

New Phytologist Supporting Information

Article title: Two bi-functional cytochrome P450 CYP72 enzymes from Olive (*Olea europaea*) catalyze the oxidative C-C bond cleavage in the biosynthesis of secoxy-iridoids - flavor and quality determinants in Olive oil

Authors: Carlos E. Rodríguez-López, Benke Hong (洪本科), Christian Paetz, Yoko Nakamura, Konstantinos Koudounas, Valentina Passeri, Luciana Baldoni, Fiammetta Alagna, Ornella Calderini, Sarah E. O'Connor.

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The following Supporting Information is available for this article:

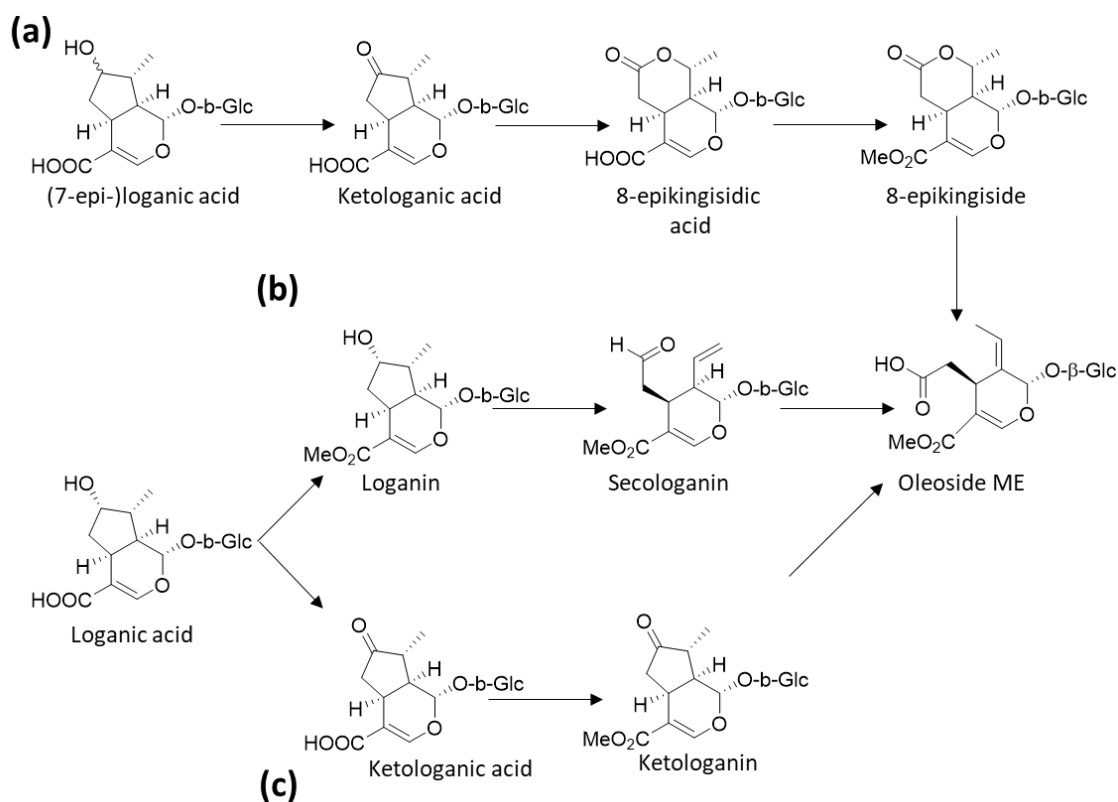


Fig. S1 Previous hypotheses on the biosynthesis of secoiridoids in Olive. Hypothesis differ on the immediate precursors: hypothesis (a), where 8-epikingiside is the immediate precursor (Inouye *et al.*, 1971; Damtoft *et al.*, 1993); hypothesis (b), where it is secologanin (Inouye *et al.*, 1971; Damtoft *et al.*, 1993; Alagna *et al.*, 2016); and hypothesis (c), being ketologanin (Alagna *et al.*, 2016). None of these hypotheses are supported by our results, but rather ketologanin is formed from 7-epiloganin, and then cleaved to form the oleoside methyl ester.

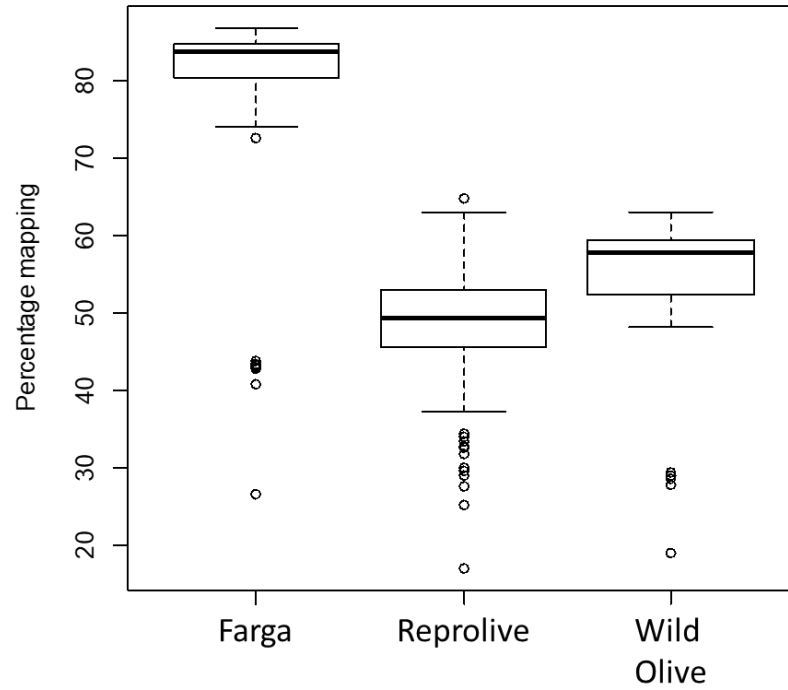


Fig. S2 Mapping of publically available datasets against available transcriptomes. Percentage mapping of the 112 runs analyzed in this article against different available transcriptomes. The box delimits the lower (25%) and upper (75%) quartiles, with the internal horizontal line depicting the median; the whiskers represent the “reasonable extremes”: 1.5 times the inter-quartile range or, if no data-point exceeds this value, the minimum or maximum of the data points for the upper and lower whisker. Data points exceeding the “reasonable extremes” are plotted as hollow circles.

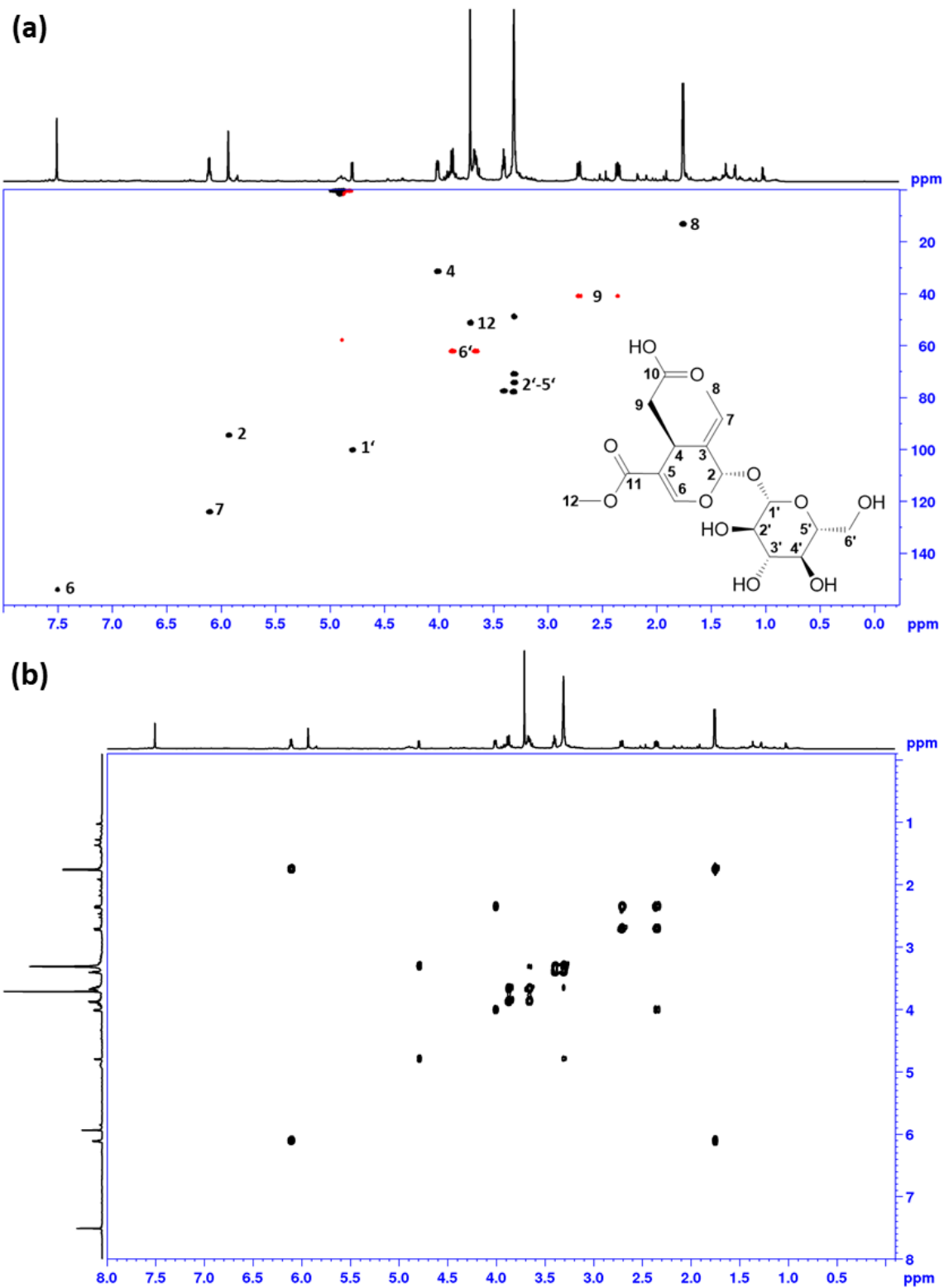


Fig. S3 NMR spectra of the oleoside methyl ester standard. The ^1H - ^{13}C HSQC (a) and ^1H - ^1H DQF-COSY (b) of oleoside methyl ester are shown.

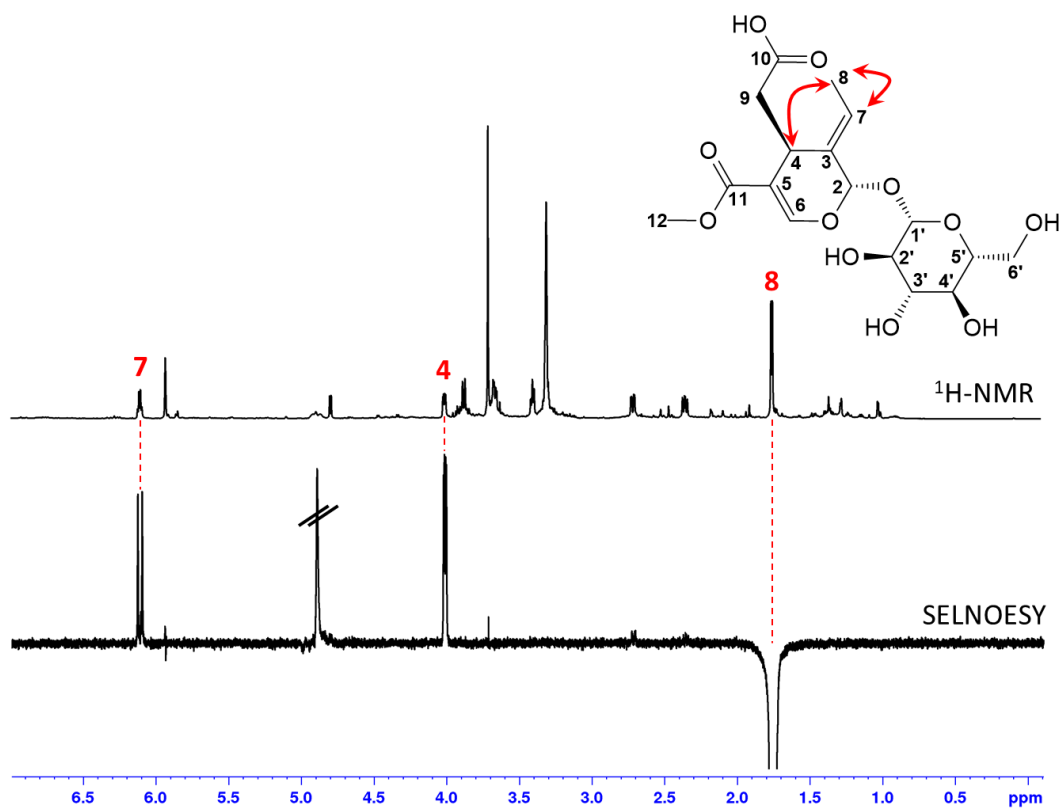


Fig. S4 Confirmation of the oleoside methyl ester configuration. The $^1\text{H-NMR}$ and selective NOESY spectra (mixing time of 600 ms) of oleoside methyl ester are shown. As transmitter frequency 1.785 ppm/1250.5 Hz was selected (methyl group in position 8). These spectra confirm that the exocyclic olefinic bond is in the (*E*)-configuration.

