Supporting Information

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SI Methods

Chlorophyll Determination. For chlorophyll determination, 200 μL of each extract was mixed with 1.8 mL acetone, incubated for 30 min at −20 °C, and centrifuged for 20 min at 21,000 × *g* (1). The absorbance A645/A663 of the supernatant was determined by UV/VIS spectrophotometry (Ultrospec 3100 pro; Fisher Scientific).

Extraction of Whole Leaves for Enzyme Assays. Fresh leaves (leaves 1–3 in Fig. 2) from greenhouse-cultivated *C. roseus* were harvested (6.0 g) and frozen in liquid nitrogen. The leaf material was pulverized and homogenized in 12 mL of extraction buffer [100 mM Tris-HCl (Sigma), pH 7.5, 14 mM β-mercaptoethanol (EMD), and 25 mM KCl (Caledon)]. The homogenate was filtered through two layers of nylon and centrifuged at 500 × *g* (21000R; Fisher Scientific) for 10 min; the supernatant was added directly to a PD-10 column (GE Healthcare) for desalting. The desalted protein was used for enzyme assays and the protein concentration was determined with Bradford assays.

Extraction of Epidermis Enriched Proteins for Enzyme Assay. Fresh leaves (leaves 1–3 in Fig. 2) from greenhouse-cultivated *C. roseus* were harvested (6.0 g). The leaf material was added batch-wise to 60 mL of extraction buffer [100 mM Tris-HCl (Sigma), pH 7.5, 14 mM β-mercaptoethanol (EMD), and 25 mM KCl (Caledon)] containing 6 g of carborundum. The leaves, carborundum, and buffer were divided equally into two 50-mL tubes (Sarstedt) and vortexed (Genie 2; Fisher Scientific) at speed level 4 for 3 min. The leaf material was removed from the buffer and the samples were then centrifuged at 4,500 × *g* (21000R; Fisher Scientific) to separate the carborundum. The buffer (25 mL each) was then filtered via vacuum filtration through a 0.45-μm PALL membrane. The filtered material was then concentrated (Millipore) to a final volume of 5 mL. The concentrate was then desalted on a PD-10 column (GE Healthcare) and was used directly for enzyme assays and for protein determination with Bradford assays.

G10H Assay. Enzyme assays contained 1 mL of epidermis-enriched or whole leaf extracts, geraniol (1 mM; Sigma), and NADPH (1 mM; Sigma), and further aliquots of NADPH (1 mM) were added at 10-min intervals. Assays were incubated at 37 °C (water bath, IsoTemp; Fisher Scientific) for 60 min. Additional control assays were performed without geraniol, without NADPH, or with denatured boiled extracts.

After terminating enzyme assays by addition of 500 μL ethyl acetate (Caledon), they were vortexed (Genie 2; Fisher Scientific) at speed leve 10 for 1 min and the organic/aqueous phases were separated by centrifuged at 10,000 × *g* (Micromax RF; Fisher Scientific). The top organic layer was harvested, and after repeating the extraction with another 500 μL ethyl acetate, the organic layers were pooled, evaporated to dryness (Speed Vacuum, Thermo Savant; Fisher Scientific) and resuspended in 200 μL methanol and analyzed by reverse-phase HPLC (2,996-photodiode array detector; Waters) using an Inertsil ODS-3 C18 column (4 × 250 mm; GL Sciences) equipped with a 3 × 4-mm guard column (Phenomenex). Reaction products were injected onto the column and were eluted (1 mL min^{-1}) with solvent A (MeOH:MeCN:5 mM ammonium acetate at 6:14:80) and solvent B (MeCN; 100) using the following linear gradient: 0–20 min 80–20% A/20–80% B; 20–25 min 20–80% A/80–20% B; 25–35min 80–80% A/ 20–20% B. Geraniol and 10-hydroxygeraniol were monitored by photodiode array detection at 210 nm.

