Supplementary Material:

Opuntisines, 14-membered cyclopeptide alkaloids from fruits of

Opuntia stricta var. dillenii isolated by high-performance
countercurrent chromatography

Frank Surup a,b *, Thu Minh Thi Tran c,f, Sebastian Pfütze, a,b Jarmo Budde c, Tamer E. Moussa-Ayoub d, Sascha Rohn e, Gerold Jerz c, *

a Helmholtz Centre for Infection Research, Department Microbial Drugs, Inhoffenstrasse 7, 38124 Braunschweig, Germany. phone: +49-531-6181-4256

b German Centre for Infection Research (DZIF), partner site Hannover-Braunschweig, 38124 Braunschweig, Germany.

c Institute of Food Chemistry, Technische Universität Braunschweig, Schleinitzstrasse 20, 38106 Braunschweig, Germany. phone: +49-531-391-7206, fax: - 7230

d Food Technology Department, Agriculture Faculty, Suez Canal University, 41522 Ismailia, Egypt.

e Institute of Food Chemistry, Hamburg School of Food Science, University of Hamburg, 20146 Hamburg, Germany.

f Food Technology and Biotechnology Department, Can Tho University of Technology, Can Tho, Vietnam.

* Corresponding authors:

 frank.surup@helmholtz-hzi.de

g.jerz@tu-braunschweig.de
* Chromatographic description and spectroscopic data of minor concentrated cyclopeptide alkaloids from *Opuntia stricta var. dillenii* 3 - 10

* Supplement Table S1 and Explanation of Parameter Calculation

Calculation of chromatographic parameters: 10 - 11


* Supplement Figure S1 11 – 12

Predicted $K_D$-values of cyclopeptide alkaloids determined by LC-ESI-MS.

*Supplement Figures S2-a – S2-g 13 – 19

1D/2D-NMR spectral analysis of Opuntisine A (1) in methanol-$d_4$.

*Supplement Figures S3-a – S3-g 20 – 26

1D/2D-NMR spectral analysis of Opuntisine B (2) in DMSO-$d_6$.

*Supplement Figures S4-a – S4-c 27 – 29

TOCSY NMR of (S)- and (R)-Mosher Esters of Opuntisine A (1) for determination of the absolute configuration of the secondary alcohol function at C-1.

*Supplement Figures S5 30 – 32

*Marfey* derivatization for determination of type and configuration of amino acids in 1 and 2.

*Supplement Figures S6 32 – 33

Fruit Analysis: Detection of Opuntisines by LC-ESI-MS in *Opuntia stricta var. dillenii* tissues.
Chromatographic description and spectroscopic data of minor concentrated cyclopeptide alkaloids from *Opuntia stricta* var. *dillenii*

*Efficiency and characterization of HPCCC separation of Opuntisines - LC-ESI-MS/MS data of unknown Opuntisine cyclopeptide metabolites*

The superior separation efficiency of preparative HPCCC for cyclopeptide alkaloids is seen in the results of very low chromatographic resolution of LC-ESI-MS analysis (cf. Suppl. Fig. S1) of the phase layer evaluation experiments. Specifically for the target ion \([\text{M+H}]^+\) at \(m/z\) 620, only two of the isobaric opuntisines were separated by analytical C18-chromatography. Applying the *off-line* ESI-MS injection approach where the preparative results of HPCCC separation were projected (cf. Fig. 1), 4 areas of isobaric metabolites (*1. 620-a, 2. 620-b and 620-c, 3. 620-d and 620-e, 4. 620-f*) were visualized by the changes in \(m/z\) 620 ion intensities of the gaussian shaped elution profile. The two-dimensional chromatography approach using LC-ESI-MS analysis securely detected in total 6 opuntisine A isobars. The results on some analysed HPCCC fractions (F33, F36, F42, F48, F54, F56 and F62) are displayed in Fig. 3a and Fig. 3b showing that the C18-LC-elution times of the separated very minor concentrated isobars are slightly different \((Rt\ 33-35\ min)\). In this specific case, HPCCC had demonstrated an excellent separation capacity to fractionate metabolite isobars (diastereomers) of \([\text{M+H}]^+\ m/z\ 620\).