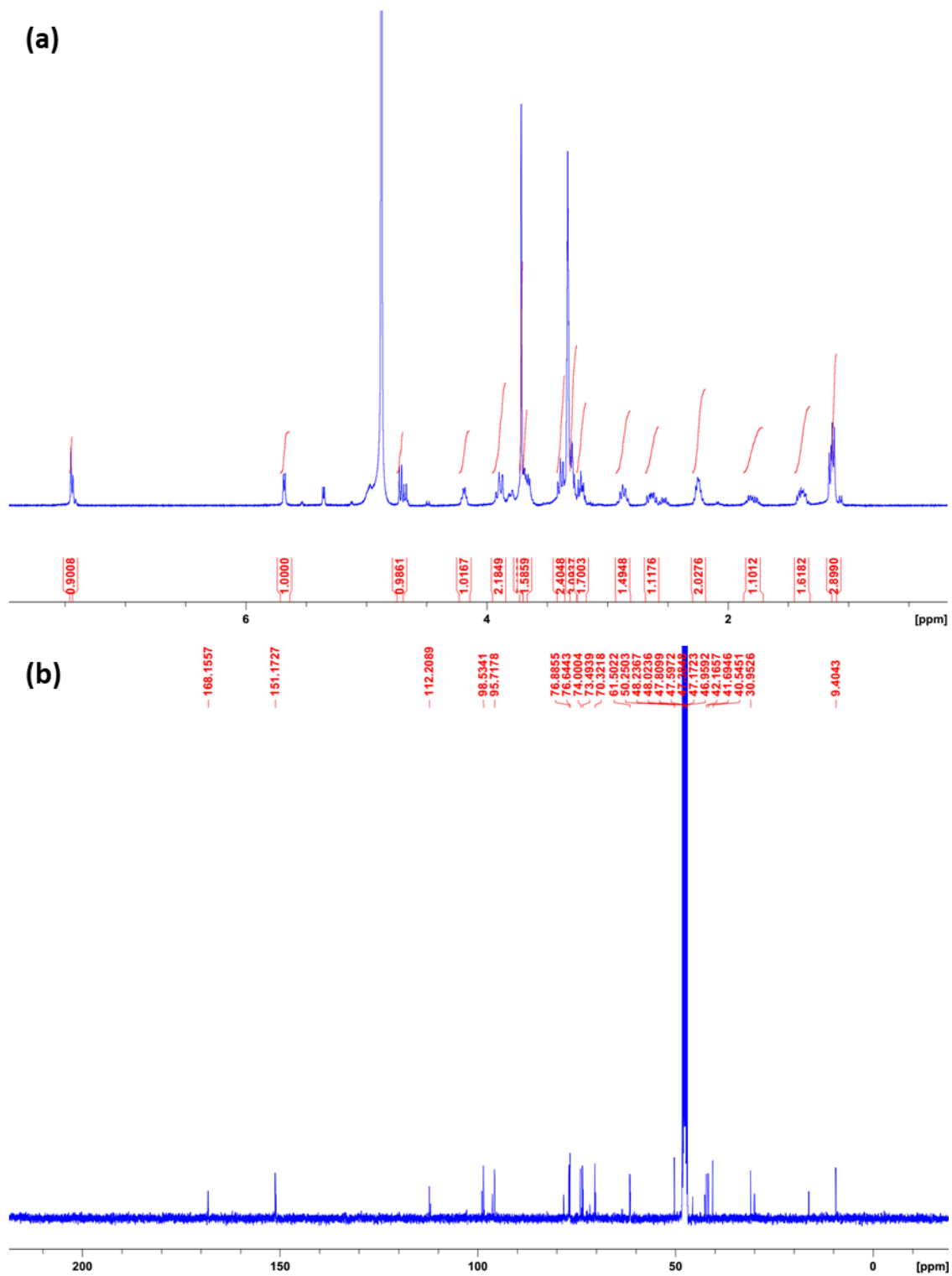


Fig. S5 NMR spectra of 7-*epi*-8-*epi*-loganin. The ^1H - (a) and ^{13}C -NMR (b) spectrum of 7-*epi*-8-*epi*-loganin is shown.

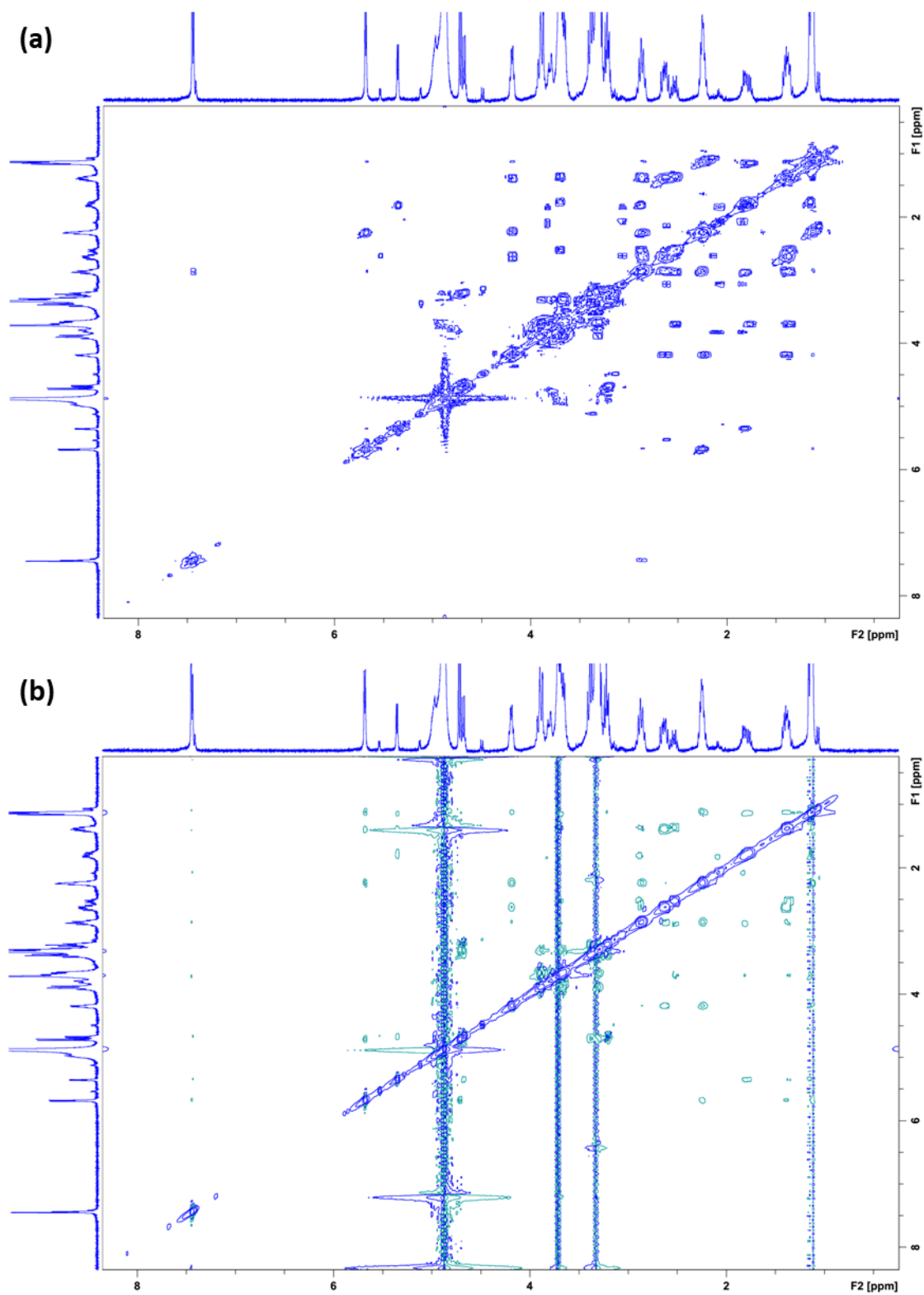


Fig. S6 COSY and NOESY spectra of 7-*epi*-8-*epi*-loganin. The ¹H-¹H COSY (a) and NOESY (b) spectrum of 7-*epi*-8-*epi*-loganin is shown.

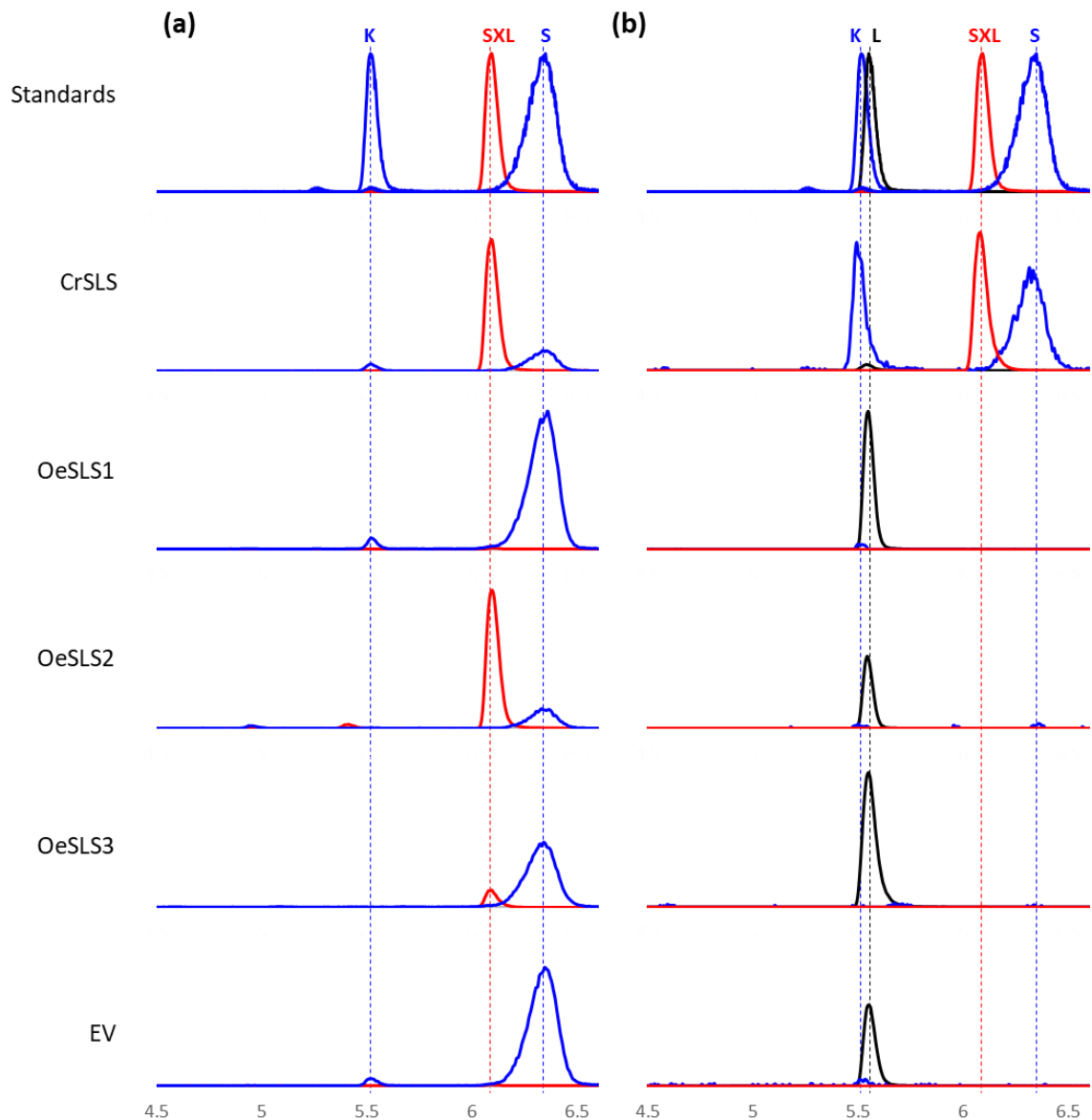


Fig. S7 Extracted Ion Chromatograms (XIC) of microsomal incubations with secologanin and loganin. XIC of the microsomal incubations with secologanin (a) and loganin (b) are shown. Three channels are depicted, corresponding to the most abundant adduct of secoxyloganin (red, $[M-H]^- = 403.1240 \pm 0.05$), secologanin (blue, $[M+FA-H]^- = 433.1346 \pm 0.05$) and loganin (black, $[M+FA-H]^- = 435.1503 \pm 0.05$). Intensities are scaled to the highest intensity of the corresponding channel in all incubations. K, ketologanin; SXL, secoxyloganin; S, secologanin.

