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Phytochemical meanings of tetrahydro-β-carboline moiety in strictosidine derivatives

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ABSTRACT

Synthesis of 13 different tetrahydro-β-carbolines (THBC) was accomplished by applying the Pictet–Spengler reaction with seven aldehydes, which have been coupled with tryptamine (**6**) and L-tryptophan methyl ester (**7**), respectively. The resulting products represent analogues of strictosidine (**1**) and carboxystrictosidine (**5**). They were investigated with respect to possible effects on herbivores in feeding bioassays upon the generalist *Spodoptera littoralis*. Maximum inhibition averages were 42% after four and 46% after six days for the most effective product (**19**) at 1000 ppm. Additionally, the frass of this particular bioassay was investigated via HPLC-UV for THBC digestion. All synthesized THBCs were also tested for their radical scavenger activity by monitoring their interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH). Compounds **16**–**20**, **24** and **25** exhibited radical scavenging activity, ranging from 50% to 74% compared to that of α-tocopherol. All results were discussed with respect to possible contributions of tetrahydro-β-carboline moieties in bioactivities of strictosidine (**1**) and its biodegradation products.

1. Introduction

The huge group of monoterpene indole alkaloids (MIAs) contains more than 2500 different substances, which have mostly been isolated from the families Loganiaceae, Apocynaceae and Rubiaceae.[1] Many of these natural products show high biological activities, which are often revealing their function *in planta*.[1] MIAs are hence interesting candidates for pharmacological purposes.[2] Most popular examples are vinblastine and vincristine as well as ajmalicine, all isolated from *Catharanthus roseus* (L.) G. Don, which are potent anti-cancer drugs and used against hypertension. Ajamaline, another MIA from *Rauvolfia sepentina* (L.) Benth. ex Kurz (both Apocynaceae) is applied in cases of cardiac arrhythmia.[3]

A wide variety of MIAs have been reported in different species of the genus *Psychotria* L., a member of the *Rubiaceae* family.[4] Plenty of those MIAs belong to the group of tryptamine-iridoid alkaloids bearing a tetrahydro-β-carboline moiety (THBC, 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole). Its most widespread representative is strictosidine (**1**, Scheme 1), generated during the strictosidine synthase (STR, E.C. 4.3.3.2) catalysed Pictet–Spengler condensation of tryptamine (**6**) and secologanin.[5] Strictosidine (**1**) is a highly functionalised key intermediate in the biosynthesis of several other tryptamine-iridoid alkaloids.[6] Moreover, even strictosidine (**1**) itself shows antifungal activity[7] and its decomposition and biodegradation products are assumed to be involved in plant defence mechanisms.[7] At an herbivore attack, where cell compartments are destroyed, the strictosidine (**1**) stock from the vacuoles uncontrollably meets the strictosidine-β-D-glucosidase (SGD, E.C. 3.2.1.105) from the nucleus. The hence catalysed deglycosylation leads to strictosidine aglycon (**2**) which very rapidly converts to a dialdehyde (**3**), as shown in Scheme 1. Multiple tests show this reactive intermediate and follow up products to possess various biological potentials, like antifungal and protein cross-linking activities.[7,8] Many efforts have been made identifying involved compounds and enzymes in the compartments of the plants.[9]

Oleuropein (**4**), isolated from leaves of *Ligustrum obtusifolium* Siebold & Zucc. (Oleaceae), also comprises a secologanin-moiety and is a described subject to comparable biodegradation (Fig. 1). The resulting products show similar protein-crosslinking activity and have an important contribution to the defence mechanism of the plant.[10] Strictosidine (**1**), however, contains the tetrahydro-β-carboline moiety, which differs from oleuropein (**4**) and its phenolic moiety. Consequently, the phytochemical meaning of this THBC moiety has not yet been described in detail.

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; MIA, monoterpene indole alkaloid; THBC, tetrahydro-β-carboline.