The dehydroxy-derivative opuntisine B (2) with \([\text{M+H}]^+\) at \(m/z\ 604\) was recovered in the final extruded fractions of the HPCCC experiment. The missing OH-function at C-1 drastically increased the lipophilic character by strong affinity to the stationary organic CCC-phase (CCC-\(K_D\)-value above 64). Predicted \(K_D\) values of co-eluting opuntisine A isobars \((620-a \ – \ 620-f)\), and opuntisine B (2) were determined by LC-ESI-MS and are displayed in Suppl. Fig. S1.
Although, the isobars were nicely separated by HPCCC (CCC-$K_D$ range 1.16 – 14) (cf. Fig. 1), the LC-ESI-MS experiment could only determine the $K_D$-mean value of all co-eluting opuntisine A derivatives (LC-$K_D$ 1.32).

In certain cases, $K_D$-prediction by LC is not guiding to accurate values which are comparable to a real CCC experiment. LC-evaluation of metabolite distributions in CCC-solvent phases are normally using lower concentrations levels of valuable crude extracts than later applied to the real preparative CCC-fractionation. This fact is highly influencing the $K_D$-values as physicochemical effects in solution such as hydrogen-bonding might strongly alter results. This effect of dimerization with polarity masking of carboxyl-groups was already observed by Nernst in case of benzoic acid in two phase systems (Nernst, 1891) and later during CCC loading studies on *Hippophaë rhamnoides* extracts containing the phenolic acid protocatechuic acid (Gutzeit, Winterhalter & Jerz, 2007).

Overall, the elution sequence by of 1 (*620-a*), and 2 achieved by HPCCC was correctly predicted by LC-ESI-MS evaluation (cf. Suppl. Table S1).

**References:**


*Opuntisine A isomers (isobars) and unknown cyclopetide alkaloid metabolites*

Finally, the off-line 2D-chromatographic approach (HPCCC x LC-ESI-MS/MS) revealed the existence of very minor concentrated isobars of opuntisine A (1) (cf. Fig. 3a). As proof for the
molecular weights of all detected cyclopeptide isobars and unknown metabolites LC-ESI-MS in the negative ionization mode was done on specific HPCCC fractions (data cf. sections below).

*Opuntisine A isomers (isobars) Mr 619*

Interestingly, solely the isobars of 620-a and 620-b (opuntisine A, 1) with [M+H]^+ at m/z 620 revealed the MS/MS fragmentation to m/z 341. MS/MS of 620-c and 620-d led to m/z 343, and m/z 359 for 620-f, respectively (Fig. 3b). In common for all of the isobars was the base-peak fragment ion at m/z 602 with the neutral loss difference Δm/z 18, suggesting the cleavage of the hydroxy-functionality at C-1. A typical fragment ion for 620-a and 620-b (1) was m/z 489, and m/z 507 for the 620-b (1) until 620-f (cf. Fig. 3b). Unfortunately, the recovered amounts and purities of the fractions containing these minor concentrated metabolite isobars were not sufficient for further NMR-spectroscopical analysis. Very minor concentrated m/z 620 isobars were 620-c and 620-f (cf. Fig. 3a).

The isobars 620-c and 620-f resulted in a similar MS/MS-fragmentation pattern with a base peak ion at m/z 359.9 suggesting a similar constitution. Although, both components were recovered with a large elution volume difference (approx. ΔV 120 mL) from the respective HPCCC fractions, the two compounds revealed a very small retention time difference (ΔRt approx. 1 min) on C18-LC (cf. Fig. 3a). This was an excellent example for the complimentary separation characteristics of CCC-methodology resulting in a highly efficient seperation for potentially very similar cyclopeptide alkaloide structures. Molecular weights of all isobars (620-a to 620-f) with Mr 619 were ascertained by abundant [M-H]^− signals at m/z 618 using LC-ESI-MS in the negative ion mode. However, the MS/MS fragment ion pattern in the negative ion mode for the observed target molecules was only in some parts consistent. Concordant ion signals revealed different ion intensities. As additional indicator for a variation
in the molecular constitutions or stereochemical situation in the six isobars, the base peak ion signals were different. As options for a structural variation in the isobars, we see a variation of amino acid units such as the exchange of isoleucin against leucin, and a 1R-configuration of the secondary alcohol in the styrylamine unit. Also a cis-configuration in the hydroxy-prolin unit with 8S/9R-configuration could be discussed.