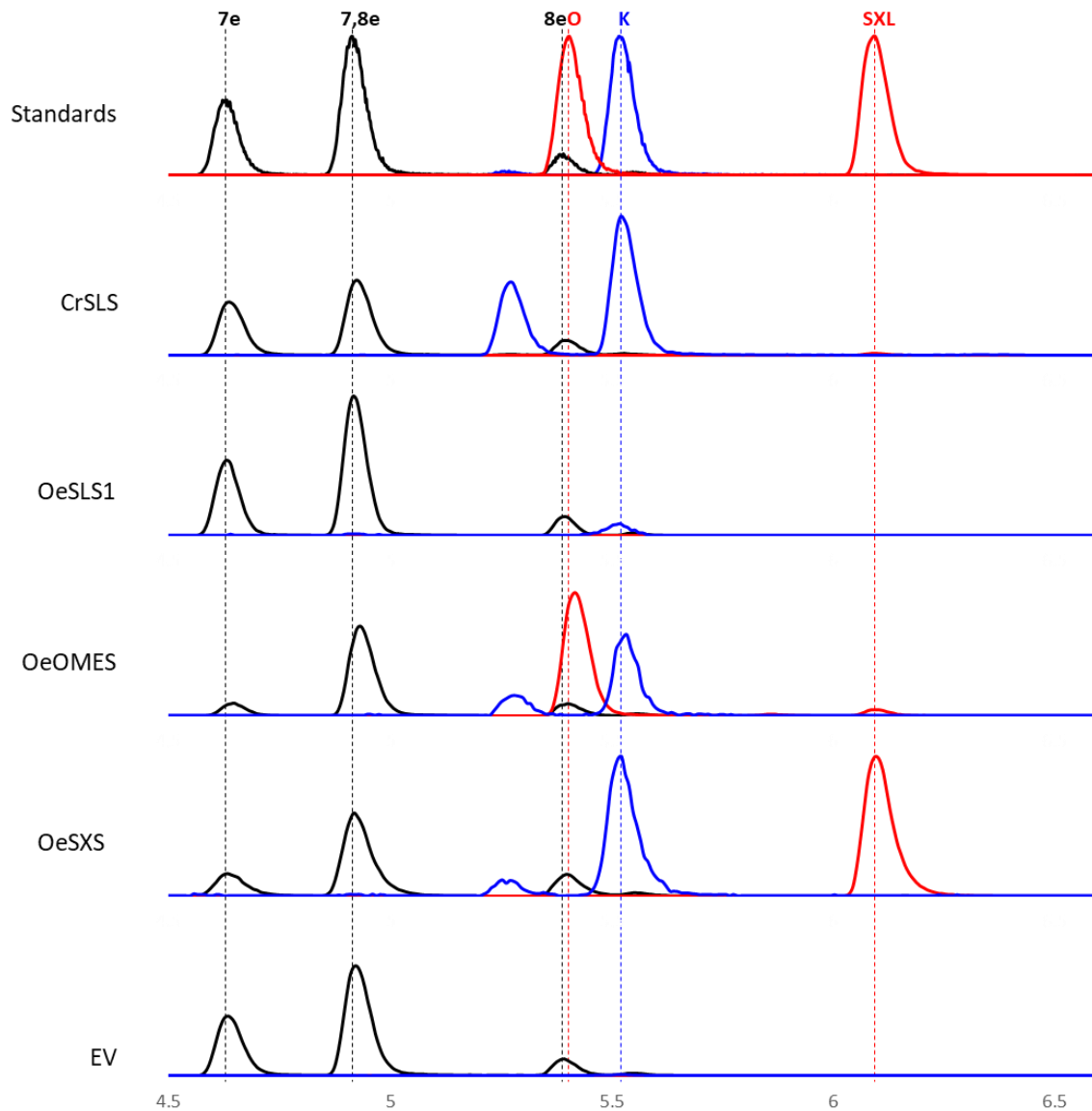


Fig. S8 Extracted Ion Chromatograms (XIC) of microsomal incubations with a mix of loganin epimers. XIC of the microsomal incubations with a mix of loganin epimers are shown. Three channels are depicted, corresponding to the most abundant adduct of OME and secoxyloganin (red, $[M-H]^- = 403.1240 \pm 0.05$), ketologanin and secologanin (blue, $[M+FA-H]^- = 433.1346 \pm 0.05$), and loganin epimers (black, $[M+FA-H]^- = 435.1503 \pm 0.05$). Intensities are scaled to the highest intensity of the corresponding channel in all incubations. 7e, 7-epi-loganin; 7,8-e, 7,8-epi-loganin; 8e, 8-epi-loganin; O, oleoside; K, ketologanin; SXL, secoxyloganin.

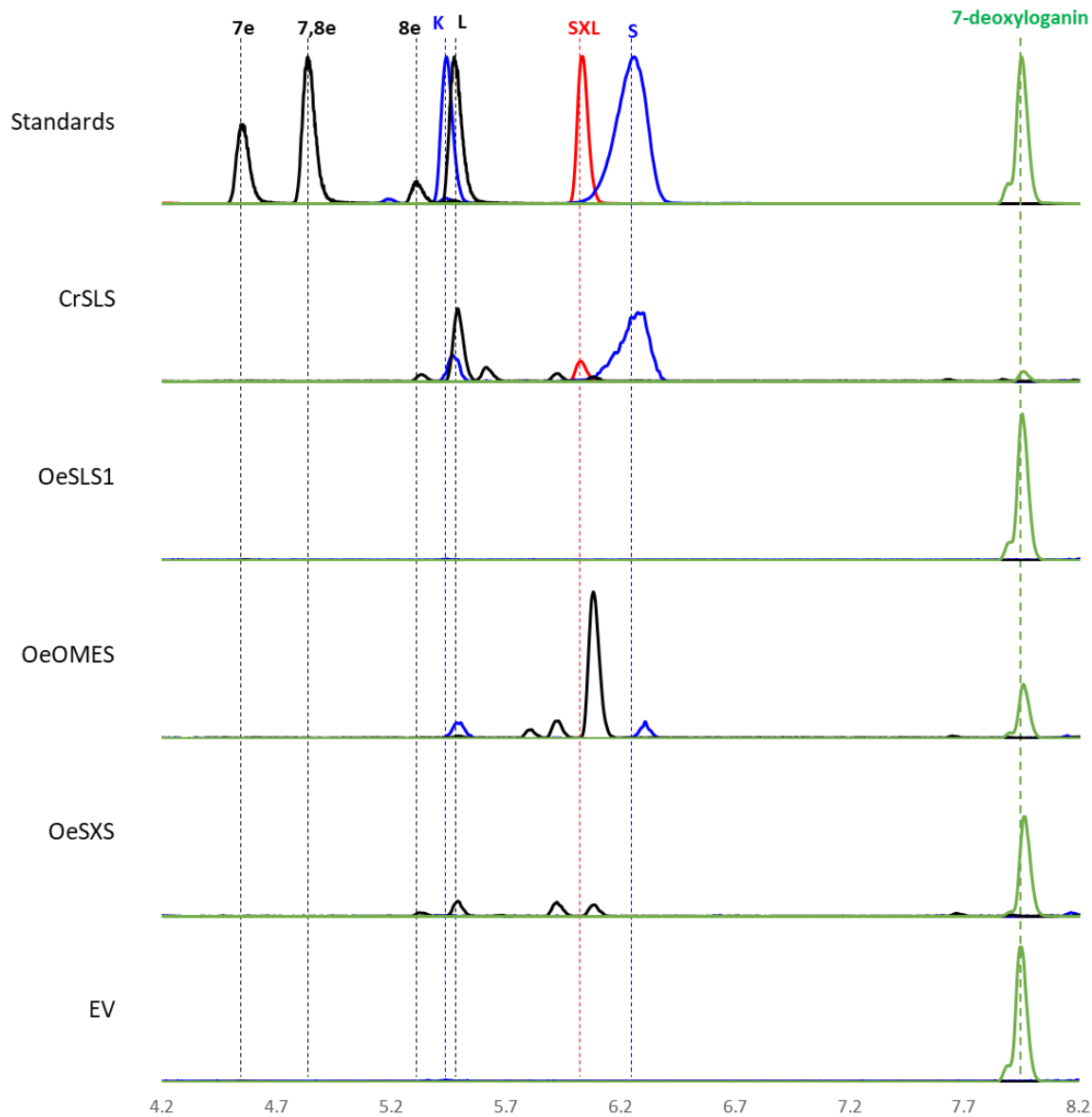


Fig. S9 Extracted Ion Chromatograms of microsome incubations with 7-deoxyloganin. XIC of

the microsomal incubations with 7-deoxyloganin are shown. Four channels are depicted: the most abundant adduct of secoxyloganin (red, $[M-H]^- = 403.1240 \pm 0.05$), ketologanin and secologanin (blue, $[M+FA-H]^- = 433.1346 \pm 0.05$), loganin epimers (black, $[M+FA-H]^- = 435.1503 \pm 0.05$) and 7-deoxy-loganin (Green, $[M+FA-H]^- = 419.1553 \pm 0.05$). Intensities are scaled to the highest intensity of the corresponding channel in all incubations. 7e, 7-epi-loganin; 7,8-e, 7,8-epi-loganin; 8e, 8-epi-loganin; L, loganin; K, ketologanin; SXL, secoxyloganin; S, secologanin.