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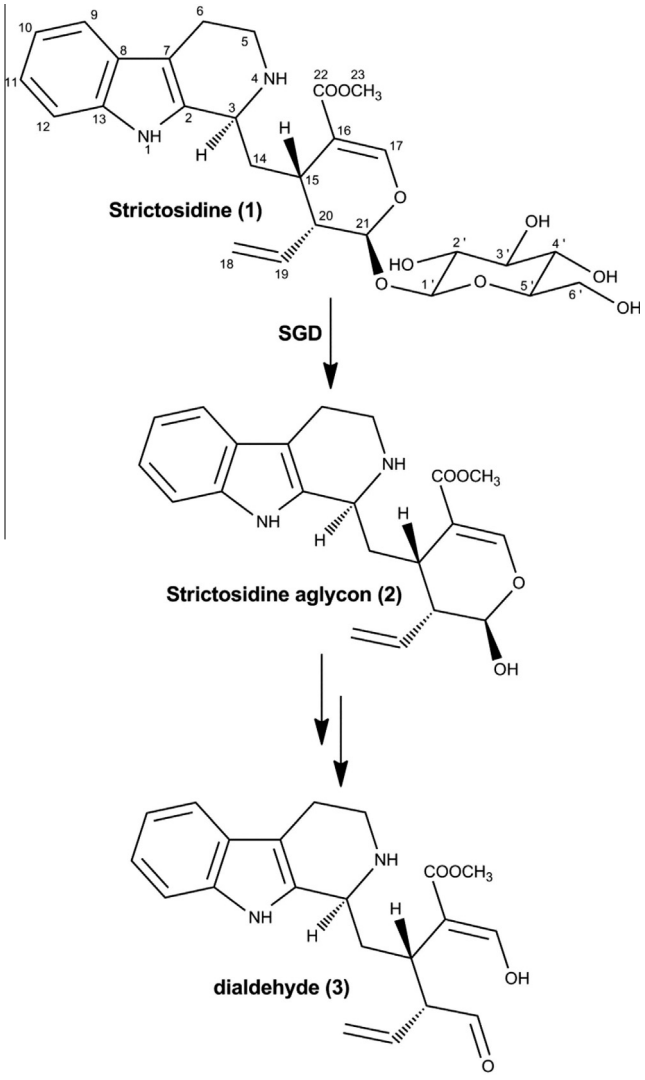

Scheme 1. Strictosidine (**1**) biodegradation induced by strictosidine β-D-glucosidase (SGD, E.C. 3.2.1.105), which catalyses deglycosylation. The intermittently occurring strictosidine aglycon (**2**) further converts to a dialdehyde (**3**), which is a chemical reactive agent in plant defence mechanism. The THBC moiety is not involved in this reaction cascade.

Hence we synthesized a small library of THBC derivatives using the Pictet–Spengler reaction. Resulting compounds (**15**–**27**) mimicked this part of the strictosidine aglycon (**2**), but did not carry reactive aldehyde groups. Their bioactivity was tested towards possible growth-retarding effects on the herbivore *Spodoptera littoralis*, as well as their potential ability as a radical scavenger. These two investigated bioactivities represent the most probable tasks of strictosidine (**1**) itself in its natural environment. The results provided several details about the meaning of the THBC moiety in strictosidine (**1**) and its decomposition products.

2. Results and discussion

First goal was the synthesis of strictosidine analogues, being tetrahydro-β-carboline (THBC) derivatives suitable for the bioactivity tests. Apart from the tricyclic THBC structure present in all compounds, different moieties replace the skeleton of the dialdehyde (**3**) which derived from the deglycosylated secologanin. These residues should not possess any significant own bioactivity and remain chemically stable. Hence mainly aliphatic and aromatic moieties were used (Scheme 2), but additionally one heterocyclic pyran skeleton was introduced. Although such structural range was rather narrow, it allowed determining the influence of these residues on bioactivities of the THBC groups. In an additional group of compounds a methoxycarbonyl group (–$COOCH_3$) was introduced at position 5. These latter compounds mimic carboxystrictosidine (**5**) compounds (Fig. 1), which also occur in species of the genus *Psychotria*.[4a,11]

2.1. Synthetic approach

To obtain the desired THBC derivatives the Pictet–Spengler reaction was utilised.[12] In the applied procedure the amine was suspended in dichloromethane and afterwards the respective aldehyde and trifluoracetic acid were added.[13] The reaction was performed with tryptamine (**6**) or L-tryptophan methyl ester (**7**), to gain the strictosidine (**1**) or carboxystrictosidine (**5**) analogues, respectively. L-Tryptophan methyl ester (**7**) was gained by esterification of L-tryptophan in high yields without significant N-methylation.[14] The seven aldehydes introducing the different side chains in position 3 were commercially available and led to the formation of **13** THBC derivatives, as shown in Scheme 2. All products were purified by column chromatography.

The achieved yields performing the Pictet–Spengler reaction ranged distinctly between 11% and 83%, mostly dependent on the applied aldehyde (Table S1). Yield differences between the use of