**Opuntisine Mr 647 (metabolite 648)**

A minor concentrated opuntisine was detected at smaller elution volumes (CCC-\(K_D\): 1.2-1.7) (Fig. 1) indicating a more polar compound from the cyclopeptide class. The even-number molecular ion signal \([M+H]^+\) at \(m/z\) 648, and the MS/MS-fragment ion \(m/z\) 342 was identical to 620-a and 620-b (1) suggested a more polar metabolite but structurally similar cyclopeptide.

**Opuntisines (metabolites 602-a, 602-b, 602-c)**

In the screening of fractions from HPCCC, the ESI-MS injection profile in the positive ionization mode detected two chromatographic sections with the ion signal \([M+H]^+\) at \(m/z\) 602 (cf. Fig. 1). LC-ESI-MS analysis of respective HPCCC fractions revealed that the lower elution volume area (\(K_D\) 1.16-1.58) presented two cyclopeptide alkaloids whereas metabolite 602-a (Rt 35.6 min) resulted in the \([M-H]^-\) signal at \(m/z\) 600, and therefore the \(Mr\) 601 was confirmed. The second signal (602-b, Rt 36.2 min) was not ionized, and therefore the \(Mr\) was tentative. In case of 602-c recovered from HPCCC (\(K_D\) 3.05-4.65), a single molecule was detected in LC-ESI-MS (neg. mode) with \([M-H]^\) at \(m/z\) 618 and also the respective ion signal \([M-H+Cl]^\) at \(m/z\) 654/656 (Rt 33.4 min) indicating the real molecular weight \(Mr\) 619, whereas a strong in-source fragmentation in the positive mode might cause the neutral loss of water from an existing hydroxyl-function.

In case of 602-c we tentatively suggest the dehydro-form of opuntisine B (2) with a double bond at C-1/2. This could be indicated by MS/MS fragment ions at \(m/z\) 584, 489 and 342 which
are $\Delta m/z$ 2 mass units lower than observed for 2 (Fig. 1). However, the lipophilic character of 602-c on HPCCC (CCC-$K_D$: 2.8-2.9) was not as strong as observed for opuntisine B (2) (Fig. 1). Unfortunately the fraction amount was not sufficient to perform NMR analysis.

**Opuntisines Mr 689 (metabolites 690-a/ 690-b)**

In the ESI-MS profile (pos. mode) of HPCCC fractions two recovery sections for the selected ion trace $[M+H]^+$ at $m/z$ 690 (CCC-$K_D$: 0.73-0.95; 690-b: 3.82-14) were observed (Fig. 1). The MS/MS fragment spectra of the isobars were completely different, but 690-b displayed the identical base peak $m/z$ 406, as already observed for metabolite 656 (Fig. 1).

**Opuntisine Mr 655 (metabolite 656)**

This cyclopeptide metabolite with $[M+H]^+$ at $m/z$ 656 had shown a similar elution $K_D$-range (CCC-$K_D$: 2.8-3.8) as 602-c. The base peak MS/MS fragment ion was $m/z$ 406 and identical to 690-b (Fig. 1). The indicative MS/MS-fragment ions in the positive ESI ion mode as seen for opuntisine A and B were absent.

**Spectroscopical data of unknown Opuntisine cyclopeptide alkaloid metabolites (cf. Fig. 1, Fig. 3a/3b)**

**Opuntisine (metabolite 648, Mr 647):**

HPCCC F28-F38 ($V_R$ 140-190 mL, $K_D$ range: 1.16 – 1.69), LC-ESI-MS (altern. +/-) from HPCCC fraction 36 (Rt 33.9 min), $[M-H]^{-}$: $m/z$ 646.3, MS/MS: $m/z$ 602 (10), 535.2 (100), 440.0 (20), 387.9 (20); off-line ESI-MS (+) from HPCCC $[M+H]^+$: $m/z$ 648.3, MS/MS: $m/z$ 630.3, 535.1, 455.0, 387.9, 341.9, 323.9, 185.9.