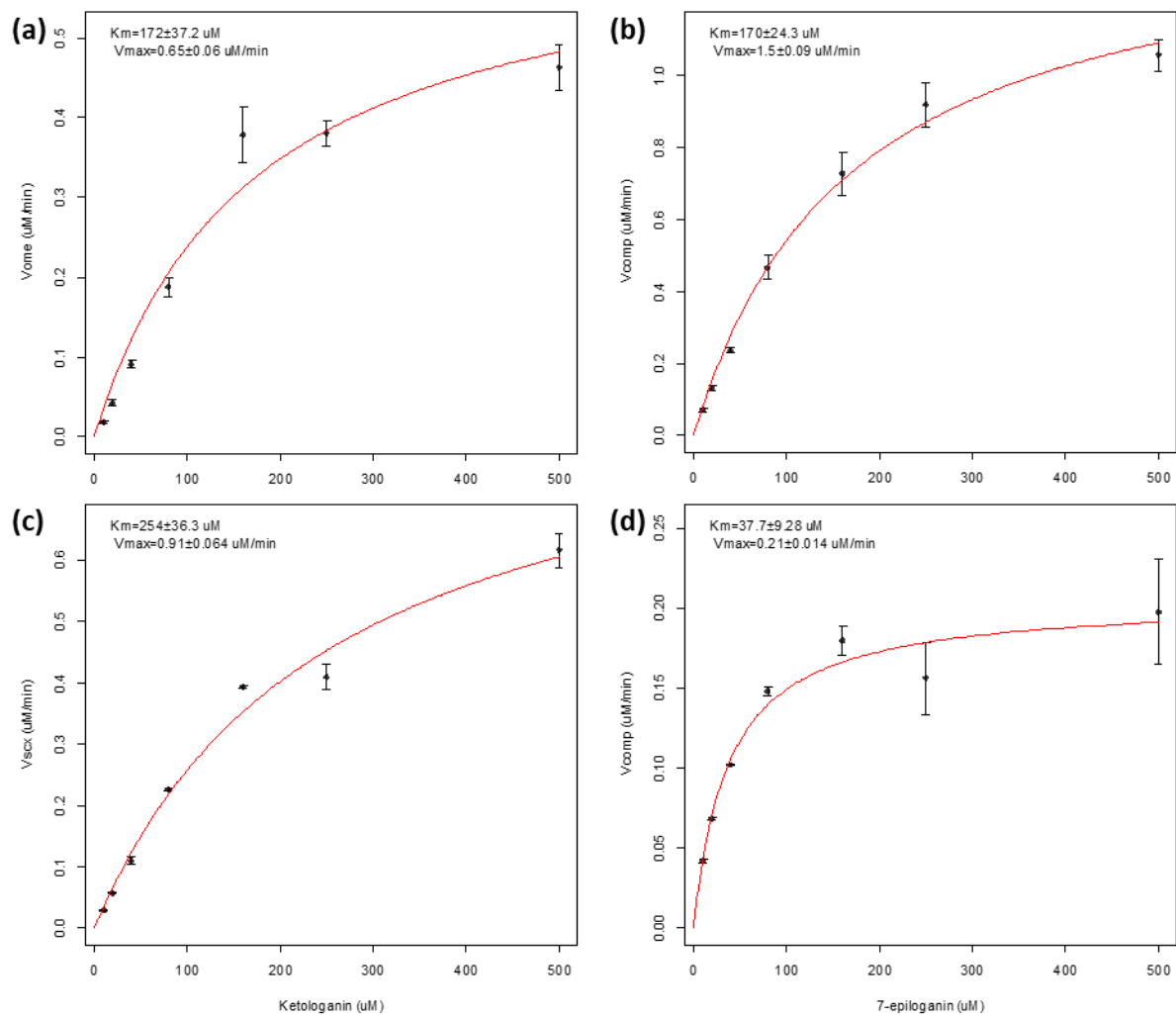


Fig. S10 Kinetic modelling of enzyme activity. Kinetics of production rate of OME by OeOMES (a) and secoxyloganin by OeSXS (c) with respect to ketologanin concentration; and scaled sum of velocities (v_{comp}) for OeOMES (b) and OeSXS (d) with respect to 7-epi-loganin concentration. Dots represent the average and error bars the standard error of three measurements, and the regression is shown in red.

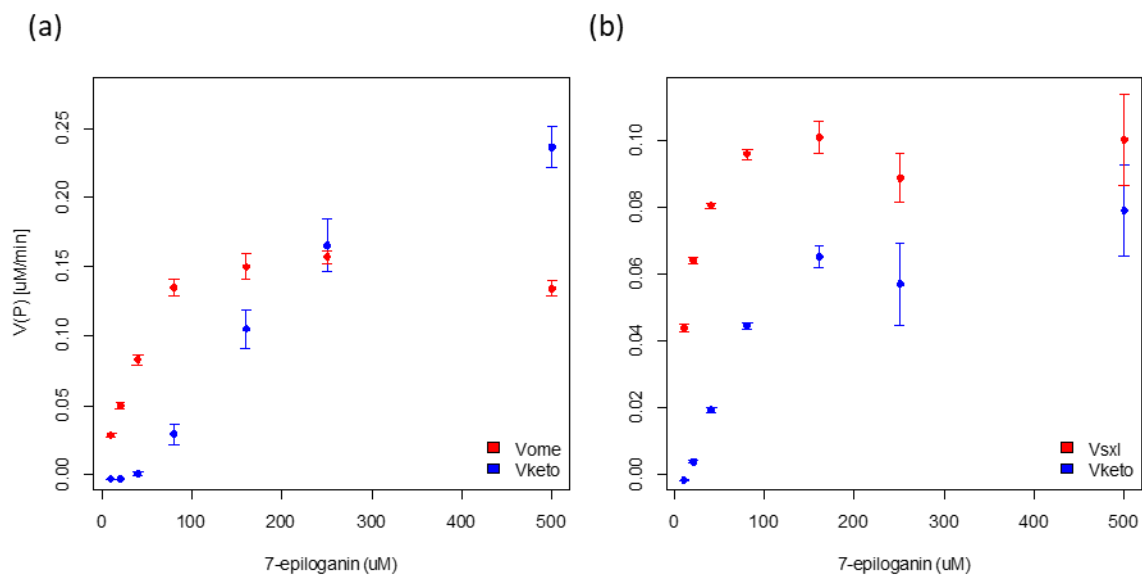
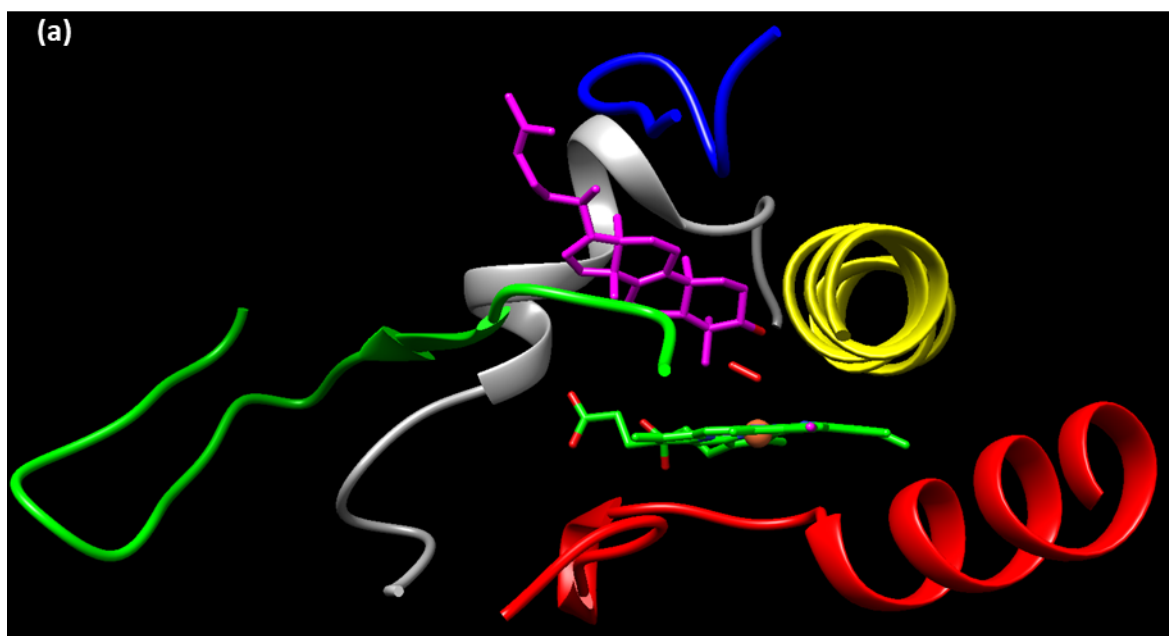


Fig. S11 Individual enzyme activities. Rates of production of OME (red) and ketologanin (blue) by OeOMES (a) and secoxyloganin (red) and ketologanin (blue) by OeSXS (b) with respect to 7-epi-loganin concentration. Dots represent the average and error bars the standard error of three measurements.



(b)

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1 MEILKTAISV VTIIIFTAAW RILKWLWLDP KKKERILRQQ GFKGNPYTFR
51 RLLFGDEKEI QKIHAEAWSK PVNIRDEVQT GKRAAPFIFR TYEKYGKKAF
101 VWAGLRPKVF IMEPDHMKTI FFNHSTFQKN FKVTNSVVQE LITGIIRFEG
151 EEWSKRRTIM SPKFQLEKLE QMIPLVLKCS DQVIIEWKKL VSNSKDGSYT
201 LDVCHDIEEL ISGVTSQFLF GIDYAKDKEI FHLITQLSEL TKQATKVSNL
251 PGSKFLPTET NRKSKRLTKE LHQRLYKLMC ERKKAIKEGK VVEDNVFNML
301 LESEIANNQD EMIGHMKGFV FNSHDTTAFV LVWNLILLCI YSEWQDRARE
351 EVFRVFGNRR PDYEGLSQLK VLPFMFMNEVL RLYPPLVELS RFLEEEEIKLG
401 EYTLPADIQV IMPTILVHRD PEFWGEDANE FKPERFAEGV LKATNGQAVF
451 FPFAWGPRIC IGYNMALLQV KLVLADLLRN FSFEISPTYE HAPRVVFTQQ
501 PQYGAPIILR NLN

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Fig. S12 Regions of interest in the homology model. (a) Homology model of OeOMES, using the backbone of Lanosterol 14- α demethylase (L14D; PDB 4LXJ; Monk et al., 2014) as a guide. Only the regions of interest, closest to the original substrate in the model of L14D, are shown. (b) OeOMES aminoacid sequence, with the regions of interest highlighted with the corresponding colors: Gray (region A), Yellow (region B), Green (region C), Red (region D) and Blue (region E).

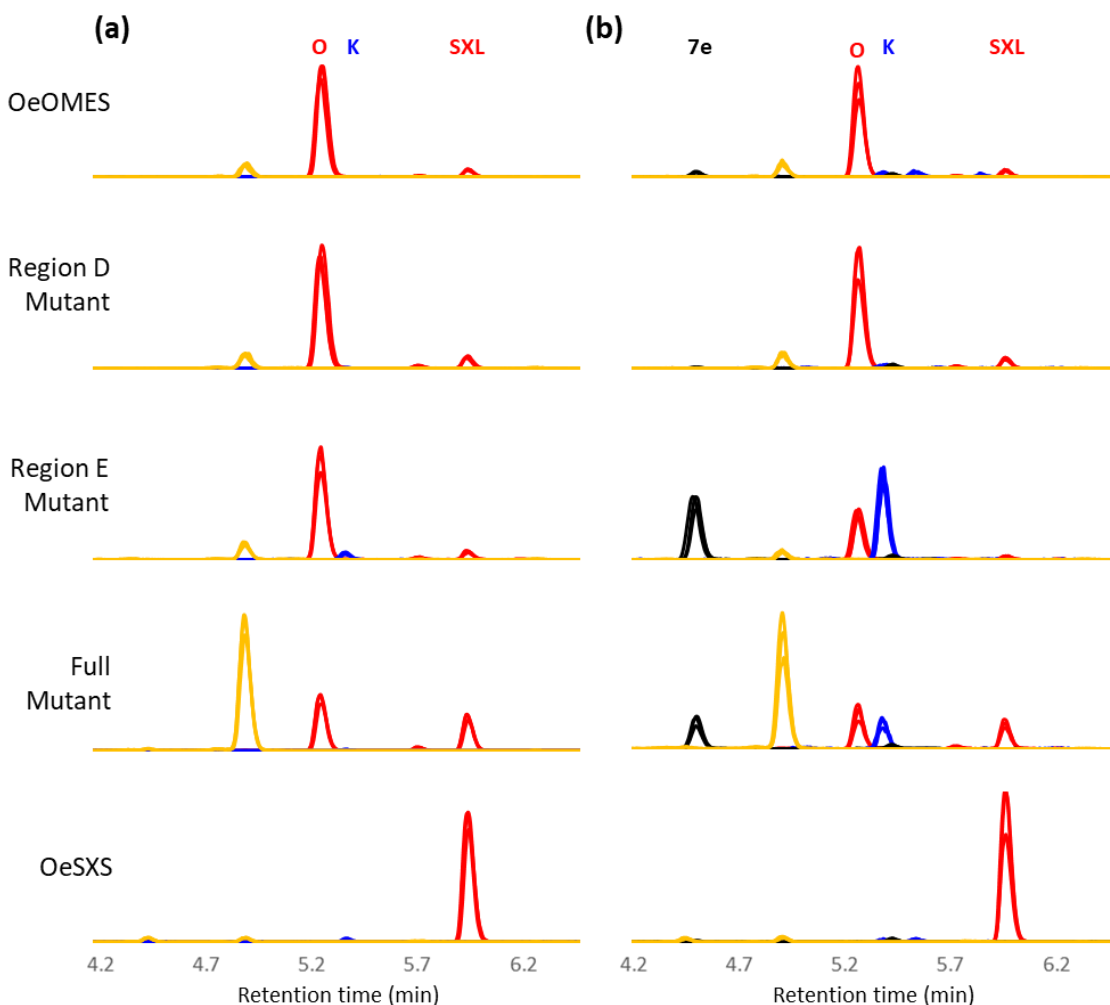


Fig. S13 Extracted Ion Chromatograms (XIC) of microsomal incubations of mutant proteins with ketologanin and 7-epiloganin. XIC of the microsomal incubations with ketologanin (a) and 7-epiloganin (b) are shown for selected mutants. Four channels are depicted, corresponding to the most abundant adduct of OME and secoxyloganin (red, $[M-H]^- = 403.1240 \pm 0.05$), ketologanin (blue, $[M+FA-H]^- = 433.1346 \pm 0.05$), 7-epiloganin (black, $[M+FA-H]^- = 435.1503 \pm 0.05$), and oxidized ketologanin (yellow, $[C_{17}H_{24}O_{11}+FA-H]^- = 449.1295 \pm 0.05$). Intensities are scaled to the highest intensity of the corresponding channel in all incubations. Three replicates are shown, overlaid, for each mutant. O, oleoside; K, ketologanin; SXL, secoxyloganin.