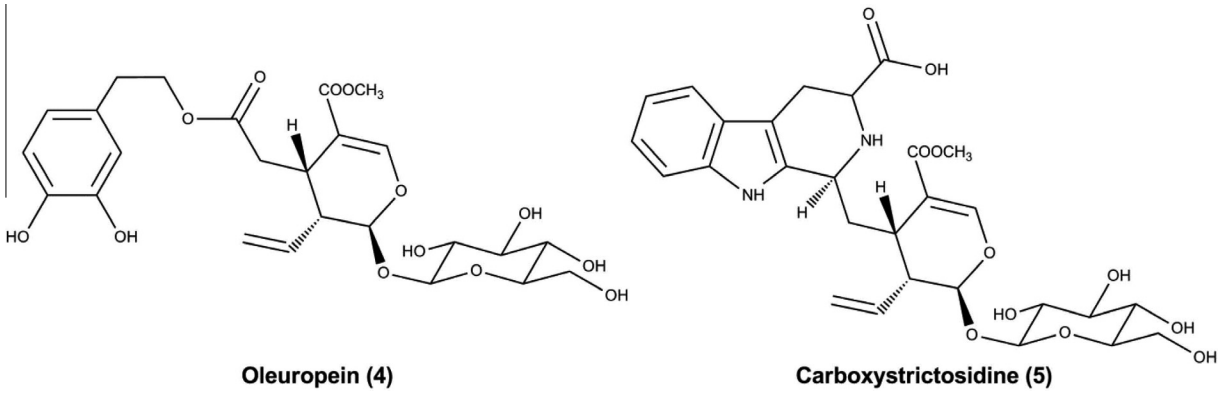

Figure 1. Oleuropein (**4**) from *Ligustrum obtusifolium* as a representative substance for being essentially involved in the defence mechanism of the plant.[10] The similarity to strictosidine (**1**) and carboxystrictosidine (**5**) lies in the secologanin moiety, which is therefore assumed to be responsible for possible biological activities within the *Psychotria* genus.

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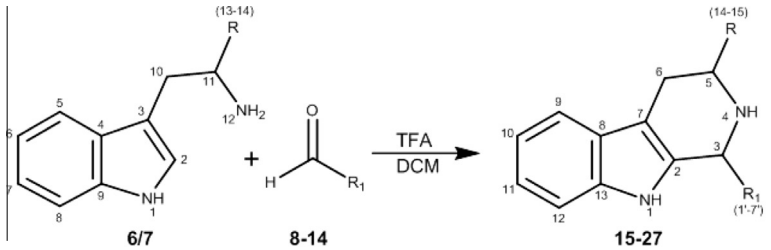

	R
6	-H
7	-$COOCH_3$

	R_1
8	-C_6H_5
9	-C_6H_{11}
10	-CH_2CH_3
11	-$CH_2CH(CH_3)_2$
12	-$CH_2C_6H_5$
13	-$(CH_2)_5CH_3$
14	-Methyltetrahydro-pyran-4-yl

	R	R_1
15/22	-H/-$COOCH_3$	-C_6H_5
16/23	-H/-$COOCH_3$	-C_6H_{11}
17/24	-H/-$COOCH_3$	-CH_2CH_3
18/25	-H/-$COOCH_3$	-$CH_2CH(CH_3)_2$
19/26	-H/-$COOCH_3$	-$CH_2C_6H_5$
20/27	-H/-$COOCH_3$	-$(CH_2)_5CH_3$
21	-H	-Methyltetrahydro-pyran-4-yl

Scheme 2. Pictet–Spengler reaction of tryptamine (**6**) and L-tryptophan methyl ester (**7**) with the respective aldehydes (**8**–**14**), to gain THBCs (**15**–**27**). Numbering of atoms in NMR data are in accordance with the numbering of strictosidine (**1**) to allow easier comparison between the compounds and the natural products.[4b,c] The numbering is hence not necessarily according to IUPAC name of the compounds.

tryptamine (**6**) and L-tryptophan methyl ester (**7**) were rather small and varied mostly in the reaction with heptanal (**13**) (21% and 47%). The lowest yield was achieved when performing the reaction with tryptamine (**6**) and (tetrahydro-2*H*-pyran-4-yl)-acetaldehyde (**14**). Nevertheless only three reactions delivered a yield less than 50% and the gained material was in all cases sufficient for structure determination, bioassays and redox activity experiments. Generally all compounds were characterized by one- and two-dimensional NMR measurements, as well as ESI-MS analysis. Exemplarily some substances also underwent elemental analysis to validate their purity concerning possible inorganic residues.

The Pictet–Spengler reaction was not stereoselective and led to enantiomeric mixtures, with respect to the newly generated chiral centre in the annulated tetrahydropyridine ring when using tryptamine (**6**) as reagent. In cases of utilising L-tryptophan methyl ester (**7**), the expected diastereomers were formed and detected in the NMR spectra. Racemisation of the chiral centre, which was introduced from L-tryptophan, was not investigated in these cases.[15] For all compounds the stereoisomers were not separated and the bioactivity as well as radical scavenging tests were performed with the mixtures of the stereoisomers.