**Isobars/ isomers of Opuntisine A (metabolites 620-a, 620-c – 620-f) characterized by LC-ESI-MS/MS (Fig 3a and 3b):**
620-a (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F33 (Rt 33.0 min), [M-H]: m/z 618.3, MS/MS 600.3 (100), 582.2 (8), 524.1 (7), 505.1 (3), 489.1 (11), 420.0 (6), 394.0 (36), 392.0 (20), 376.0 (3), 357.9 (3), 341.9 (1), 263.8 (16), [M-H+Cl]: m/z 654/656; [M-H+HCOOH]: m/z 664. [M+H]^+: m/z 620.3, MS/MS: m/z 602.2 (100), 584.3 (15.5), 574.3 (5.3), 489.2 (5.2), 359.9 (14.4), 341.9 (62.9), 260.9 (4.0), 232.9 (8.5).

620-c (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F36 (Rt 34.4 min), [M-H]: m/z 618.3, MS/MS 600.3 (5), 574.2 (3), 507.2 (25), 489.1 (100), 422.0 (32), 394.0 (84), 376.0 (35), 265.8 (32), [M-H+Cl]: m/z 654/656; [M-H+HCOOH]: m/z 664. [M+H]^+: m/z 620.3, MS/MS: m/z 602.2 (73), 507.1 (11.3), 359.9 (100), 342.9 (11.5), 222.8 (9.0).

620-d (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F54 (Rt 33.1 min), [M-H]: m/z 618.3, MS/MS 600.3 (90), 574.2 (4), 557 (7), 535.2 (45), 523.2 (35), 507.2 (85), 438.1 (60), 412.1 (90), 410.0 (100), 394.0 (25), [M-H+Cl]: m/z 654/656; [M-H+HCOOH]: m/z 664. [M+H]^+: m/z 620.3, MS/MS: m/z 602.3 (52.4), 507.1 (15.0), 465.1 (3.5), 343.9 (91.0), 326.9 (12.7), 206.9 (8.0).

620-e (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (Rt 33.8 min), [M-H]: m/z 618.3, MS/MS 600.3 (15), 574.2 (3), 535.2 (20), 523.2 (25), 507.2 (40), 482.2 (100), 438.1 (15), 412.1 (15), 410.0 (40), 394.0 (10), [M+H]^+: m/z 620.3, MS/MS: m/z 602.3 (9.4), 507.1 (9.4), 465.1 (1.7), 343.9 (100), 326.9 (14.9), 308.9 (4.1), 206.9 (9.2).

620-f (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F62 (Rt 35.4 min), [M-H]: m/z 618.3, MS/MS: m/z 600.3 (35), 574.3 (10), 535.2 (20), 523.2 (25), 507.2 (70), 482.1 (5), 438.1 (20), 412.0 (100), 410 (80), 394.0 (20), 376.0 (10); [M-H+Cl]: m/z 654/656; [M-H+HCOOH]: m/z 664. [M+H]^+: m/z 620.3, MS/MS: m/z 602.3 (35.7), 507.1 (6.7), 449.1 (7.8), 421.0 (1.2), 359.9 (100), 342.9 (3.0), 319.9 (10.0), 206.9 (4.6).

Opuntisine (metabolites 602-a – 602-c):

602-a (Mr 601): LC-ESI-MS (altern. +/-) from HPCCC fraction F33 (Rt 35.6 min), [M-H]: m/z 600.3, MS/MS: 582.2 (15), 489 (5), 420 (17), 394.0 (100), 377.9 (35), 265.8 (65). [M+H]^+: m/z 620.3, MS/MS: 600.3 (100), 582.2 (8), 524.1 (7), 505.1 (3), 489.1 (11), 420.0 (6), 394.0 (36), 392.0 (20), 376.0 (3), 357.9 (3), 341.9 (1), 263.8 (16).
m/z 602.3, MS/MS: m/z 584.2 (7.2), 566.2 (12.0), 557.3 (14.5), 491.1 (1.0), 455.1 (5.7), 396.0 (77.9), 350.9 (100), 284.9 (16.9).