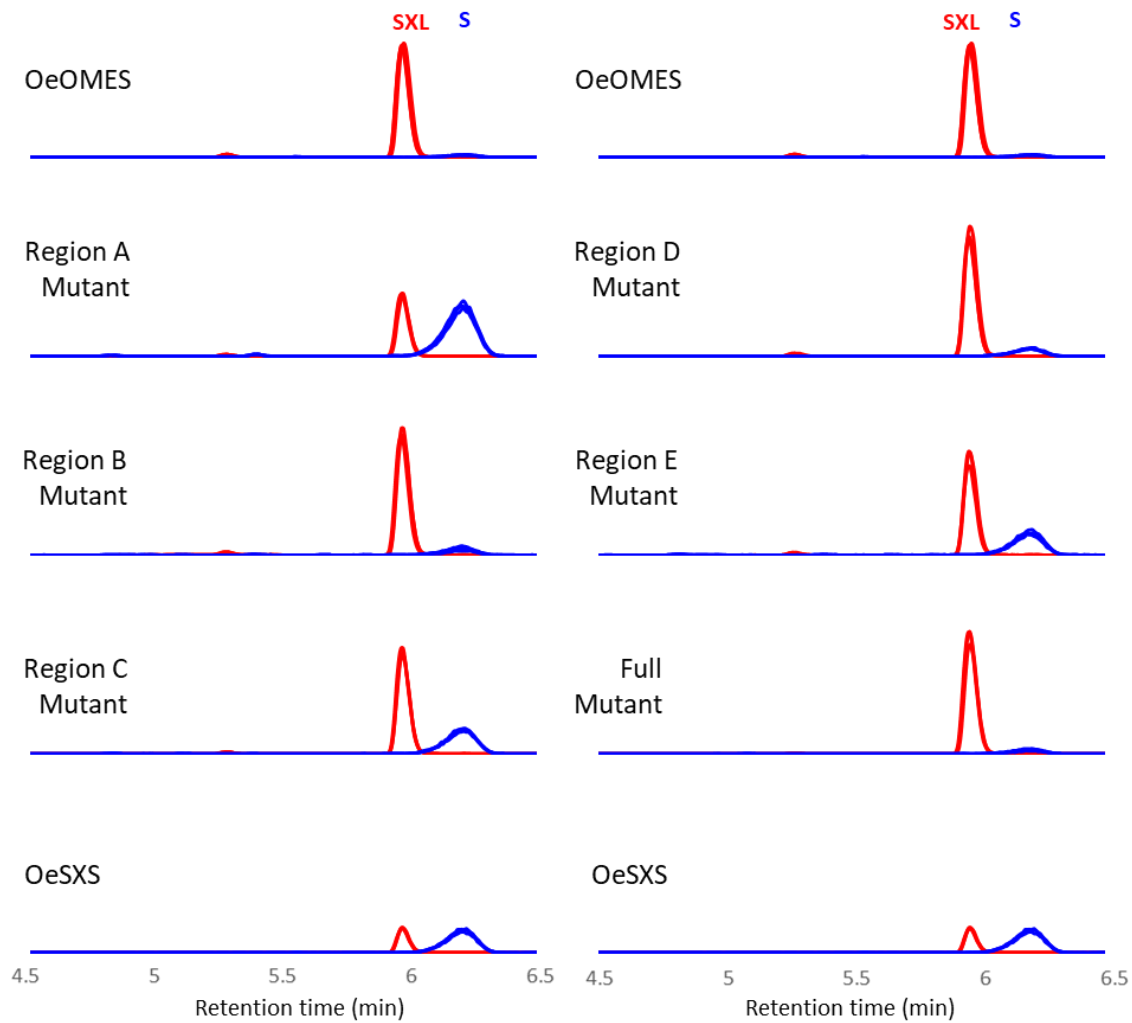


Fig. S14 Extracted Ion Chromatograms (XIC) of microsomal substrate assays. XIC of the microsomal incubations with secoyanin are shown for all mutants. Two channels are depicted, corresponding to the most abundant adduct of secoxyloganin (red, $[M-H]^- = 403.1240 \pm 0.05$), and ketologanin (blue, $[M+FA-H]^- = 433.1346 \pm 0.05$). Intensities are scaled to the highest intensity of the corresponding channel in all incubations. Three replicates are shown, overlaid, for each mutant. SXL, secoxyloganin; S, secoyanin.

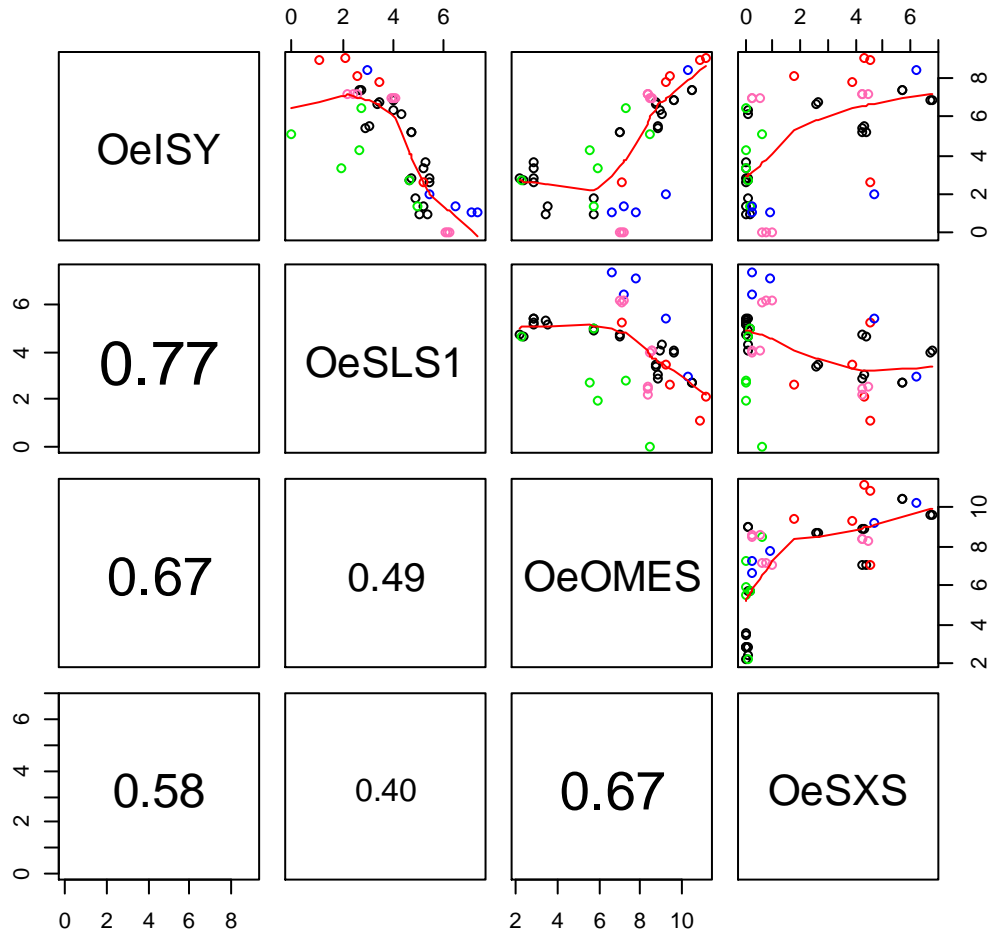


Fig. S15 Coexpression Analysis of Olive secologanin synthase homologues with Iridoid Synthase. A correlogram is shown of the coexpression of *OeISY*, *OeSLS1*, *OeOMES* and *OeSXS* in different BioProjects. The upper diagonal shows scatter plots of the logarithm, base 2, of the expression in Transcripts Per Million (TPM) of each gene, with a lowest regression on the data (red line). The lower diagonal shows the absolute of Pearson's product-moment correlation coefficient. Each point corresponds to an RNA-seq run of projects PRJNA256033 (black), PRJNA378602 (green), PRJNA401310 (red), PRJNA514943 (blue), and PRJNA596876 (pink)

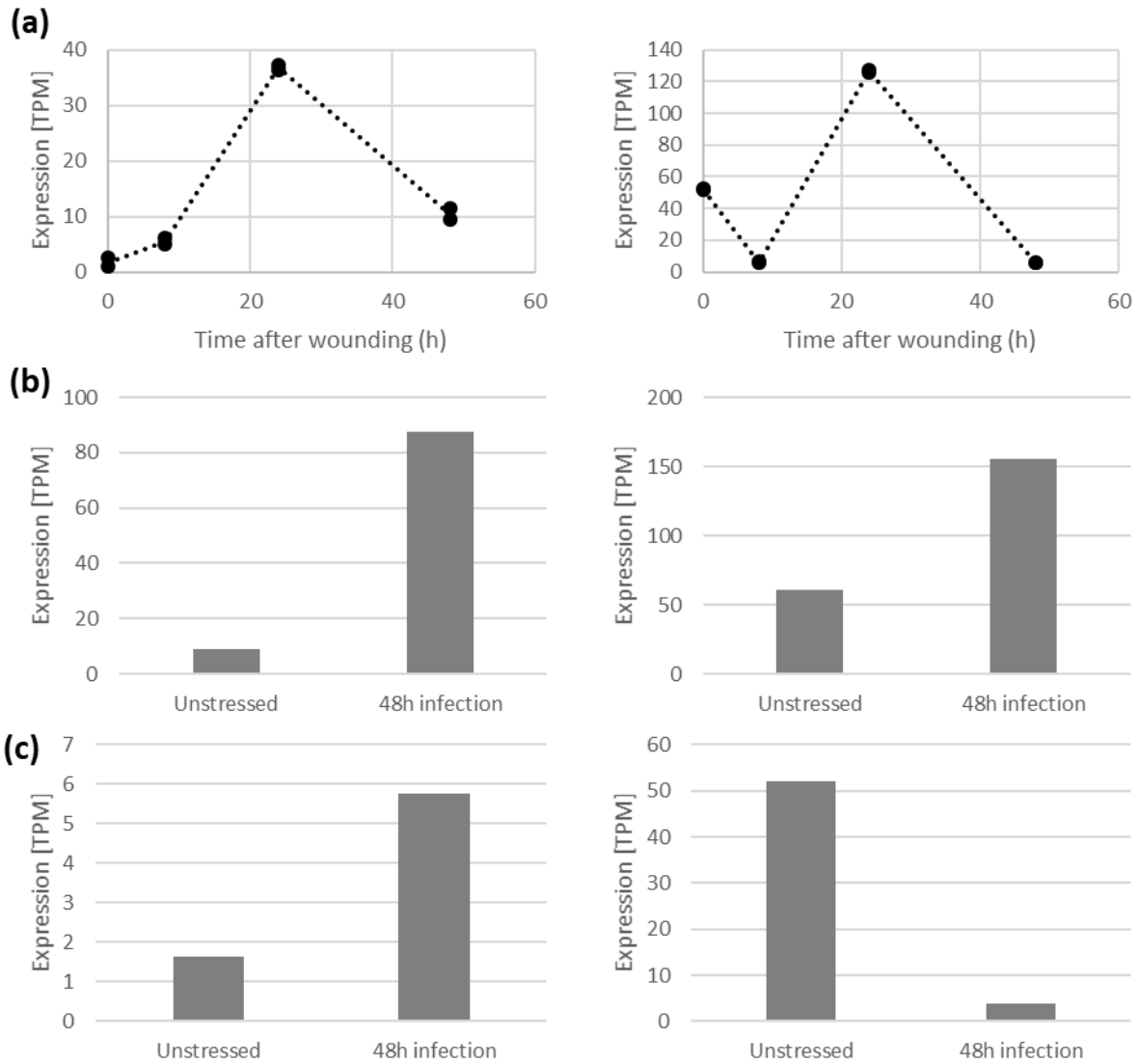


Fig. S16 Effect of different stresses in OeISY and OeOMES expression. Expression is shown for OeISY (left) and OeOMES (right) Picual roots after mechanical wounding (a), and Frantoio (b) and Picual (c) roots after infection with *Verticillium dahliae*. The last two experiments, (b) and (c) had a single replicate, while (a) was done in duplicate. For the latter, each point is a replicate.