LAMT, 16-OMT, NMT, and DAT Enzyme Assays. LAMT, 16-OMT, and NMT radioactive assay measurements were performed as described previously (1). Enzyme assays (120 μL) contained LAMT (1.3 mM; Chromadex), 16-hydroxytabersonine (60 μM) or 2,3-dihydro-3-hydroxy-*N*-methyltabersonine (60 μM), 3.7×10^{-3} kBq [$^{14}CH_3$]-S-adenosyl-L-methionine (2.00 GBq/mmol; GE Healthcare), 100 μL of epidermis-enriched or whole leaf extracts, and were incubated at 37 °C for 60 min. DAT assays contained (120 μL) deacetylvindoline (60 μM), 3.7×10^{-3} kBq [14-C]-acetyl-CoA (2.18 GBq/mmol; GE Healthcare), 100 μL of epidermis-enriched or whole leaf extracts and were incubated at 37 °C for 60 min. After LAMT assays were stopped by quick-freezing the assays in liquid nitrogen, they were lyophilized and dissolved in methanol (15 μL). Samples were submitted to silica gel TLC (Polygram Sil G/UV_{254}; Macherey-Nagel) and radiolabeled loganin was resolved in (7:3 chloroform:methanol). Radioactive products were stored on a phosphor screen (GE Healthcare) for 16 h and emissions were detected using a phosphorimager (FLA-3000 equipped with multigauge version 3.0 software; Fujifilm). After harvesting the radioactive loganin spot, its concentration was measured by liquid scintillation counting (Beckman Coulter). Enzyme assays for 16-OMT, NMT, and DAT were stopped with 20 μL of 10 M NaOH and alkaloids were extracted and processed as described for the G10H assay to produce evaporated residues. Reaction products from each assay were dissolved in methanol (10 μL). Samples were submitted to silica gel TLC (Polygram Sil G/UV_{254}; Macherey-Nagel) and reaction products were resolved in ethyl acetate: methanol at a 9:1 ratio. The radioactive products were detected and their concentrations were measured as described for LAMT.

Effect of Catharanthine on Growth on *P. nicotianeae* Zoospores. *P. nicotianeae* zoospores were prepared by aseptically removing a mycelia plug from a stock plate to fresh V8 agar medium. This medium was prepared by mixing well 10% vol/vol V8 juice, 1 g/L calcium carbonate, and 1.45% wt/vol agar, followed by autoclaving at 121 °C for 20 min, cooling to 40 °C, and pouring into sterile Petri plates that were stored at 4 °C until required. Mycelia were cultivated under continuous light for 7 d at 26 °C (TC19; Enconair Ecological Chamber). The culture was then treated with 0.5 to 1.0 mL of 0.01 M KNO_3 and incubated for a further 8 d under the same conditions. The plate containing mycelia was rinsed with sterile distilled water (10 mL) and air bubbles on the agar surface were dislodged by gently scraping the surface with a sterile plastic rod. After discarding the water, the washing procedure was repeated a second time. Fresh sterile distilled water was added and the plate was transferred to continuous light for at least 2 h at room temperature. The plate was chilled on ice for 30 min and then placed at room temperature for a further 30 min to release zoospores. The liquid containing zoospores was transferred to a sterile test tube to be shaken vigorously on a vortex (Genie 2; Fischer Scientific) at speed level 5 for 2 min and then allowed to settle by gravity for 3 to 5 min. The concentration of zoospores in the supernatant was determined by counting them under a light microscope using a hemacytometer. Zoospores of a given concentration were transferred to 1.5 mL Eppendorf tubes containing 1 mL of half strength Gamborg B5 media (2), sucrose (20 g/L), myoinositol (50 mg/L), nicotinic acid (0.5 mg/L), pyridoxine (0.5 mg/L), thiamine (5 mg/L; Sigma), and various concentrations of catharanthine to be cultivated under continuous light at 26 °C. The growth and multiplication of zoospore was determined in triplicate on days 0, 2, and 4 after the beginning of the experiment.

