15.6, 4.8, 4.8 Hz, H-3), 2.44 (1H, dd, *J*=15.6, 8.1 Hz, H-3), 2.55 (1H, m, H-4′), 2.84 (1H, d, *J*=3.9 Hz, H-13), 3.14 (1H, d, *J*=3.9 Hz, H-13), 3.58 (1H, d, *J*=4.5 Hz, H-11), 3.73 (1H, ddd, *J*=11.8, 11.8, 3.0 Hz, H-5′), 3.80–4.00 (3H, m, H-2, 4′, 5′), 3.85 (1H, d, *J*=12.8 Hz, H-15), 4.22 (1H, q, *J*=6.7 Hz, H-13′), 4.54 (1H, d, *J*=12.8 Hz, H-15), 5.29 (1H, s, H-12′), 5.44 (1H, br d, *J*=4.5 Hz, H-10), 5.90 (1H, dd, *J*=8.1, 4.8 Hz, H-4), 5.92 (1H, d, *J*=10.1 Hz, H-10′), 6.02 (1H, d, *J*=2.0 Hz, H-2′), 6.08 (1H, d, *J*=17.3 Hz, H-7′), 6.61 (1H, dd, *J*=10.1, 10.1 Hz, H-9′), 7.41 (1H, dd, *J*=17.3, 10.1 Hz, H-8′); ^{13}C NMR ($CDCl_3$, $CDCl_3$=77.00): δ 7.63 (C-14), 15.30 (C-14′), 20.25 (C-7), 21.04 (OCO*Me*), 23.34 (C-16), 25.82 (C-4′), 27.45 (C-8), 34.28 (C-3), 43.28 (C-6), 48.08 (C-13), 48.89 (C-5), 60.40 (C-5′), 64.15 (C-15), 65.44 (C-12), 68.05 (C-11), 69.24 (C-13′), 73.25 (C-12′), 74.10 (C-4), 79.06 (C-2), 80.22 (C-6′), 118.78 (C-10), 120.64 (C-10′), 122.14 (C-2′), 131.04 (C-7′), 134.25 (C-8′), 140.38 (C-9), 142.65 (C-9′), 149.97 (C-3′), 166.04 (C-1′), 167.06 (C-11′), 169.61 (O*C*OMe)

3.3.6. Satratoxin H 13′-acetate (6). A colorless amorphous powder; R_f=0.49 (2:3 hexane–ethyl acetate); mp 152–155°C (not recrystallized); HRFABMS *m/z*: positive: 571.2577 $[M+H]^+$, calcd for $C_{31}H_{39}O_{10}$, 571.2544; $[\alpha]_D^{31}$=+71 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ): 228 (4.29), 257 (4.10); IR (CH_2Cl_2) ν_{max} cm^{-1}: 3695, 3602, 3063, 2962, 1720, 1605, 1376, 1242, 1191, 1079, 964; ^1H NMR ($CDCl_3$, $CHCl_3$=7.26): δ 0.83 (3H, s, H-14), 1.22 (3H, d, *J*=6.2 Hz, H-14′), 1.73 (3H, br s, H-16), 1.86–2.12 (4H, m, H-7, 8), 2.10 (3H, s, 13′-OAc), 2.20 (1H, ddd, *J*=15.3, 5.4, 5.4 Hz, H-3), 2.38 (1H, d, *J*=8.6 Hz, 12′-OH), 2.45 (1H, dd, *J*=15.3, 8.2 Hz, H-3), 2.62 (1H, m, H-4′), 2.83 (1H, d, *J*=4.1 Hz, H-13), 3.14 (1H, d, *J*=4.1 Hz, H-13), 3.60 (1H, d, *J*=4.0 Hz, H-11), 3.66 (1H, ddd, *J*=11.8, 11.8, 3.1 Hz, H-5′), ~3.85 (2H, m, H-4′, 5′), 3.85 (1H, d, *J*=5.1 Hz, H-2), 3.86 (1H, d, *J*=12.3 Hz, H-15), 3.95 (1H, d, *J*=8.6 Hz, H-12′), 4.55 (1H, d, *J*=12.3 Hz, H-15), 5.45 (1H, br d, *J*=4.0 Hz, H-10), 5.54 (1H, q, *J*=6.2 Hz, H-13′), 5.85 (1H, d, *J*=2.2 Hz, H-2′), 5.90 (1H, dd, *J*=8.2, 5.4 Hz, H-4), 5.93 (1H, d, *J*=10.1 Hz, H-10′), 6.04 (1H, d, *J*=16.7 Hz, H-7′), 6.64 (1H, dd, *J*=10.1, 10.1 Hz, H-9′), 7.33 (1H, ddd, *J*=16.7, 10.1, 1.0 Hz, H-8′); ^{13}C NMR ($CDCl_3$, $CDCl_3$=77.00): δ 7.58 (C-14), 15.13 (C-14′), 20.28 (C-7), 21.42 (OCO*Me*), 23.36 (C-16), 25.20 (C-4′), 27.57 (C-8), 34.33 (C-3), 43.33 (C-6), 48.10 (C-13), 48.87 (C-5), 60.24 (C-5′), 64.14 (C-15), 65.47 (C-12), 68.13 (C-11), 71.83 (C-13′), 73.33 (C-12′), 74.17 (C-4), 79.07 (C-2), 80.72 (C-6′), 118.78 (C-10),† 119.21 (C-2′),† 120.71 (C-10′), 131.98 (C-7′), 134.59 (C-8′), 140.40 (C-9), 142.57 (C-9′), 154.68 (C-3′), 166.19 (C-1′), 167.01 (C-11′), 170.35 (O*C*OMe).

3.4. Lethal toxicity

An ethanol (0.05 ml) solution of each compound (**1**, **3**–**6**: each 0.5 mg) was diluted with 0.2 ml of physiological saline (0.9 wt% of aqueous NaCl solution). This was immediately injected into the abdominal cavity of female ddY strain mice, weighing 25–30 g. Lethal activity was observed one day after these injections.

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