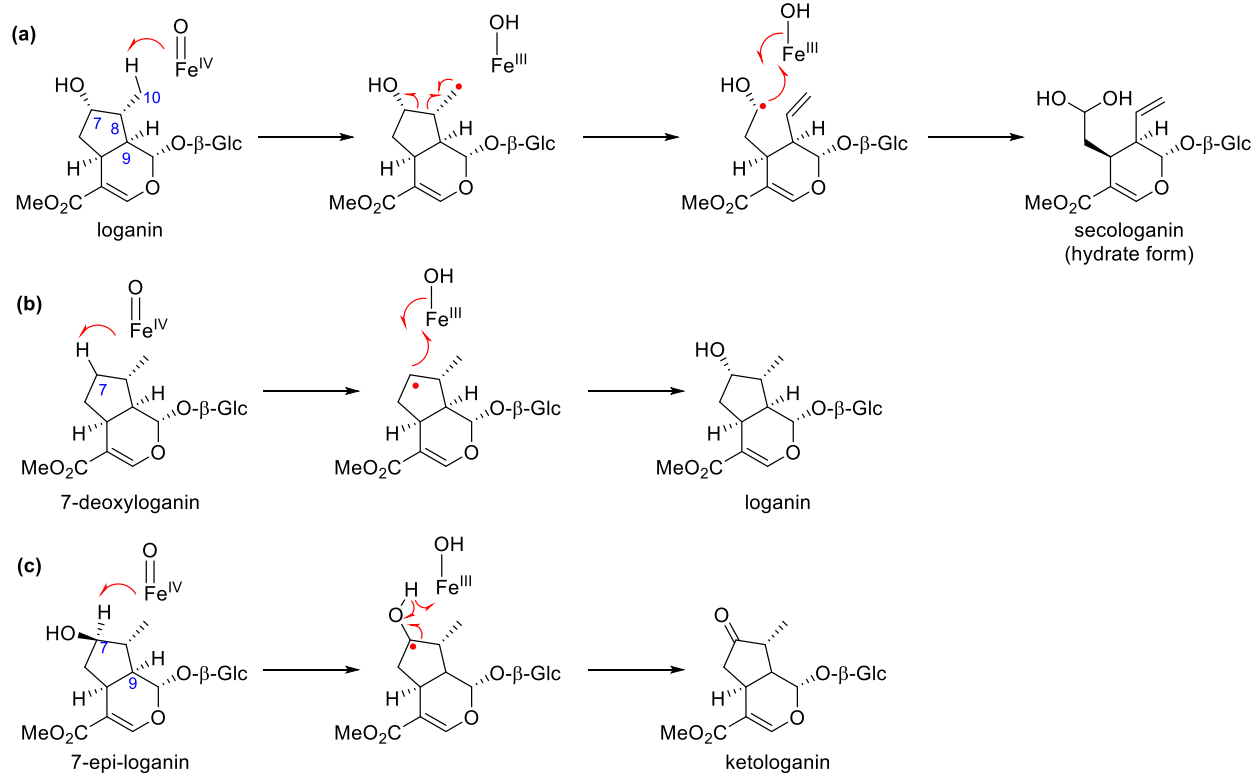


Fig. S17 Proposed mechanisms of CrSLS catalyzed reactions. A summary of the proposed mechanisms for production of secologanin (a), loganin (b) and ketologanin (c) by CrSLS.

Mechanism (a) was adapted from (Yamamoto *et al.*, 2000).

Table S1. Amino acid shifts in each of the selected regions.

| Mutant | Amino acid shifts |
|---------------|--|
| Region A | 123 N→K 126 T→N 136 S→N 137 V→I 140 E→D |
| Region B | 320 V→F 323 S→A 329 F→V 330 V→L |
| Region C | 386 L→V 392 F→L 393 L→V 404 L→I |
| Region D | 450 F→Y 454 A→G 459 I→V 463 Y→Q 465 M→F 470 V→A |
| Region E | 494 R→H 496 V→I 499 Q→L |

Notes S1 Steady-State kinetics of a coupled reaction.

Assuming that enzyme regeneration by the CPR is much quicker than product oxidation, which is reasonable under excess NADPH, we can assume we have a coupled reaction of the type:



Where S is 7-epiloganin, E is the enzyme, C_1 is the enzymatic complex of Enzyme and 7-epiloganin, C_2 is the enzymatic complex of enzyme and ketologanin, and P is the final product (either secoxyloganin or the Oleoside Methyl Ester). Applying the law of mass action:

$$\frac{dC_1}{dt} = v_{C1} = k_1SE - k_{-1}C_1 - k_2C_1 \quad (\text{Eq. S1.2})$$

$$\frac{dC_2}{dt} = v_{C2} = k_2C_1 - k_4C_2 \quad (\text{Eq. S1.3})$$

$$\frac{dP}{dt} = v_P = k_4C_2 \quad (\text{Eq. S1.4})$$

Assuming quasi-steady-state, $v_{C1} = 0$, and thus, substituting in Eq. S1.2:

$$v_{C1} = k_1SE - (k_{-1} + k_2)C_1 = 0$$
$$\frac{(k_{-1} + k_2)C_1}{k_1} = K_{MS}C_1 = SE \quad (\text{Eq. S1.5})$$

Where $K_{MS} = \frac{(k_{-1} + k_2)}{k_1}$; the Michaelis-Menten constant of epiloganin. By mass balance, the free enzyme equals the initial concentration, minus the complexed enzyme: $E = E_0 - C_1 - C_2$; then:

$$K_{MS}C_1 = SE_0 - SC_1 - SC_2$$
$$(K_{MS} + S)C_1 = SE_0 - SC_2$$
$$C_1 = \frac{SE_0}{(K_{MS} + S)} - \frac{SC_2}{(K_{MS} + S)} \quad (\text{Eq. S1.6})$$

Now, substituting in (Eq. S1.3):

$$v_{C2} = k_2 C_1 - k_4 C_2 = \frac{k_2 S E_0}{(K_{MS} + S)} - \frac{k_2 S C_2}{(K_{MS} + S)} - k_4 C_2$$

And substituting with (Eq. S1.4):

$$v_{C2} = \frac{k_2 S E_0}{(K_{MS} + S)} - \frac{k_2 S C_2}{(K_{MS} + S)} - v_P$$

Since we can measure v_P , the secoxyloganin or OME production rate, and v_{C2} , given that the enzyme and ketologanin dissociate during the methanol extraction, we can group:

$$v_{C2} + v_P = \frac{k_2 S E_0}{(K_{MS} + S)} - \frac{k_2 S C_2}{(K_{MS} + S)}$$

$$v_{C2} + v_P = \frac{k_2 S}{(K_{MS} + S)} (E_0 - C_2) = \frac{k_2 E_0 S}{(K_{MS} + S)} \left(1 - \frac{C_2}{E_0}\right)$$

Given the maximum velocity of C_2 formation is $V_S = k_2 E_0$, and by (Eq. S1.4) $C_2 = \frac{v_P}{k_4}$ then:

$$v_{C2} + v_P = \frac{V_S S}{(K_{MS} + S)} \left(1 - \frac{v_P}{k_4 E_0}\right)$$

$$\frac{v_{C2} + v_P}{\left(1 - \frac{v_P}{k_4 E_0}\right)} = \frac{V_S S}{(K_{MS} + S)}$$

Since we can measure the maximum velocity of secoxyloganin or OME formation by assaying microsomal preparations with ketologanin, then we can obtain $k_4 E_0$ as the $V_X = k_4 E_0$, as long as the initial amount of enzyme is the same in both experiments. Then:

$$\frac{v_{C2} + v_P}{\left(1 - \frac{v_P}{V_X}\right)} = \frac{V_S S}{(K_{MS} + S)} \quad (\text{Eq. S1.7})$$

Which is equivalent to (Eq. 1) in the main text, when $v_x = 0$, since in this model there is no dissociation of ketologanin from the enzyme complex.

Notes S2 Steady-State kinetics of a dissociative, sequential reaction.

Assuming that enzyme regeneration by the CPR is much quicker than product oxidation, which is reasonable under excess NADPH, we can assume we have a sequential reaction of the type:



Where S is 7-epiloganin, E is the enzyme, C_1 is the enzymatic complex of Enzyme and 7-epiloganin, X is ketologanin, C_2 is the enzymatic complex of enzyme and ketologanin, and P is the final product (either secoxyloganin or the Oleoside Methyl Ester). By mass balance:

$$S_0 = S + C_1 + X + C_2 + P$$

Then:

$$\frac{dS_0}{dt} = \frac{dS}{dt} + \frac{dC_1}{dt} + \frac{dX}{dt} + \frac{dC_2}{dt} + \frac{dP}{dt} = 0$$
$$v_S + v_{C_1} + (v_X + v_{C_2}) + v_P = 0 \quad (\text{Eq. S2.3})$$

Since we can measure v_P , and the sum of $(v_X + v_{C_2})$, given than the enzyme and ketologanin dissociate during the methanol extraction, we can group (Eq. S2.3) as:

$$(v_X + v_{C_2}) + v_P = -(v_S + v_{C_1}) \quad (\text{Eq. S2.4})$$

Applying the law of mass action:

$$v_S = -k_1SE + k_{-1}C_1 \quad (\text{Eq. S2.5})$$

$$v_{C_1} = k_1SE - k_{-1}C_1 - k_2C_1 \quad (\text{Eq. S2.6})$$

$$v_P = k_4C_2 \quad (\text{Eq. S2.7})$$

Then, adding (Eq. S2.5) + (Eq. S2.6):

$$(v_S + v_{C1}) = -k_2 C_1 \quad (\text{Eq. S2.8})$$

And substituting in (Eq. S2.4)

$$(v_X + v_{C2}) + v_P = -(v_S + v_{C1}) = k_2 C_1 \quad (\text{Eq. S2.9})$$

Assuming quasi-steady-state in the beginning of the incubation, $v_{C1} = 0$, and thus, substituting in Eq. S1.6:

$$\begin{aligned} v_{C1} &= k_1 S E - (k_{-1} + k_2) C_1 = 0 \\ \frac{(k_{-1} + k_2) C_1}{k_1} &= K_{MS} C_1 = S E \end{aligned} \quad (\text{Eq. S2.10})$$

Where $K_{MS} = \frac{(k_{-1} + k_2)}{k_1}$; the Michaelis-Menten constant of epiloganin. By mass balance, the free enzyme equals the initial concentration, minus the complexed enzyme: $E = E_0 - C_1 - C_2$; then:

$$\begin{aligned} K_{MS} C_1 &= S E_0 - S C_1 - S C_2 \\ (K_{MS} + S) C_1 &= S E_0 - S C_2 \\ C_1 &= \frac{S E_0}{(K_{MS} + S)} - \frac{S C_2}{(K_{MS} + S)} \end{aligned} \quad (\text{Eq. S2.11})$$

Now, substituting in (Eq. S2.9):

$$\begin{aligned} (v_X + v_{C2}) + v_P &= k_2 C_1 = \frac{k_2 S E_0}{(K_{MS} + S)} - \frac{k_2 S C_2}{(K_{MS} + S)} \\ (v_X + v_{C2}) + v_P &= \frac{k_2 S}{(K_{MS} + S)} (E_0 - C_2) = \frac{k_2 E_0 S}{(K_{MS} + S)} \left(1 - \frac{C_2}{E_0}\right) \end{aligned} \quad (\text{Eq. S2.12})$$

Given the maximum velocity of ketologanin formation is $V_S = k_2 E_0$, and by (Eq. S2.7) $C_2 = \frac{v_P}{k_4}$ then:

$$(v_X + v_{C2}) + v_P = \frac{V_S S}{(K_{MS} + S)} \left(1 - \frac{v_P}{k_4 E_0}\right)$$

$$\frac{(v_X + v_{C2}) + v_P}{\left(1 - \frac{v_P}{k_4 E_0}\right)} = \frac{V_S S}{(K_{MS} + S)}$$

Since we can measure the maximum velocity of secoxyloganin or OME formation by assaying microsomal preparations with ketologanin, then we can obtain $k_4 E_0$ as the $V_X = k_4 E_0$, as long as the initial amount of enzyme is the same in both experiments. Then:

$$\frac{(v_X + v_{C2}) + v_P}{\left(1 - \frac{v_P}{V_X}\right)} = \frac{V_S S}{(K_{MS} + S)} \quad (\text{Eq. S2.13})$$

Which equals (Eq. 1) in the main text and is practically indistinguishable from (Eq. S1.7), since we cannot determine which of the measured ketologanin is complexed and which is free.

