SUPPORTING INFORMATION

Substrate Specificity and Diastereoselectivity of Strictosidine Glucosidase, a Key Enzyme in Monoterpene Indole Alkaloid Biosynthesis

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I. High resolution mass data

The samples were diluted in methanol and then ionized by ESI using a Micromass LCT Premier TOF Mass Spectrometer. The LC was performed on an Acquity Ultra Performance BEH C18 column. MassLynx 4.1 was used for analysis, and accurate mass measurements were obtained in W-mode, with reserpine as a reference.

Masses of the purified strictosidine analog substrates:

1: [M+H]^+ expect. 531.2343, obsd. 531.2327
2: [M+H]^+ expect. 545.2499, obsd. 545.2482
3: [M+H]^+ expect. 545.2499, obsd. 545.2474
4: [M+H]^+ expect. 545.2499, obsd. 545.2482
5: [M+H]^+ expect. 545.2499, obsd. 545.2498
6: [M+H]^+ expect. 561.2448, obsd. 561.2452
7: [M+H]^+ expect. 561.2448, obsd. 561.2430
8: [M+H]^+ expect. 549.2248, obsd. 549.2220
9: [M+H]^+ expect. 549.2248, obsd. 549.2240

Masses of the observed deglycosylated strictosidine analogs:

Deglycosylated 1: [M+H]^+ expect. 351.1709, obsd. 351.1711
Deglycosylated 2: [M+H]^+ expect. 365.1865, obsd. 365.1887
Deglycosylated 3: [M+H]^+ expect. 365.1865, obsd. 365.1887
Deglycosylated 4: [M+H]^+ expect. 365.1865, obsd. 365.1863
Deglycosylated 5: [M+H]^+ expect. 365.1865, obsd. 365.1885
Deglycosylated 6: [M+H]^+ expect. 381.1814, obsd. 381.1823
Deglycosylated 7: [M+H]^+ expect. 381.1814, obsd. 381.1802
Deglycosylated 8: [M+H]^+ expect. 369.1614, obsd. 369.1623
Deglycosylated 9: [M+H]^+ expect. 369.1614, obsd. 369.1623

Pentynyl secologanin: [M+H]^+ expect. 441.1761, obsd. 441.1787
Pentynyl strictosidine 10: [M+H]^+ expect. 583.2656, obsd. 583.2687
Deglycosylated 10: [M+H]^+ expect. 403.2022, obsd. 403.2047
Vincoside 11: [M+H]^+ expect. 531.2343, obsd. 531.2356
Deglycosylated 11: [M+H]^+ expect. 351.1709, obsd. 351.1722
Vincoside 11 (deuterated): [M+H]^+ expect. 533.2499, obsd. 533.2514
Deglycosylated deuterated vincoside 11: [M+H]^+ expect. 353.1865, obsd. 353.1868
Vincoside lactam 12: [M+H]^+ expect. 499.2080, obsd. 499.2079
II. Deglycosylation of vincoside 11

*LC-MS assay of deuterated vincoside with SGD*

![LC-MS traces](image)

**Figure S1.** LC-MS traces of deuterated vincoside with SGD, with retention times marked above the peaks.
A: Deuterated deglycosylated vincoside at m/z 353.
B: Deuterated vincoside at m/z 533.
C: Total ion count of the reaction.

*LC-MS data of strictosidine and vincoside mixture with SGD*

A 1:1 mixture of strictosidine 1 and vincoside 11 was incubated with SGD. Strictosidine (m/z 531) has a retention time of 6.87 minutes, and vincoside (m/z 531) has a retention time of 7.48 minutes (Figure S2, bottom trace). No deglycosylated strictosidine or vincoside (m/z 351) is observed in the absence of SGD (Figure S2, top trace, m/z 351).
Figure S2. Strictosidine 1 and vincoside 11 in the absence of SGD. Shown are extracted traces at m/z 531 (bottom) corresponding to 1 and 11 and at m/z 351 (top) corresponding to the deglycosylated products. No deglycosylated products were observed in the absence of enzyme.