Effect of Catharanthine on Growth of Insect larvae. ***Cultivation of insect larvae.*** The larvae of *S. littoralis* Boisd (Lepidoptera, Noctuidae) and *S. eridania* were reared with a 14 to 16 h photoperiod at 22 °C to 24 °C in plastic boxes using an artificial diet [300 g/L agar, 400 g/L bean flour, 3 g sodium ascorbate, 3 g ethyl p-hydroxybenzoate, 1 g formaldehyde (3), and added with 0.3 g β-sitosterol, 0.3 g leucine, 3.3 g vitamin mixture]. The larvae of *H. armigera* were reared on artificial diet containing wheat germ and soybean powder between 24 °C and 27 °C with a 16:8 (L:D) photoperiod. The leaf bettles, *P. cochleariae*, were reared in the laboratory at 18 °C to 20 °C and 16:8 L:D period, fed on *Brassica rapa* spp. Chinensis (4). The fifth-instar larvae of *B. mori* (Lepidoptera) were reared at 14 to 16 h photophase between 22 °C and 24 °C, kept in plastic boxes, grown on artificial diet (mulberry leaf powder, potato starch, soybean powder, sucrose, agar, mineral salts, and vitamin B complex).

Feeding of Catharanthus leaves to insects. Four larvae of each species were placed in a closed Petri dish containing one *C. roseus* leaf pair in third developmental stage attached to its stem that was placed and sealed in an Eppendorf tube containing 1 mL of water.

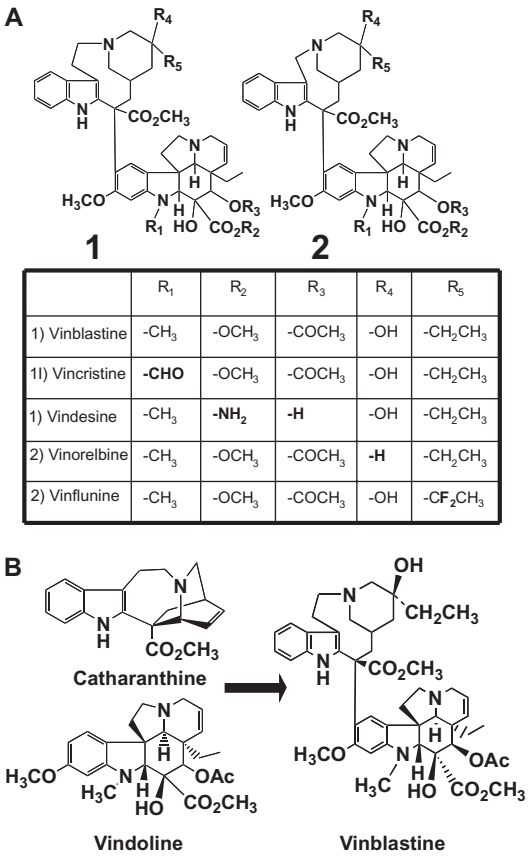
***Feeding of mulberry artificial diets to* Bombyx mori.** *C. roseus* cv. Little Delicata leaves (2/3, 1, or 2 leaves) from the third developmental stage were frozen in liquid nitrogen and ground into a fine powder, then mixed with 2.8 g of Mulberry artificial diet that was used in feeding studies. Leaves from the same developmental stage were dipped in chloroform to obtain catharanthine-enriched extracts from the surface and the remaining leaf was extracted in methanol to obtain MIAs and other metabolites occurring inside the leaf. After evaporating both extracts to dryness, the MIAs were dissolved in 2.8 g of Mulberry artificial diet. In additional experiments, mulberry artificial diet was supplemented in a similar manner with various concentrations of pure catharanthine for performing the feeding studies. Each of these diets was then analyzed for alkaloid content by analytical UPLC as described in *Methods* in the main text. Individual larvae were placed in a closed Petri dish and fed with a fresh 0.2 g of refrigerated prepared diet containing different test components at 24-h intervals. The feeding study was continued for 7 d unless the larvae died before the end of the experiment. Each day, dead larvae were removed and stored in a 20 °C freezer for further dissection at the end of the experiment.

Collection of regurgitates. The regurgitates were collected and mixed with methanol at a 1:1 ratio after 2 and 16 h, centrifuged at 13,000 rpm for 15 min at 4 °C (Micromax RF; Thermo-IEC).

Collection of feces. The feces were collected after 30 h and pulverized with mortar and pestle in 1.5 mL of methanol.