**602-b (tentative Mr 601):** LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (Rt 36.2 min), [M-H]−: no ionization. [M+H]+: m/z 602.3, MS/MS: m/z 585.3 (18.6), 567.3 (42.6), 523.2 (5.1), 479.1 (15.0), 435.1 (9.1), 391.1 (32.8), 347.0 (12.6), 302.9 (35.4), 258.9 (50.3), 240.9 (7.8), 214.9 (100).

**602-c (Mr 619):** HPCCC F53-F58 (V_R 265-290 mL, K_D range: 3.05-4.65), LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (Rt 33.4 min), [M-H]−: m/z 618.3, MS/MS: m/z 654/656, MS/MS: m/z 618.3 (10), 482.1 (50), 273.8 (2). [M+H]+: m/z 602, MS/MS: m/z 584.2 (52.5), 574.2 (6.4), 557.3 (4.3), 490.1 (4.0), 455.1 (7.7), 341.9 (100), 260.8 (9.3), 232.9 (19.2).

**Opuntisine (metabolite 690-a, Mr 689):**
HPCCC F20-F24 (V_R 100-120 mL, K_D range: 0.73 – 0.95), LC-ESI-MS MS (altern. +/-) from HPCCC fraction F22 (Rt 36.5 min), [M-H]−: no ionization. [M+H]+: m/z 690.4, MS/MS: see below; off-line ESI-MS (+) from HPCCC [M+H]+: m/z 690, MS/MS: m/z 673.4, 655.4, 611.4, 549.1, 523.3, 479.2, 435.1, 411.0, 391.1, 347.0, 302.9, 258.9, 214.9.

**Opuntisine (metabolite 690-b, Mr 689):**
HPCCC F56-F64 (V_R 280-320 mL, K_D range: 3.82 – 14.0), LC-ESI-MS (altern. +/-) from HPCCC fraction F62 (Rt 33.6 min), [M-H]−: m/z 602.3 (40), 575.2 (5), 421.0 (5), 507.2 (10), 359.9 (100). [M+H]+ m/z 690.3, MS/MS: m/z 430.1 (10), 406.0 (100), 388.0 (10), 292.9 (10); off-line ESI-MS (+) from HPCCC [M+H]+: m/z 648, MS/MS: m/z 527.12, 430.0, 405.9, 388.0, 292.8.

**Opuntisine (metabolite 656, Mr 655):**
HPCCC F52-F56 (V_R 260-280 mL, K_D range: 2.80 – 3.82), LC-ESI-MS (altern. +/-) from HPCCC fraction F53 (Rt 32.7 min), [M-H]−: m/z 654.4, MS/MS: m/z 636.3 (15), 548.2 (5),
421.0 (10), 394.0 (100) 248.9 (10). [M+H]$^+$ m/z 656.4, MS/MS: m/z 493.1 (7), 406.0 (100), 388.0 (15), 258.9 (20); off-line ESI-MS (+) from HPCCC [M+H]$^+$: m/z 656, MS/MS: m/z 569.2, 493.2, 406.0, 387.9, 281.8, 258.9.

**Supplement Table S1.**

Calculation of chromatographic parameters of opuntisine A (1) and B (2) from the HPCCC experiment.