2.2. Bioassays on herbivores *Spodoptera littoralis*

The herbivores chosen for the different feeding bioassays were neonate larvae of *S. littoralis* (Lepidoptera, Noctuidae). This noctuid moth is a large, polyphagous insect which can ruin crops of more than 100 other different plant species from various families by feeding from leaves.[16] Nevertheless, it is also utilised as a model organism for bioassays, especially for its features of the larvae being very polyphagous and for the insensitivity towards multiple pesticides.[17] An additional advantage is the rather uncomplicated way to culture a laboratory colony of *S. littoralis*.[18] We used these insects to investigate the phytochemical potential of the synthesized THBCs. In particular, their growth inhibitory effects against neonate larvae were tested.

2.2.1. Short term growth tests

All 13 substances (**15**–**27**) were screened in feeding bioassays over a period of 6 d to study their possible activity against the growth of the neonate larvae. Three different concentrations (250, 500 and 1000 ppm) were added to the food and in parallel a control group was fed without addition of the THBC derivatives. After 4 d and 6 d the average mass of the larvae in each experiment was measured and compared to those of the larvae in the control group. Most of the substances did not show a significant impact on the growth of the larvae; in some cases the larvae even gained more weight compared to the control. However, the substances **15**, **18**, **19** and **20** showed inhibitory activity in the range 22–34% of the gained mass. All those compounds were tryptamine (**6**) based and mimicked the tetrahydro-β-carboline moiety of strictosidine (**1**). The carboxystrictosidine analogues which derived from L-tryptophan methyl ester (**7**), however, did not show any significant inhibitory effect. Most effective inhibition of 34% after 4 d and 6 d was gained by **20** in a concentration of 1000 ppm. The further three substances **15**, **18** and **20** were most efficient at 500 ppm after 6 d, with inhibitory effects of 22%, 28% and 26%, respectively. Summed inhibition rates are listed in Tables 1 and S2.

The four most active compounds were all tryptamine-derived and possessed different substituents, namely a linear hexyl, a branched *iso*-butyl, a phenyl and a benzyl group (**15**, **18**, **19** and **20**, Scheme 2). All these moieties were rather bulky and had a non-polar aliphatic or aromatic character. The lipophilic compounds with an unsubstituted THBC moiety had a moderate but significantly larger influence on growth compared to the more hydrophilic carboxystrictosidine analogues. This implied a gentle effect on the growth rate of the herbivore, likely caused by the structural features, for example, by protein binding. The bioactivity of

Table 1
Selected activities of tetrahydro-β-carbolines on neonate larvae of *S. littoralis*. Growth inhibition is indicated in [% m/m] against growth of a control group

Compound	Concentration [ppm]	4 d [% m/m]	6 d [% m/m]
15	1000	12	16
	500	15	22
	125	4	10
18	1000	12	6
	500	8	28
	125	4	−20
19	1000	17	16
	500	20	26
	125	1	5
20	1000	34	34
	500	13	13
	125	−9	−10

Negative values mean that the larvae gained more mass compared to the control.

Table 2
Further investigation of the three most active substances (**18–20**) after the screening (S1), to examine the biological activity in closer detail

Compound	Concentration [ppm]	4 d [% m/m]	6 d [% m/m]
	1000	15	12
	800	28	14
	600	18	10
	500	4	−6
18	400	13	6
	300	9	2
	250	4	10
	200	−1	0
	100	0	7
	50	1	−3
	1000	42	46
	800	30	38
	600	37	38
	500	39	33
19	400	19	21
	300	12	16
	250	17	18
	200	16	8
	100	11	7
	50	11	3
	1000	35	30
	800	20	18
	600	9	0
	500	28	24
20	400	22	13
	300	6	6
	250	16	10
	200	10	−2
	100	4	−4
	50	4	−7

Negative values mean that the larvae gained more mass compared to the control.

strictosidine (**1**) against herbivores is hence not only based on the reactivity of the dialdehyde (**3**) follow up product shown in Scheme 1, but in a minor amount caused by the THBC moiety.

The three THBCs **18**–**20** with the highest inhibitory effect were further investigated by triple-fold repetition of the 6 d lasting feeding experiments using ten concentrations between 50 ppm and 1000 ppm. The results were slightly different, but principally in good agreement with those of the screening experiment. Here, compound **19** showed the highest inhibition on the *S. littoralis* larvae growths, with an effect of 42% after 4 d and 46% after 6 d (1000 ppm), while **18** and **20** caused a slightly smaller inhibition of growth. The averaged results summarised in Table 2 showed a correlation between the concentration of the THBC derivatives and the reduced gain in weight. However, compounds **18** and **20** showed higher effect after 4 d, compared to those after 6 d, which implied a recovery of the larvae on habituation after a short time period. This could be explained due to optimised digestion and improved nitrogen incorporation.