After incubation with SGD, strictosidine 1 and vincoside 11 were completely consumed (Figure S3, bottom trace m/z 531) and deglycosylated products (peaks at 7.82 and 9.48 minutes) were observed (Figure S3, top trace m/z 351).

Figure S3. Strictosidine 1 and vincoside 11 in the presence of SGD. Extracted traces at m/z 531 (bottom) corresponding to 1 and 11 and at m/z 351 (top) corresponding to the deglycosylated products are shown. All 1 and 11 was consumed by the SGD enzyme.
III. Representative NMR data

$^1$H-NMR data for 11-methyl-strictosidine 4

$^1$H-NMR (d$_4$-methanol), 500 MHz, $\delta$ 7.81 (1H, s), 7.35 (1H, d, $J = 8.1$), 7.11 (1H, s), 6.9 (1H, d, $J = 8.0$), 5.86 (2H, m), 5.37 (1H, d, $J = 17.3$), 5.28 (1H, d, $J = 10.7$), 4.81 (1H, d, $J = 7.9$), 4.6 (1H, d, $J = 11.5$), 4.0 (1H, m), 3.80 (3H, s), 3.73 (1H, dt, $J = 12.2$, 4.9), 3.67 (1H, dd, $J = 7.2$, 11.8), 3.47-3.34 (3H, m), 3.24 (1H, d, $J = 3.6$), 3.22 (1H, dd, $J = 1.9$, 9.2), 3.1 (3H, m), 2.75 (1H, td, $J = 4.9$, 8.3), 2.4 (3H, s), 2.32 (1H, m), 2.2 (1H, m).

$^{13}$CNMR (d$_4$-methanol) δ 171.3, 156.9, 138.7, 135.5, 133.4, 129.5, 125.4, 122.4, 119.8, 118.9, 112.2, 109.0, 107.0, 100.5, 97.3, 78.9, 78.0, 74.7, 71.8, 63.1, 53.1, 52.6, 45.4, 42.7, 34.8, 32.7, 21.9, 19.6.

$^1$H-NMR data for vincoside 11

$^1$H-NMR (d$_4$-methanol), 500 MHz, $\delta$ 7.56 (1H, s), 7.49 (1H, d, $J = 7.5$), 7.38 (1H, d, $J = 8$), 7.18 (1H, t, $J = 7$), 7.08 (1H, t, $J = 7.5$), 6.02 (1H, m), 5.70 (1H, d, $J = 7.5$), 5.45 (1H, d, $J = 16.5$), 5.39 (1H, d, $J = 11.5$), 4.80 (3H, m), 4.01 (1H, dd, $J = 2$, 12), 3.79 (1H, m), 3.68 (5H, m), 3.54 (1H, m), 3.40 (3H, m), 3.35 (4H, m), 3.12 (4H, m), 2.84 (1H, q, $J = 8$), 2.52 (1H, m), 2.17 (1H, m).
IV. Glucose detection assay

The Amplex® Red Glucose/Glucose Oxidase Assay Kit from Invitrogen was used as further verification that vincoside is deglycosylated by SGD. Glucose formation was monitored at 560 nm using a reaction solution made from glucose oxidase, horseradish peroxidase, and Amplex® Red according to the manufacturer’s instructions. Upon deglycosylation a coupled assay reaction converted Amplex® Red to a fluorescent compound (see Zhou, M.; Diwu, Z.; Panchuk-Voloshina, N.; Haugland, R. Anal. Biochem. 1997, 253, 162-168).

![UV-visible spectra at 560 nm](image)

**Figure S4.** UV-visible spectra at 560 nm.  **A.** Vincoside 11 incubated with SGD and all components of the Amplex® Red assay.  **B.** Vincoside 11 and all components of the enzyme assay without SGD.  **C.** Vincoside 11 and SGD without components of the Amplex® Red assay (Amplex® red, glucose oxidase and horseradish peroxidase).