Supplementary Methods

Authentic standard compounds

The authentic standard compounds used in this study were obtained from the following: rutin (Nacalai Tesque), protodioscin (Toronto Research Chemicals, Canada), and asparaptine (isolated form asparagus spears, Nakabayashi et al., 2015).

Asparagus spears

Spears of asparagus were harvested in the Medicinal Plant Garden at Hoshi University on 27th March, 2015. The harvested spears for deriving the callus and suspension cell line were stored in a refrigerator until use. The spears for the untargeted metabolome analysis were frozen with liquid nitrogen and lyophilized at −55°C.

Deriving the callus and suspension cell line from asparagus

The callus and suspension cell line were derived from the green asparagus spears. The green asparagus spears were sterilized using 80% ethanol for 2 min and 2% sodium hypochlorite solution for 30 min. The sterilized spears were cut to obtain explants sized 0.5–1 cm. For callus induction, the explants were cultured in an MS medium (Murashige and Skoog 1962) containing 3% sucrose, 0.2% gelrite, 5 μM 1-naphthaleneacetic acid (NAA), and 5 μM kinetin at 25°C under dark condition. After four weeks, yellow callus was induced from the explants. The induced callus was maintained by subculturing for a month under the identical condition, with the incubation temperature changed to 22°C.

Suspension cells were obtained by subculturing the callus in the MS liquid medium containing 3% sucrose, 5 μM NAA, and 5 μM kinetin at 22°C on a rotary shaker (125 rpm) under dark condition. The callus and suspension cells were maintained by culturing every month and 10 days under the same conditions, respectively.

Preparing the calluses and suspension cells for untargeted metabolome analysis

All calluses were subcultured thrice every month in a MS medium containing 3% sucrose, 0.2% gelrite, 1 μM NAA, and 1 μM kinetin at 25°C under dark condition. And then, the calluses were harvested and frozen with liquid nitrogen.

The subcultured suspension cells were harvested using a 50 ml tube after cultured for 10 days under the dark condition. After centrifugation at 10,000 × *g* for 10 min, the liquid medium was removed and then water (40 ml) was added to the tube. The centrifugation was performed again to harvest the suspension cells. The harvested cells were frozen with liquid

nitrogen.

Preparing the suspension cells and its liquid medium treated with phytohormones for untargeted metabolome analysis

The subcultured suspension cells were cultured in a new liquid MS medium. Five days later, the MS medium was refreshed and each phytohormone dissolved in dimethylsulfoxide (DMSO) was added for final concentration, 100 μM. After two days, the suspension cells were harvested and lyophilized at −55°C. The suspension cells and liquid medium were harvested using the method above.

Extraction of metabolites

The freeze-dried samples were completely powdered using a mixer mill with stainless beads for 7 min at 18 Hz and 4°C (MM300, Retsch) for the untargeted metabolome analysis. The powdered samples (3 mg dry weight) were extracted with 50 μl of 80% MeOH containing 2.5 μM lidocaine per mg dry weight using a mixer mill with zirconia beads for 7 min at 18 Hz and 4°C (MM300, Retsch). Following centrifugation at 17,800 × *g* for 10 min, the supernatant was obtained using an HLB μElution plate (Waters).

The medium (3 ml) was loaded on the HLB 3 cc cartridge (Waters) that have been conditioned with 3 ml of MeOH and been equilibrated with 3 ml of 0.1% acetic acid. The cartridge was washed with 3 ml of 0.1% acetic acid and was eluted with 3 ml of 90% MeOH containing 0.1 % acetic acid. The elution was then evaporated to dryness. The residues were dissolved in 100 μl of 80%MeOH containing 2.5 μM lidocaine, and then filtered using a 0.20 μm Ultrafree MC centrifugal filter (Millipore).

Untargeted metabolome analysis by LC–MS/MS

Extracts (1 μl) were analyzed using LC–QTOF–MS instrument (LC, Waters Acquity UPLC system; MS, Waters Xevo G2 Q-Tof). Analytical conditions were as follows LC: column, Acquity bridged ethyl hybrid (BEH) C18 (1.7 μm, 2.1 mm × 100 mm, Waters); solvent system, solvent A (water including 0.1% formic acid) and solvent B (acetonitrile including 0.1% formic acid); gradient program, 99.5%A/0.5%B at 0 min, 99.5%A/0.5%B at 0.1 min, 20%A/80%B at 10 min, 0.5%A/99.5%B at 10.1 min, 0.5%A/99.5%B at 12.0 min, 99.5%A/0.5%B at 12.1 min and 99.5%A/0.5%B at 15.0 min; flow rate, 0.3 ml/min at 0 min, 0.3 ml/min at 10 min, 0.4 ml/min at 10.1 min, 0.4 ml/min at 14.4 min and 0.3 ml/min at 14.5 min; column temperature, 40 °C; MS detection: polarity, positive; capillary voltage, +3.00 kV (positive); cone voltage, 25.0 V; source temperature, 120 °C; desolvation temperature, 450 °C; cone gas flow, 50 l/h;

desolvation gas flow, 800 l/h; collision energy, 6 V; mass range, *m/z* 50–1500; scan duration, 0.1 sec; inter-scan delay, 0.014 sec; data acquisition, centroid mode; lockspray, leucine enkephalin; scan duration, 1.0 sec; inter-scan delay, 0.1 sec. MS/MS data was acquired as the following analytical conditions: (1) MS: polarity, positive; mass range, *m/z* 50–1500; scan duration, 0.1 sec; inter-scan delay, 0.014 sec; data acquisition, centroid mode and (2) MS/MS: polarity, positive; mass range, *m/z* 50–1500; scan duration, 0.02 sec; inter-scan delay, 0.014 sec; data acquisition, centroid mode. In this mode, MS/MS spectra of the top 10 ions (> 1000 counts) in an MS scan were automatically obtained. If the ion intensity was less than 1000, MS/MS data acquisition was not performed and moved to of next top 10 ions. The LC−MS/MS analysis was performed in three biological replicates.