Extraction of larval intestinal tracts, larval bodies, and remaining artifical diet mix. Larvae were dissected to separate their intestinal tracts from the rest of the body. Intestinal tracts were pulverized in 1 mL of methanol in 1.5 mL Eppendorf tubes and the supernatant was collected for analysis after centrifugation (4). The larval body and remaining *Catharanthus* mixed artificial diet were homogenized individually using a mortar and pestle in presence of 3 and 6 mL of methanol, respectively. The homogenate was transferred to 15-mL sealed plastic tubes and extracts were incubated on an Innova 2000 shaker (New Brunswick Scientific) at 125 rpm at room temperature for 1 h. An aliquot of 200 μL was then filtered through (0.22 μm) PALL filter (VWR) before being analyzed by UPLC/single-quadrupole MS (Waters).

1. Murata J, Roepke J, Gordon H, De Luca V (2008) The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization. *Plant Cell* 20:524–542.
2. Gamborg OL, Murashige T, Thorpe TA, Vasil IK (1976) Plant tissue culture media. *In Vitro* 12:473–478.
3. Bergomaz B, Boppré M (1986) A simple insect diet for rearing Arctiidae and other moths. *J Lepid Soc* 40:131–137.
4. Kunert M, et al. (2008) De novo biosynthesis versus sequestration: A network of transport systems supports in iridoid producing leaf beetle larvae both modes of defense. *Insect Biochem Mol Biol* 38:895–904.



	R_1	R_2	R_3	R_4	R_5
1) Vinblastine	$-CH_3$	$-OCH_3$	$-COCH_3$	-OH	$-CH_2CH_3$
1I) Vincristine	**-CHO**	$-OCH_3$	$-COCH_3$	-OH	$-CH_2CH_3$
1) Vindesine	$-CH_3$	**$-NH_2$**	**-H**	-OH	$-CH_2CH_3$
2) Vinorelbine	$-CH_3$	$-OCH_3$	$-COCH_3$	**-H**	$-CH_2CH_3$
2) Vinflunine	$-CH_3$	$-OCH_3$	$-COCH_3$	-OH	$-CF_2CH_3$

Fig. S1. Commercially relevant dimeric MIAs (*A*) are derived by further modification of a dimer produced from the chemical or enzymatic coupling of catharanthine and vindoline (*B*).