2.2.2. Growth experiment until metamorphosis

A long term investigation, lasting from hatching of the larvae until the beginning of metamorphosis, was carried out with compound **15** applying 125 and 250 ppm concentrations. During the first week the higher concentrated feed led to an inhibitory impact of ca. 39% mass decrease compared to the control group. However, the caterpillars were afterwards able to recover successfully, showing no negative long term effects on growth rate after 14 d. At that time most of the larvae had already pupated and the other were on their way to metamorphosis. Afterwards, hatching of the nymphs was also observed, indicating no significant long term effects on their development. The investigated strictosidine analogue hence influenced the growth of *S. littoralis* in an early growth phase, but the moths were able to accustom themselves to the exposition and continue their lifecycle. The moderate effect on the growth rate of the herbivore caused by the structural features was hence not sufficient to sustainably affect this herbivore.

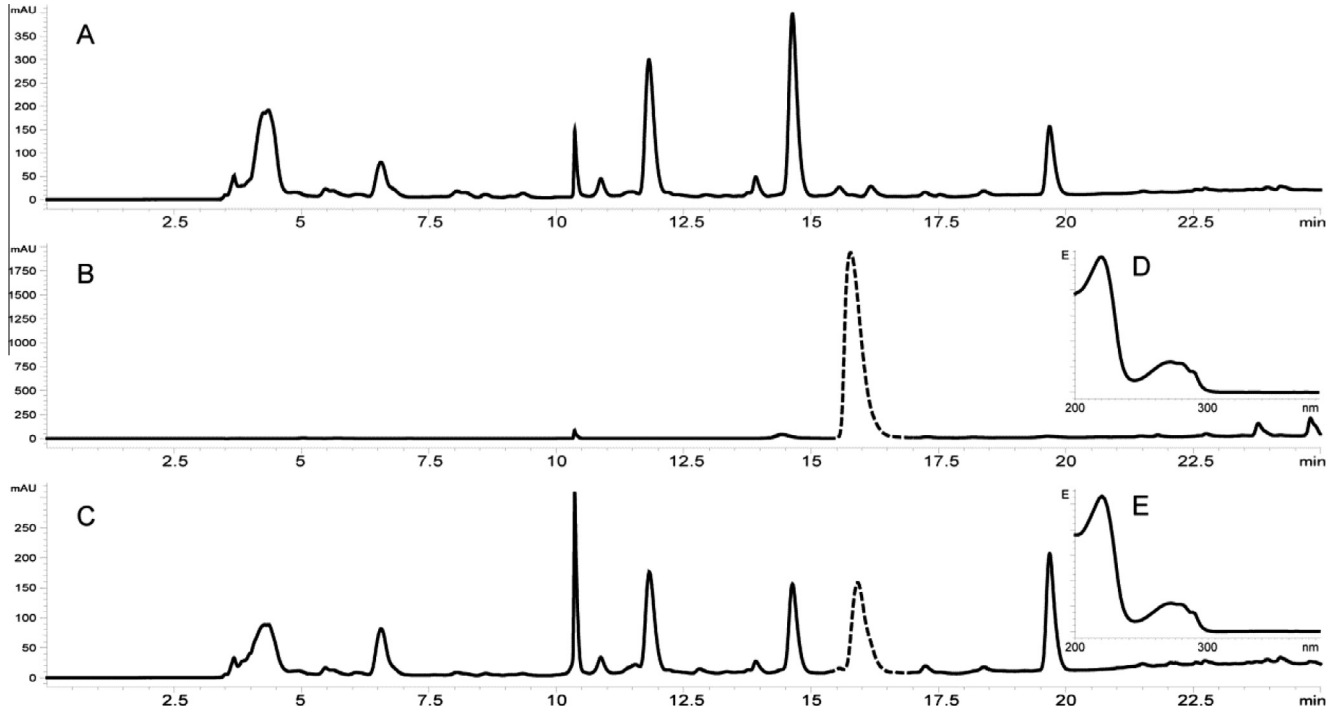

Figure 2. HPLC of frass collected from neonate larvae of *S. littoralis*. Panel A shows the chromatogram of an extract from frass collected after larvae were fed with diet not containing any synthesized THBC. In panel B a chromatogram of pure compound **19** is shown. The significant peak is indicated as dashed section. Inlet D shows the UV spectrum recorded from this peak. Panel C presents a chromatogram of frass collected from larvae fed with diet containing compound **19**. The peak of **19** is also indicated as dashed section and inlet E shows the UV spectrum recorded of this peak. Concurrent retention times and UV absorption maxima indicate that **19** has not been digested by the larvae.