Methods S1. Standard compound characterization

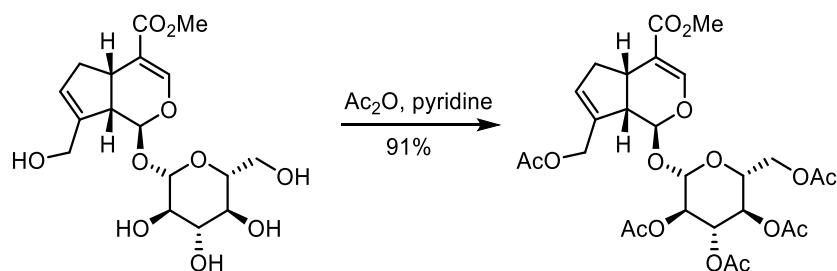
Stereochemistry confirmation of oleoside methyl ester

NMR spectra were measured on a 700 MHz Bruker Avance III HD (Bruker Biospin GmbH, Rheinstetten, Germany), equipped with a cryoplatfom and a 1.7 mm TCI cryoprobe. MeOH- d_3 was used as a solvent. NMR spectra were referenced to the residual solvent signals at δ_H 3.31 and δ_C 49.0. For spectrometer control and data processing Bruker TopSpin ver. 3.6.1 was used. For the selective NOESY experiment a mixing time of 600 ms was set and 4096 scans were accumulated. As transmitter frequency 1.785 ppm/1250.5 Hz was selected (methyl group adjacent to the exocyclic double bond in the oleoside methyl ester).

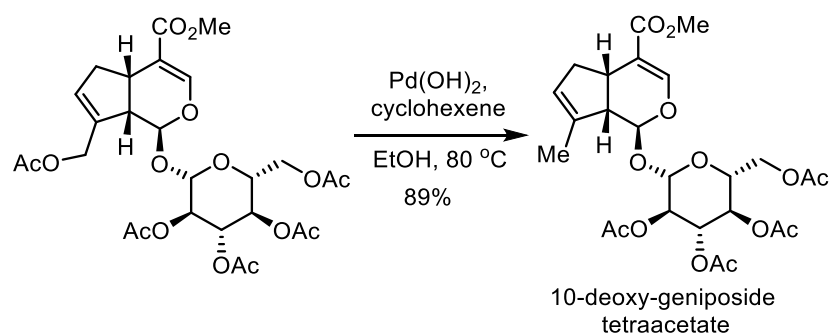
Characterization of synthesized compounds

NMR spectra were measured on a 400 MHz Bruker Avance III HD spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). CD₃OD was used as a solvent. NMR spectra were referenced to the residual solvent signals at δ_H 3.33 and δ_C 47.6. For spectrometer control and data processing Bruker TopSpin ver. 3.6.1 was used.

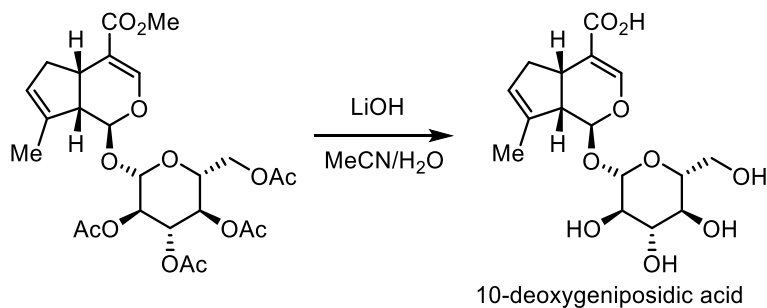
Methods S2. Chemical Synthesis of Iridoid Standards.



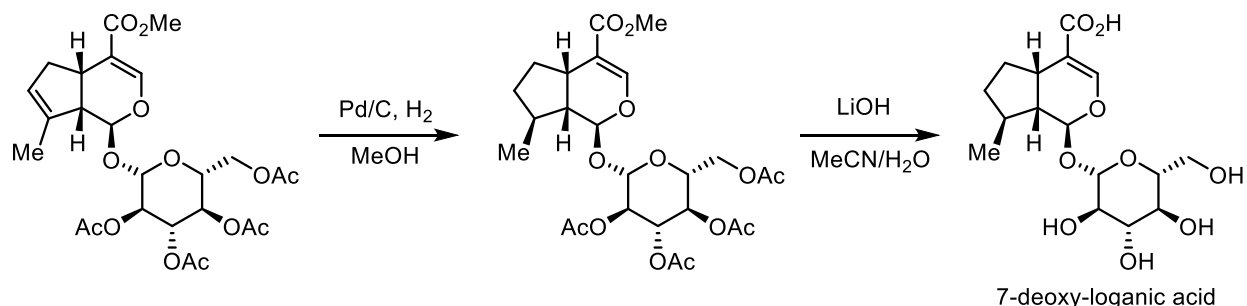
Geniposide (1g, 2.58 mmol, purchased from *Biosynth Carbosynth*.) was added to a mixed solvent of pyridine (6 mL) and acetic anhydride (3 mL), the mixed solution was stirred for 4 h at room temperature. Then the reaction mixture was concentrated in vacuum and purified by column chromatography (PE/EA = 1/1) to afford geniposide pentaacetate (1.4g, 91%). The spectroscopic data are in accordance with the literature values reported (Zhang *et al.*, 2013).



Geniposide pentaacetate (900 mg) and Pd(OH)₂/C (90 mg) was added to a mixed solvent of cyclohexene (1.25 mL) and EtOH (2.5 mL). The mixture was refluxed for 5 h and the catalyst was filtered off. The filtrate was concentrated in vacuum and purified by column chromatography (PE/EA = 2/1) to afford 10-deoxy-geniposide tetraacetate (720 mg, 89%). The spectroscopic data are in accordance with the literature values reported (Inoue *et al.*, 1992).

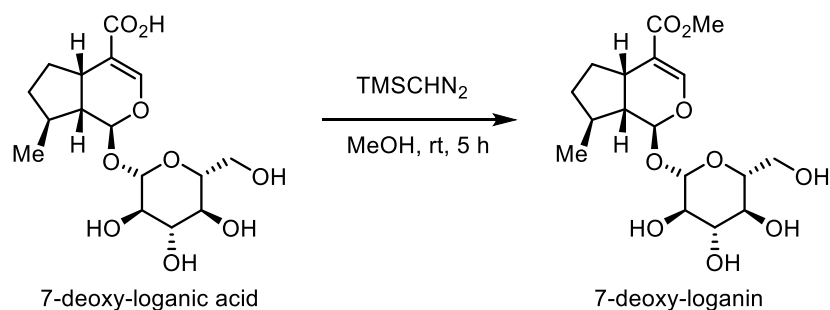


To a stirred solution of 10-deoxygeniposide tetraacetate (38 mg, 0.703 mmol) in MeCN (2.5 mL) and H₂O (1 mL) was added LiOH (25 mg, 1.06 mmol) at room temperature and the resulting mixture was stirred for 3 h at 40 °C. Then the reaction was purified by column chromatography (Dowex®50WX8-200 ion-exchange resin, deionized water) directly to afford 10-deoxygeniposidic acid (27 mg, quant.). The spectroscopic data are in accordance with the literature values reported (Inoue *et al.*, 1992).



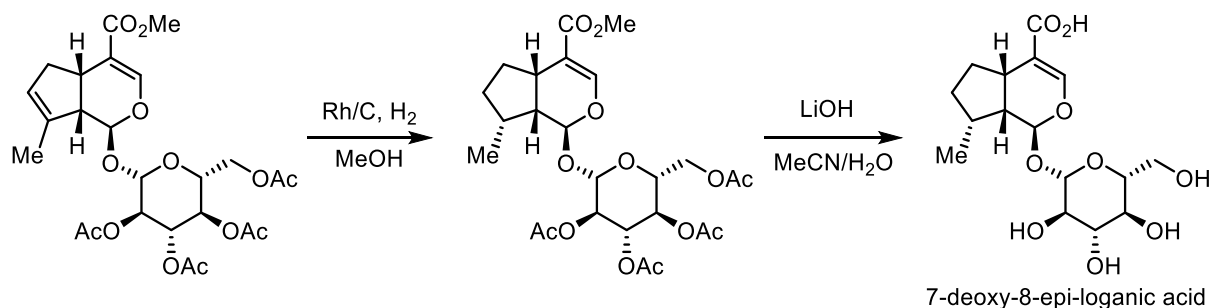
10-Deoxygeniposide (15 mg) was dissolved in MeOH (2 mL), Pd/C (1.5 mg, 5 mol%) was added and the flask was connected to a balloon filled with hydrogen and evacuated. After stirring for 1 h at room temperature under hydrogen atmosphere, the catalyst was filtered off and the solvent was evaporated. The resulted residue was used in next step directly.

To a stirred solution of above residue in MeCN (1 mL) and H₂O (0.4 mL) was added LiOH (10 mg, 0.417 mmol) at room temperature and the resulting mixture was stirred for 3 h at 40 °C. Then the reaction was purified by column chromatography (Dowex®50WX8-200 ion-exchange resin, deionized water) directly to afford 7-deoxy-loganic acid (10 mg, quant.). The spectroscopic data are in accordance with the literature values reported (Inoue *et al.*, 1992).



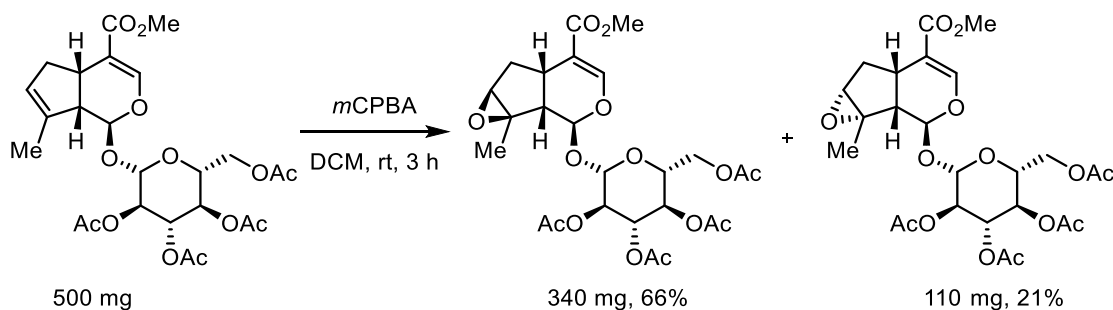
To a stirred solution of 7-deoxy-loganic acid (2.5 mg, 0.00694 mmol) in MeOH (1 mL) was added TMSCHN₂ (58 μL, 0.0348 mmol, 0.6 M in hexane) at room temperature and the resulting mixture

was stirred for 5 h at the same temperature. Then the reaction was purified by column chromatography (Dowex®50WX8-200 ion-exchange resin, deionized water) directly to afford 7-deoxy-loganin (2.2 mg, 85%). The spectroscopic data are in accordance with the literature values reported (Inoue *et al.*, 1992).



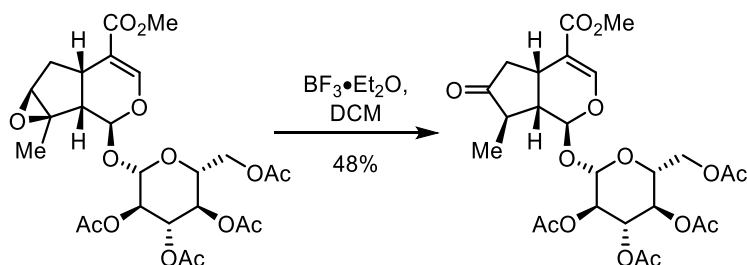
10-Deoxygeniposide (25 mg) was dissolved in MeOH (4 mL), Rh/C (5 mg, 5 mol%) was added and the flask was connected to a balloon filled with hydrogen and evacuated. After stirring for 2 h at room temperature under hydrogen atmosphere, the catalyst was filtered off and the solvent was evaporated. The resulted residue was used in next step directly.

To a stirred solution of above residue in MeCN (2.0 mL) and H₂O (1.0 mL) was added LiOH (17 mg, 0.692 mmol) at room temperature and the resulting mixture was stirred for 3 h at 40 °C. Then the reaction was purified by column chromatography (Dowex®50WX8-200 ion-exchange resin, deionized water) directly to afford 7-deoxy-8-*epi*-loganic acid (16 mg, 96%). The spectroscopic data are in accordance with the literature values reported (Nakamura *et al.*, 2000).