The metabolite elution times of HPCCC were converted over identified elution/retention volumes $V_R$ into their respective partition ratio values $K_D$ (cf. equations 1 - 7). The $V_R$ values for the elution of the respective cyclopeptides were determined with high accuracy by the off-line ESI-MS injection experiment (cf. Fig. 1). The calculation of elution/retention volumes $V_R$, stationary phase retention $S_F$, partition ratio values $K_D$ is described by the following equations:

**Retention volume $V_R = \text{elution time [min]} \times \text{flow rate [mL/min]} \quad (1)**

The value for $S_F$ of the used solvent system was determined by equation (3) using $V_C$ (125 mL), and $V_M$ (37.63 mL) resulting in a $S_F$ value of 69.9 %.

$$V_S = (V_C - V_M) \quad (2) \quad S_F = V_S / V_C \quad (3)$$

The $S_F$-value was re-calculated by use of the extra column volume $V_{Ext}$ (7 mL) existing for the connecting periphery tubings in the HPCCC set-up, using equations (2 - 5)

**corrected $V_M = V_M - V_{Ext} = 37.63 \text{ mL} - 7 \text{ mL} = 30.63 \text{ mL} \quad (4)**

**Corrected $V_S = V_C - \text{corrected } V_M = 94.4 \text{ mL} \quad (5)**

The stationary phase retention factor $S_F$ was re-calculated as 75.5% (corrected value), and is the real percentage of stationary phase being present on the system during the HPCCC-experiment. A high $S_F$-value directly correlates to a higher resolution and efficiency in a CCC-separation.

**corrected $S_F = \text{corrected } V_S / V_C \quad (6)**

For the elution-mode, the metabolite and solvent system specific partition ratio $K_D$ values were calculated by the equation (7).

**corrected $K_D = (V_R - \text{corrected } V_M) / \text{corrected } V_S \quad (7)**

From the begin of single solvent phase recovery in the extrusion-mode (cf. Fig. 2: F48, $V_M = 240 \text{ mL}$), equation (8) will be applied:
Corrected $K_D = V_{CM} / (V_{CM} + V_C - V_R)$ \hspace{1cm} (8)

$V_S$: retained experimental stationary phase volume
$V_C$: coil column volume/capacity
$V_M$: volume of mobile phase take up to the coil at equilibrium of HPCCC
$S_F$: stationary phase retention
$K_D$: partition ratio
$V_{CM}$: volume of switch volume to extrusion (end of classical mode elution)
$V_{ext}$: external periphery volume

Corrected chromatographic CCC values $V_M$, $V_S$, $K_D$: re-calculation by use of external volume ($V_{ext}$) of periphery

| No. | Selected ion traces compounds $m/z$: [M+H]$^+$ | Prediction of partition ratio $K_D$ by LC-ESI-MS $K_D$ = Area (upper layer) / Area (lower layer) | HPCCC Peak range Tube fractions Retention volume $V_R$ ~ [mL] $K_D$ range $K_D$ width Mean value | $\Delta K_D$ = Predicted $K_D$ – HPCCC $K_D$ |
|-----|---------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 1   | $m/z$ 620                                     | 1.32 Derivatives 1a – 1f                         | F36 – F52 / 180 - 260                              | 1.58 – 2.80                                     | 2.19                                            | 0.87 |
|     | and 620                                      |                                                   | only derivative 1b                                 |                                                  |                                                 |      |
|     | a – f                                        |                                                   |                                                  |                                                 |                                                 |      |
| 2   | $m/z$ 604                                     | 96                                               | F64 - F66 / 320 - 330                              | 14 – 42                                         | 28                                              | 68   |

**Supplement Figure S1:**

Predicted $K_D$-values of cyclopetide alkaloids determined by LC-ESI-MS (pos. ionization), and calculated from integrated areas of selected ion traces in the HPCCC solvent system phase layers by equation ($K_D = A_{upper}/ A_{lower}$) (Ito, 1996).