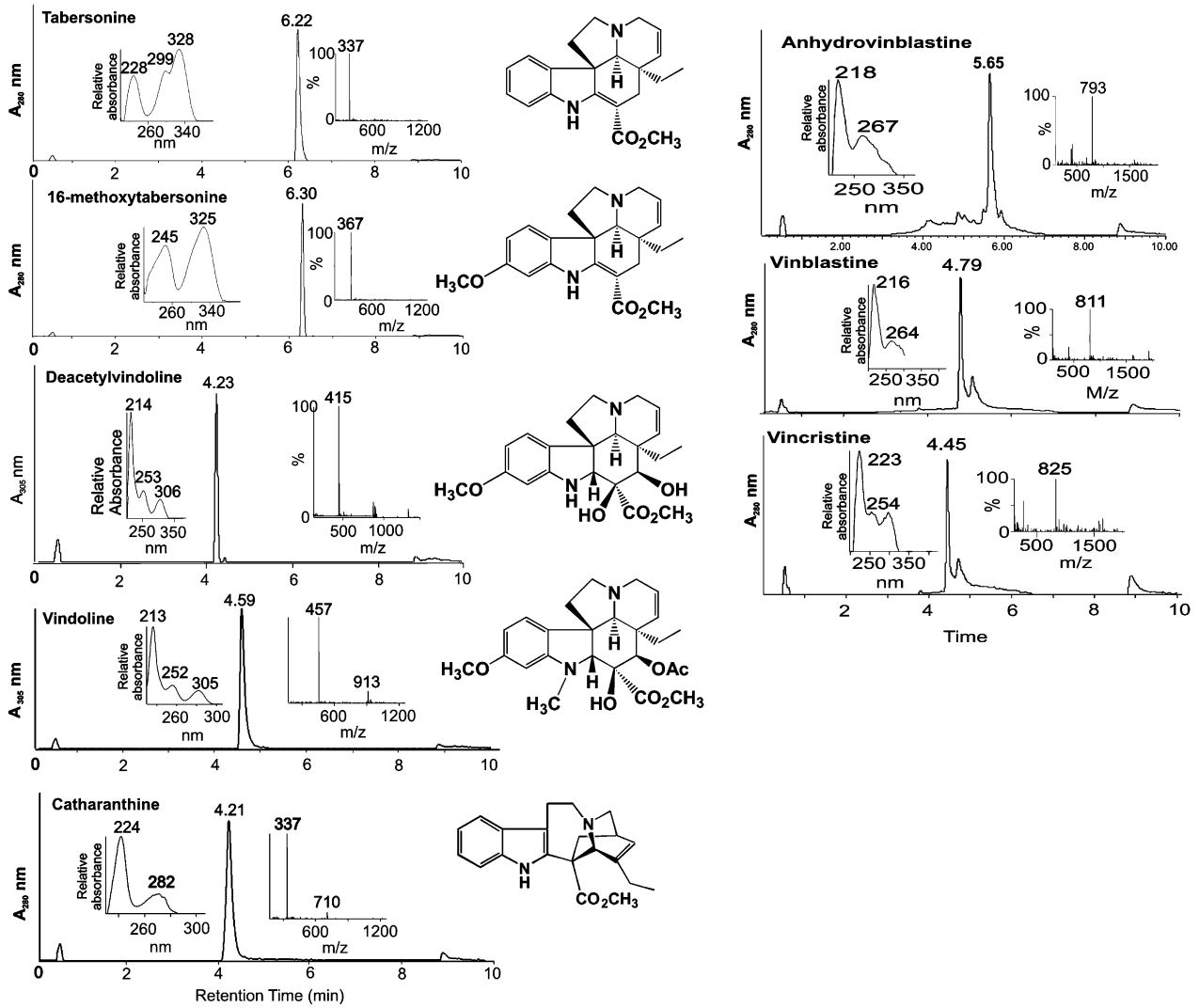


Fig. S2. UPLC-DAD-MS profiles of tabersonine, 16-methoxytabersonine, deacetylvindoline, vindoline, catharanthine, anhydrovinblastine, vinblastine, and vincristine. The retention time of each standard is indicated above each absorption peak.

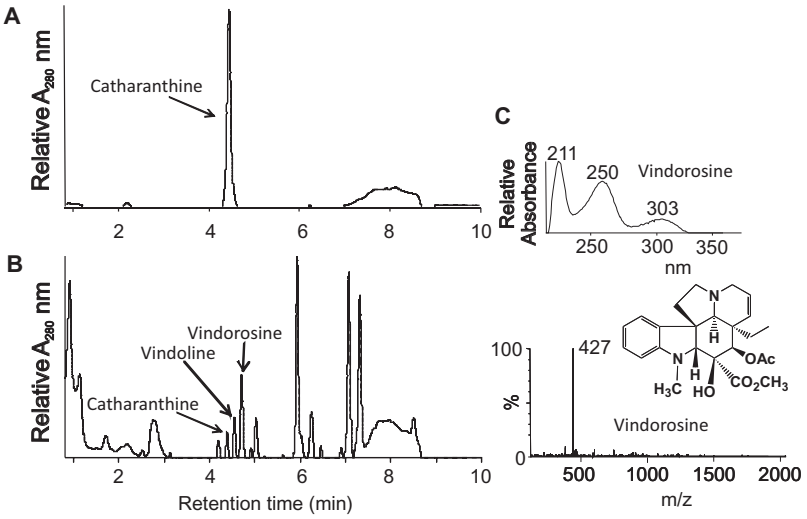


Fig. S3. MIAs extracted in chloroform after incubation of *C. roseus* (Pacifica Peach) leaves for 1 h were separated and identified and their concentrations were measured by UPLC-MS (*Upper*). After incubation in chloroform for 1 h, MIAs from leaves were extracted, separated, and identified, and their concentrations were measured by UPLC-MS (*Lower*). The peaks of catharanthine, vindoline, and vindorosine were identified by their retention times, their absorption spectra their respective masses. The absorption and mass spectra of vindorosine are displayed in Fig. S3*C*.