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Table 3
Radical scavenging activity of the synthesized tetrahydro-β-carboline derivatives

Compound	5 min		30 min	
	EC_{50}	%*	EC_{50}	%*
15	2.36	43	2.31	44
16	3.17	32	1.64	62
17	3.86	26	1.97	51
18	3.48	29	2.00	50
19	9.17	11	1.47	68
20	7.33	14	1.36	74
21	4.69	21	3.96	25
22	35.13	3	15.37	7
23	57.89	2	31.95	3
24	1.83	55	5.89	17
25	1.82	55	23.81	4
26	5.28	19	2.54	40
27	2.97	34	4.93	20
α-Tocopherol	1.00	100	1.00	100

Listed are EC_{50} values and relative activity compared to those of α-tocopherol.
* Relative antioxidant activity compared to those of α-tocopherol.

2.2.3. THBC derivatives in the frass

During the more detailed feeding experiments of compounds **15**, **18**, **19** and **20** the frass of the larvae was collected, extracted with methanol, and examined to study possible decomposition products after digestion. The investigated faeces of compounds **15**, **18** and **19** showed only one additional significant peak during the HPLC analysis, which appeared at the same retention time as the synthesized substance fed to the caterpillars. Also the UV spectra of the originally fed compounds and the recovered substances from faeces did match in all cases. Figure 2 shows the congruence exemplary for compound **19**. In case of compound **20**, significant line broadening was observed in the chromatogram, indicating their incomplete solution. Therefore no distinct conclusion can be made for this compound. However, the compliances in the other three cases allow the assumption, that the tetrahydro-β-carboline derivatives were passing the insects without being metabolized by digestion. The moderate effects on growth rates can hence be ascribed to structural moieties of the originally fed THBC strictosidine analogues.

2.3. Antioxidant activity of THBCs

To evaluate the possible radical scavenging activity of the synthesized tetrahydro-β-carboline derivatives (**15**–**27**) the DPPH method (2,2-diphenyl-1-picrylhydrazyl) was applied.[19] Data collected after 5 min and 30 min were used to calculate the according EC_{50} values for each tested substance in comparison to those of the additionally tested α-tocopherol, the most active antioxidant of the vitamin E family.[1a,20] Seven investigated THBC derivatives (**16**–**20**, **24** and **25**) exhibited rather high radical scavenging activities, ranging from 50% to 74% compared to those of α-tocopherol. Results of all compounds are summarised in Table 3.

Most of the investigated substances showed highest radical scavenging activities after ca. 30 min when reaching their equilibrium. At that point in time the activities of the strictosidine analogues were averagely higher compared to those of the carboxystrictosidine analogues, independent from the structure of the modified alkyl or aryl moieties. The activity after 5 min, however, was highest for carboxystrictosidine analogues **24** and **25**, both carrying small alkyl chain residues, one linear and the other branched. These two compounds had about half the efficiency of α-tocopherol after 5 min and showed a distinctly lower activity after 30 min. Compound (**27**) showed similar behaviour, but not as differentiated and with lower activity.

3. Conclusion

Tetrahydro-β-carboline moieties are present in strictosidine (**1**) as well as in related MIAs and biodegradation products. Model compounds bearing this structural building block were easily synthesized in moderate to good yields by Pictet–Spengler reactions. The resulting products mimicked the THBC moiety and were used for bioassays to investigate its phytochemical meaning in the natural products. Only moderate growth-retarding effects were determined during the feeding experiments with neonate larvae of *S. littoralis*. The THBC skeleton was hence supposed to contribute only moderately to strictosidine (**1**) derived input in plant defence mechanisms against herbivores. As opposed to this, the substances with THBC moiety showed a good ability as radical scavengers in investigations using the DPPH method. This indicates a significant contribution to antioxidant activity in THBC bearing MIAs.

These findings indicated that the functions of strictosidine (**1**) and related natural products of genus *Psychotria* are not limited to their known contribution in plant defence or to act as a key intermediate in biosynthesis for further tryptamine-iridoid alkaloids.[5,8] In particular the quite distinct radical scavenging activity of the THBC skeleton was a not yet thoroughly investigated feature, with respect to its phytochemical meaning of this strictosidine moiety. It is hence of interest to further study the importance and functions of strictosidine derivatives in *Psychotria* with respect to its successful pantropic distribution.

4. Experimental

4.1. General

All substances and solvents were purchased from Sigma Aldrich or Alfa Aesar and used without further purification. For column chromatography between 30 and 50 g of silica gel (Silicagel 60 M (40–63 μm) from Macherey-Nagel was used with different ratios of DCM/MeOH as mobile phases. TLC was performed on silica gel plates (0.25 mm), Macherey-Nagel (ALUGRAM® Xtra SIL G/UV 254) developed in different organic solvents.