*Predicted partition ratio* $K_D = \frac{\text{Area selected single ion trace (upper layer)}}{\text{Area selected single ion trace (lower layer)}}$ $K_D = A_{upper}/ A_{lower}$
**Target cyclopeptide alkaloids**

**LC-prediction**

\[ K_D (620) = 1.32 \]

\[ K_D (604) = 96 \]

**CCC-experiment**

\[ K_D (620) = 1.16 - 14 \]

\[ K_D (604) = > 14 \]
Supplement Figures S2–a: $^1$H NMR spectrum (700 MHz, methanol-$d_4$) of opuntisine A (1).
Supplement Figures S2–c: COSY NMR spectrum (700 MHz, methanol-$d_4$) of opuntisine A (1).
Supplement Figures S2–d: TOCSY NMR spectrum (700 MHz, methanol-$d_4$) of opuntisine A (1).
Supplement Figures S2–e: ROESY NMR spectrum (700 MHz, methanol-\(d_4\)) of opuntisine A (1).
Supplement Figures S2–f: HSQC-DEPT NMR spectrum (700 MHz, methanol-$d_4$) of opuntisine A (1).
Supplement Figures S2–g: HMBC NMR spectrum (700 MHz, methanol-\textit{d}_4) of opuntisine A (1).
Supplement Figures S3 – a: $^1$H NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S3 – b : $^{13}$C NMR spectrum (125 MHz, DMSO-d$_6$) of opuntisine B (2).
Supplement Figures S3 – c : COSY NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S3 – d: TOCSY NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S3 – e:
ROESY NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S3 – f: HSQC-DEPT NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S3 – g: HMBC NMR spectrum (700 MHz, DMSO-$d_6$) of opuntisine B (2).
Supplement Figures S4 – a: TOCSY NMR spectrum (700 MHz, pyridine-\textit{d}_5) of opuntisine A (1).
Supplement Figures S4 – b : TOCSY NMR spectrum (700 MHz, pyridine-$d_5$) of the $(S)$-MTPA ester of opuntisine A (1).
Supplement Figures S4 – c: TOCSY NMR spectrum (700 MHz, pyridine-$d_5$) of the ($R$)-MTPA ester of opuntisine A (1).
Supplement Figures S5: Marfey derivatization for determination of type and configuration of amino acids in 1 and 2.

Detection of L-isoleucine; a) hydrolysate of 1 after derivatisation with FDAA; b) FDAA derivative of d-allo-isoleucine standard, c) FDAA derivative of D-isoleucine standard, d) FDAA derivative of L-allo-isoleucine standard, e) FDAA derivative of L-isoleucine standard.

Detection of L-glutamate; a) hydrolysate of 1 after derivatisation with FDAA; b) FDAA derivative of D-glutamate and c) L-glutamate standards, respectively.

Detection of L-phenylalanin; a) hydrolysate of 1 after derivatisation with FDAA; b) FDAA derivative of D-phenylalanin and c) L-phenylalanin standards, respectively.
Supplement Figures S5: *Marfey* derivatization for determination of type and configuration of amino acids in 1 and 2.

Detection of L-isoleucine; a) hydrolysate of 2 after derivatisation with FDAA; b) FDAA derivative of D-allo-isoleucine standard, c) FDAA derivative of D-isoleucine standard, d) FDAA derivative of L-allo-isoleucine standard, e) FDAA derivative of L-isoleucine standard.

Detection of L-glutamate; a) hydrolysate of 2 after derivatisation with FDAA; b) FDAA derivative of D-glutamate and c) L-glutamate standards, respectively.
Detection of L-phenylalanin; a) hydrolysate of 2 after derivatisation with FDAA; b) FDAA derivative of D-phenylalanin and c) L-phenylalanin standards, respectively.
Supplement Figure S6: Fruit Analysis: Detection of Opuntisines in fruit tissues

a. HPLC-UV/VIS chromatogram (190-600 nm) of pulp extract; b. HPLC-extracted ion mass (620.3 Da) chromatogram of pulp extract; c. HPLC-ESI-extracted ion (604.3 Da) chromatogram of pulp extract; d. HPLC-UV/VIS chromatogram (190-600 nm) of seeds; e. HPLC-extracted ion mass (620.3 Da) chromatogram of seed extract; f. HPLC-extracted ion mass (604.3 Da) chromatogram of seed extract; g. HPLC-UV/VIS chromatogram (190-600 nm) of peel extract; h. HPLC-extracted ion mass (620.3 Da) chromatogram of peel extract; i. HPLC-extracted ion mass (604.3 Da) chromatogram of peel extract.