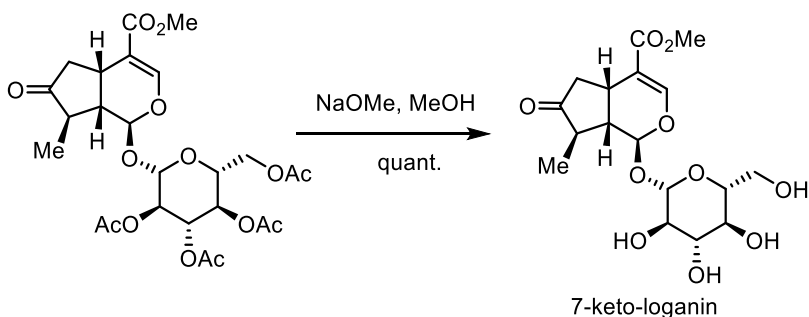


To a stirred solution of 10-deoxygeniposide tetraacetate (500 mg, 0.925 mmol) in DCM was added *m*CPBA (250 mg, 1.02 mmol) at 0 °C and the resulting mixture was stirred for 3h at room temperature. The reaction was quenched with 2N aqueous NaOH, extracted with DCM, dried over anhydrous Na₂SO₄ and concentrated in vacuum. The resulted residue was purified by

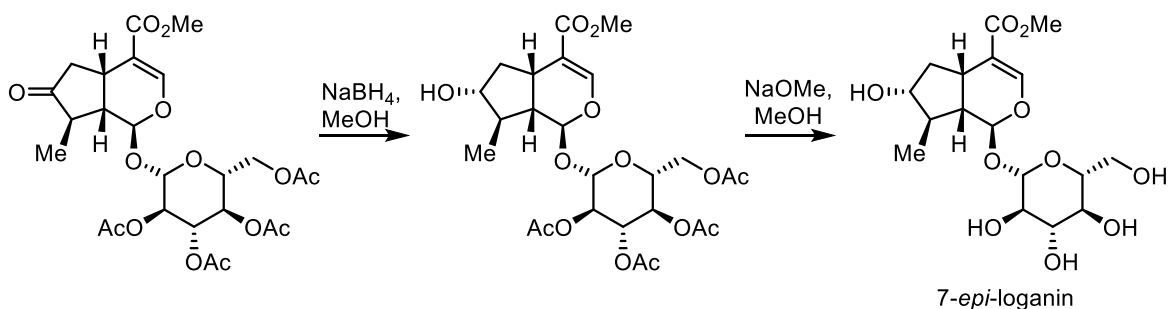
column chromatography (PE/EA = 4/1) to afford epoxide 1 (340 mg, 66%) and epoxide 2 (110 mg, 21%). The spectroscopic data are in accordance with the literature values reported (Inouye et al., 1970).



To a stirred solution of epoxide (50 mg, 0.0899 mmol) in DCM (2 mL) was added $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.2 mL) at room temperature and the resulting mixture was stirred for 10 min at the same temperature. The reaction was quenched with saturated NaHCO_3 , extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The resulted residue was purified by column chromatography (PE/EA = 3/1) to afford ketone (24 mg, 48%). The spectroscopic data are in accordance with the literature values reported (Inouye et al., 1970).

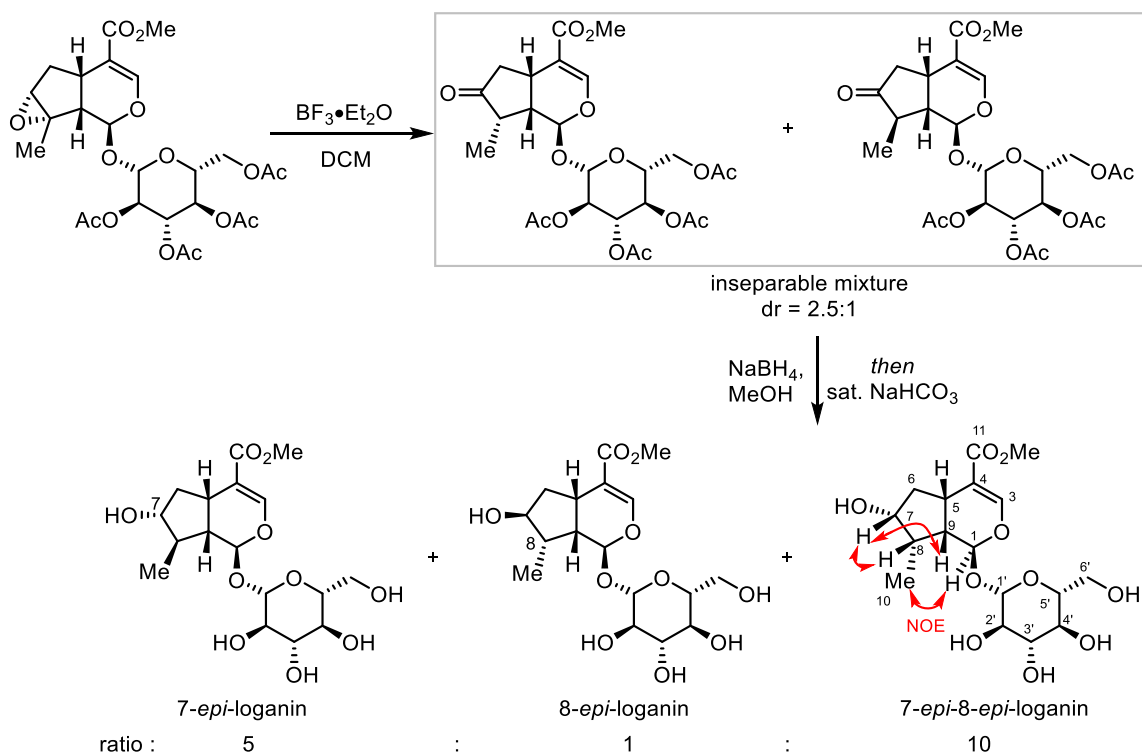


To a stirred solution of ketone (8 mg, 0.0144 mmol) in MeOH was added NaOMe (5.4 M, 2.7 μL , 0.0144 mmol) at room temperature and the resulting mixture was stirred for 3 h at the same temperature. The reaction was purified by column chromatography (Dowex®50WX8-200 ion-exchange resin, deionized water) directly to afford 7-keto-loganin (5 mg, 90%). The spectroscopic data are in accordance with the literature values reported (Gross *et al.*, 1986).



To a stirred solution of ketone (7 mg, 0.0126 mmol) in MeOH (1 mL) was added NaBH₄ (0.5 mg, 0.0126 mmol) at room temperature and the resulting mixture was stirred for 30 min at the same temperature. The reaction was quenched with saturated NaHCO₃, extracted with DCM, dried over anhydrous Na₂SO₄ and concentrated in vacuum. The resulted residue was used in the next step directly.

To a stirred solution of above residue in MeOH (1 mL) was added NaOMe (5.4 M, 2.3 μ L, 0.0126 mmol) at room temperature and the resulting mixture was stirred for 3 h at the same temperature. Then the reaction was purified by column chromatography (Dowex[®] 50WX8-200 ion-exchange resin, deionized water) directly to afford 7-*epi*-loganin (4.0 mg, 82%). The spectroscopic data are in accordance with the literature values reported (Itoh *et al.*, 2005).



To a stirred solution of epoxide (30 mg, 0.054 mmol) in DCM (4 mL) was added $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (160 μL) at room temperature and the resulting mixture was stirred for 10 min at the same temperature. The reaction was quenched with saturated NaHCO_3 , extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The resulted residue was used in the next step directly.

To a stirred solution of above residue in MeOH (2 mL) was added NaBH_4 (2 mg, 0.054 mmol) at room temperature and the resulting mixture was stirred for 30 min at the same temperature. The reaction was quenched with saturated NaHCO_3 (4 mL) and stirred overnight. Then the reaction was purified by column chromatography (Dowex[®]50WX8-200 ion-exchange resin, deionized water) to afford a mixture of *7-epi*-loganin, *8-epi*-loganin, and *7-epi-8-epi*-loganin (ratio = 5 : 1 : 10, 11 mg, 52% for two steps.).

Methods S3. NMR spectroscopical data for 7-*epi*-8-*epi*-loganin

Data of 7-*epi*-8-*epi*-loganin: ^1H NMR (400 MHz, CD_3OD) δ 7.43 (s, 1H, H-3), 5.66 (d, $J = 5.6$ Hz, 1H, H-1), 4.70 (d, $J = 7.9$ Hz, 1H, H-1'), 4.17 (dd, $J = 12.7, 5.7$ Hz, 1H, H-7), 3.89-3.84 (m, 1H), 3.69 (s, 3H, OMe), 3.68-3.61 (m, 1H), 3.39-3.34 (m, 1H), 3.24-3.17 (m, 3H), 2.84 (dd, $J = 18.0, 8.4$ Hz, 1H, H-5), 2.61 (ddd, $J = 14.2, 9.8, 7.4$ Hz, 1H, H-6), 2.22 (td, $J = 12.1, 6.6$ Hz, 2H, H-8, H-9), 1.42-1.32 (m, 1H, H-6), 1.10 (d, $J = 7.0$ Hz, 3H, H-10);

^{13}C NMR (100 MHz, CD_3OD) δ 168.2 (C-11), 151.2 (C-3), 112.2 (C-4), 98.5 (C-1'), 95.7 (C-1), 76.9 (C-7), 76.6, 74.0, 73.5, 70.3, 61.5 (C-2'-6'), 50.3 (OMe), 42.2 (C-9), 41.7 (C-8), 40.5 (C-6), 30.9 (C-5), 9.4 (C-10);

References

- Alagna F, Geu-Flores F, Kries H, Panara F, Baldoni L, O'Connor SE, Osbourn A. 2016.** Identification and characterization of the iridoid synthase involved in oleuropein biosynthesis in olive (*Olea europaea*) fruits. *J. Biol. Chem.* 291(11): 5542-5554.
- Damtoft S, Franzyk H, Jensen SR. 1993.** Biosynthesis of secoiridoid glucosides in Oleaceae. *Phytochemistry* 34 (5): 1291-1299.
- Gross G, Sticher O., Anklin C. 1986.** Ein neues Esteriridoidglycosid aus *Sambucus ebulus* L. (Caprifoliaceae). 1. Mitteilung über die Inhaltsstoffe der Zwergholunderwurzel. *Helv Chim Acta.* 69: 156-162.
- Inoue K, Ono M, Nakajima H, Fijie I, Inouye H, Fujita T. 1992.** Radioimmunoassay of Iridoid Glucosides: Part 1. General Method for Preparation of the Haptens and the Conjugates with a Protein of This Series of Glucosides. *Heterocycles.* 33(2):673-695.
- Inouye H, Yoshida T., Tobita S, Okigawa M. 1970.** Studies on monoterpene glucosides—IX: Chemical correlation between asperuloside and loganin. *Tetrahedron.* 26(16): 3905-3915.
- Inouye H, Ueda S, Inoue K, Takeda Y. 1971.** Über die biosynthese der oleuropein-typ-secoiridoidglucoside der Oleaceae. *Tetrahedron Lett.* 12 (43): 4073-4076.
- Itoh A, Kumashiro T, Yamaguchi M, Nagakura N, Mizushina Y, Nishi T, Tanahashi T. 2005.** Indole alkaloids and other constituents of *Rauwolfia serpentina*. *J Nat Prod.* 68(6): 848-52.
- Monk BC, Tomasiak TM, Keniya MV, Huschmann FU, Tyndall JD, O'Connell JD, Cannon RD, McDonald JG, Rodriguez A, Finer-Moore JS, et al. 2014.** Architecture of a single membrane spanning cytochrome P450 suggests constraints that orient the catalytic domain relative to a bilayer. *Proc Natl Acad Sci U S A.* 111: 3865-3870.
- Nakamura M, Kido K, Kinjo J, Nohara T. 2000.** Antinociceptive substances from *Incarvillea delavayi*. *Phytochemistry* 53(2): 253-256.
- Yamamoto H, Katano N, Ooi A, Inoue K. 2000.** Secologanin synthase which catalyzes the oxidative cleavage of loganin into secologanin is a cytochrome P-450. *Phytochemistry.* 53(1): 7-12.
- Zhang H, Shi T, Wang J, Li R, Tang W. 2013.** Protective effect of penta-acetyl geniposide on acute liver injury induced by D-galactosamine in mice. *Br. J. Pharmacol.* 4(6):256-261.