4.2. Spectroscopic an analytical techniques

The ^{1}H, ^{13}C and 2D NMR-spectra were recorded on Bruker DRX-400 (400.13 MHz) or on Bruker AVIII-600 (600.13 MHz), using Topspin 1.3 and Topspin 3.1 software, respectively. 1D spectra were recorded by acquisition of 32 k data points. ^{13}C NMR have been recorded using the SEFT (Spin-Echo Fourier Transform) technique. After zero filling to 64 k data points Fourier transformation spectra were performed with a range of 14 ppm (^{1}H) and 240 ppm (^{13}C), respectively. To determine the 2D COSY, TOCSY, NOESY, HMQC and HMBC spectra 128 experiments with 2048 data points each were recorded and Fourier transformed to 2D-spectra with a range of 10 ppm and up to 220 ppm for ^{1}H and ^{13}C, respectively. Measurement temperature was 298 ± 0.05 K. To perform the measurements, each compound was dissolved in $CDCl_3$ (~5.0 mg in 0.7 mL) and transferred into 5 mm high precision NMR sample tubes. All NMR spectral data were expressed as δ values and the residual $CDCl_3$ was used as an internal standard for ^{1}H (δ_H 7.26) and for ^{13}C (δ_C 77.02) measurements.

Mass spectra were recorded by direct infusion electrospray ionisation (ESI) in positive ionisation mode (mass accuracy ± 5 ppm). The analyser was a high resolution time-of-flight (TOF) mass spectrometer (maXis, Bruker Daltonics). TOF measurements have been performed within the selected mass range of m/z 100–2500. ESI was achieved by applying a capillary voltage of 4 kV to uphold a (capillary) current between 30 and 50 nA. The flow rate was

adjusted to 4.0 mL/min at a nitrogen temperature of 180 °C, whereas the nebulizer gas pressure of N_2 was 0.3 bar.

Elemental analyses were performed at the Microanalytical Laboratory of the University of Vienna with a Eurovector EA3000 elemental analyser and are within ±0.4% of the calculated values, confirming their ⩾95% purity.

The HPLC measurements were performed on Agilent 1100 series with using a Hypersil BDS-C18 column (250 mm × 4.6 mm, 5 μm particle size) with UV diode array detection at 230 nm. The eluent contained MeOH (B) and an aqueous buffer containing 15 mM *ortho*-phosphoric acid and 1.5 mM tetrabutylammonium hydroxide (A). The method applied a linear MeOH gradient 0–15 min:15–60% (B) followed by 5 min 60–100% and (B) hold for 5 min. The flow rate was 0.8 mL/min and the injection volume 10 μL.

An incubator (WTB, Binder) was used for rearing the larvae. The antioxidant activity was measured by employing a Tecan Sunrise-Basic plate reader.

4.3. Esterification of L-tryptophan

A suspension of L-tryptophan (200 mg, 0.98 mmol) in MeOH (5.0 mL) was cooled to 0 °C before thionyl chloride (350 mg, 12.94 mmol) was added slowly. The solution was stirred for 3 h at rt. MeOH was evaporated, water (3.0 mL) added to the residue and the pH adjusted to 9–10 with aq NaOH (10%). The solution was extracted with ethyl acetate and the organic phase was washed with brine and dried over $MgSO_4$. After removal of the solvent column chromatography was performed using DCM/MeOH (9:1). Yield: 60% of L-tryptophan methyl ester (**7**, 129 mg, 0.59 mmol). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.03 (s, 2H, H-12), 3.05 (dd, J = 14.4, 7.8 Hz, 1H, H-10), 3.29 (dd, J = 14.4, 4.8 Hz, 1H, H-10), 3.71 (s, 3H, H-14), 3.83 (dd, J = 7.8, 4.8 Hz, 1H, H-11), 6.96 (s, 1H, H-2), 7.12 (dd, J = 7.4, 7.8 Hz, 1H, H-7), 7.19 (dd, J = 7.4, 7.8 Hz, 1H, H-6), 7.31 (d, J = 7.8 Hz, 1H, H-8), 7.60 ppm (d, J = 7.8 Hz, 1H, H-5), 8.85 (s, H-1); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 30.5 (C-10), 51.9 (C-14), 54.7 (C-11), 110.3 (C-3), 111.2 (C-8), 118.4 (C-5), 119.2 (C-7), 121.8 (C-6), 123.1 (C-2), 127.2 (C-9), 136.2 (C-4), 175.5 ppm (C-13) (Fig. S1); ESI-MS ($C_{12}H_{14}O_2N_2$): calculated m/z 219.1128 $[M+H]^+$, observed m/z 219.1120 $[M+H]^+$.

4.4. General procedure of the Pictet–Spengler reaction

To a cooled solution (0 °C) of tryptamine (**6**, 100 mg, 0.62 mmol)/L-tryptophan methyl ester (**7**, 100 mg, 0.46 mmol) in DCM (1.5 mL) the respective aldehyde (**8**–**14**, 1.2 mol equiv) and the trifluoracetic acid (1.5 mol equiv) were added slowly. The reaction was stirred for 24 h at room temperature and then the solvent was evaporated. The residue was triturated with aq K_2CO_3 (5%), extracted with DCM and dried over $MgSO_4$. After removal of the solvent column chromatography was performed using variable DCM/MeOH mixtures.