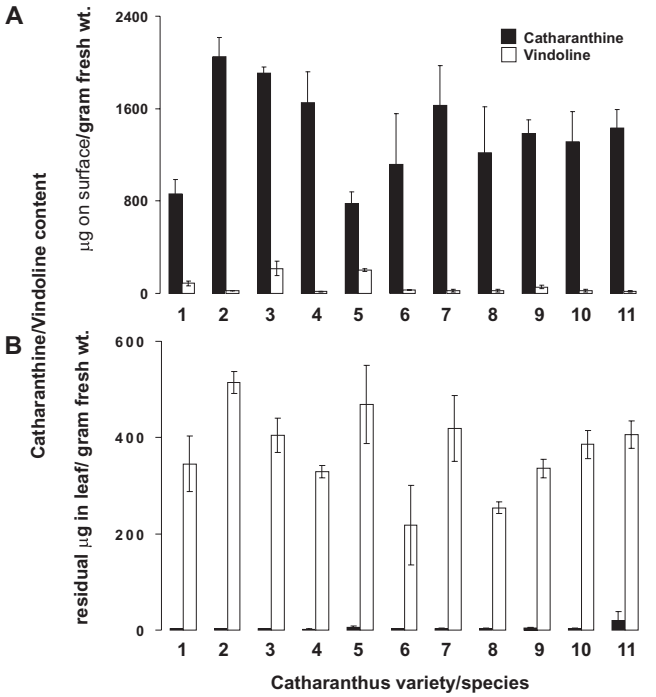


Fig. S4. Distribution of catharanthine and vindoline on the surface of and within the third leaf of *C. roseus* (L.) G. Don cv. Little Delicata (1), *C. roseus* Pacifica Peach (2), *Catharanthus longifolius* (Pichon) Pichon (3), *Catharanthus ovalis* (4), *Catharanthus trichophyllus* (Baker) Pichon (5), plus *C. roseus* hybrids (tolerant to the fungal pathogen *P. nicotianiae* var. Parasitica) Cora Deep Lavender (6), Cora White (7), Cora Lavender (8), Cora Violet (9), Cora Apricot (10), and Cora Burgundy (11). The experiment for each time point was performed in triplicate.

Table S1. Relative fresh *C. roseus* leaf consumption by various insect species during 1-wk feeding

Insect species	Relative leaf consumption	Days before death	Detection of MIAs in insect feces		
			Catharanthine	Vindoline	Anhydrovinblastine
S. littoralis third-instar larvae	++	ND	+	—	—
S. littoralis fourth- instar larvae	+++	ND	+	—	—
S. eridania third-instar larvae	—	ND	NS	NS	NS
S. eridania fourth-instar larvae	+	ND	NS	NS	NS
H. armigera third-instar larvae *a*	+	ND	NS	NS	NS
P. cochleariae third-instar larvae	—	5	NS	NS	NS
B. mori fifth-instar larvae	—	5	NS	NS	NS

All feeding studies were performed with *C. roseus* cv Roseus leaves except for those conducted with *Bombyx mori* where cv Little Delicata leaves were used. ND, none detected; NS, not studied (these insects did not eat or they fed poorly on Catharanthus leaves and produced no feces).

Table S2. Distribution of each alkaloids detected in various samples extracted from fifth-instar *B. mori* larvae fed with *C. roseus* leaves mixed mulberry artificial diet in triplicate

Samples	Catharanthine, %	Vindoline, %	Anhydrovinblastine, %
Regurgitates	0.21 ± 0.02	0.31 ± 0.08	0
Feces	6.35 ± 1.38	6.14 ± 1.99	0
Gut	0.71 ± 0.06	1.18 ± 0.14	0
Body	4.07 ± 0.48	6.55 ± 1.51	0
Remaining prepared diet	75.19 ± 10.91	81.37 ± 7.92	0