Numbering of atoms in NMR data are in accordance with the numbering of strictosidine (**1**) to allow easier comparison.[4b,c] The numbering is shown in Schemes 1 and 2.

4.4.1. 1-Phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (15)

Yield 83% (128 mg, 0.52 mmol), mobile phase ratio 9:1 (DCM/MeOH), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.87 (m, 1H, H-6), 2.94 (m, 1H, H-6), 3.12 (m, 1H, H-5), 3.31 (m, 1H, H-5), 5.39 (s, 1H, H-3), 7.13 (m, 1H, H-11), 7.16 (m, 1H, H-10), 7.22 (m, 1H, H-12), 7.30 (m, 5H, H-2′-6′), 7.52 ppm (m, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 21.3 (C-6), 41.7 (C-5), 57.4 (C-3), 109.9 (C-7), 111.0 (C-12), 118.3 (C-9), 119.6 (C-11), 122.1 (C-10), 127.0 (C-13), 128.7 (C-4′), 128.9 (C-3′/5′), 129.0 (C-2′/6′), 132.3 (C-2), 136.1 (C-8), 139.3 ppm (C-1′) (Fig. S2); ESI-MS ($C_{17}H_{16}N_2$): calculated m/z 249.1386 $[M+H]^+$, observed m/z 249.1387 $[M+H]^+$.

4.4.2. 1-Cyclohexyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (16)

Yield 81% (128 mg, 0.50 mmol), mobile phase ratio 8:2 (DCM/MeOH), ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.14 (m, 1H, H-2′), 1.19 (m, 2H, H-3′/4′), 1.32 (m, 1H, H-5′), 1.42 (m, 1H, H-6′), 1.54 (m, 1H, H-2′), 1.71 (m, 1H, H-4′), 1.75 (m, 1H, H-3′), 1.77 (m, 1H, H-6′), 1.81 (m, 1H, H-1′), 1.84 (m, 1H, H-5′), 2.69–2.79 (m, 2H, H-6), 3.00 (m, 1H, H-5), 3.38 (m, 1H, H-5), 3.99 (m, 1H, H-3), 7.10 (m, 1H, H-11), 7.15 (m, 1H, H-10), 7.32 (d, J = 8.0 Hz, 1H, H-12), 7.49 ppm (d, J = 7.7 Hz, 1H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 22.5 (C-6), 26.4 (C-4′), 26.5 (C-3′), 26.8 (C-5′), 27.5 (C-2′), 30.2 (C-6′), 42.2 (C-1′), 42.9 (C-5), 57.7 (C-3), 109.8 (C-7), 110.6 (C-12), 117.9 (C-9), 119.2 (C-11), 121.4 (C-10), 127.5 (C-13), 135.0 (C-2), 135.6 ppm (C-8) (Fig. S3); ESI-MS ($C_{17}H_{22}N_2$): calculated m/z 255.1857 $[M+H]^+$, observed m/z 255.1855 $[M+H]^+$.

4.4.3. 1-Ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (17)

Yield 50% (62 mg, 0.31 mmol), mobile phase ratio 8:2 (DCM/MeOH), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.07 (t, J = 7.3 Hz, 3H, H-2′), 1.72 (m, 1H, H-1′), 1.94 (m, 1H, H-1′), 2.75 (m, 2H, H-6), 3.04 (m, 1H, H-5), 3.38 (m, 1H, H-5), 4.02 ppm (m, 1H, H-3), 7.10 (m, 1H, H-11), 7.15 (m, 1H, H-10), 7.31 (d, J = 7.5 Hz, 1H, H-12), 7.49 ppm (d, J = 7.5 Hz, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 10.2 (C-2′), 22.7 (C-6), 27.7 (C-1′), 42.6 (C-5), 53.9 (C-3), 109.2 (C-7), 110.7 (C-12), 118.0 (C-9), 119.3 (C-11), 121.5 (C-10), 127.6 (C-13), 135.6 (C-2), 136.1 ppm (C-8) (Fig. S4); ESI-MS ($C_{13}H_{16}N_2$): calculated m/z 201.1386 $[M+H]^+$, observed m/z 201.1385 $[M+H]^+$.

4.4.4. 1-(2-Methyl-propyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (18)

Yield 67% (95 mg, 0.42 mmol), mobile phase ratio 8:2 (DCM/MeOH), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.01 ppm (m, 3H, H-3′), 1.04 (m, 3H, H-4′), 1.63 (m, 2H, H-1′), 1.98 (m, 1H, H-2′), 2.75 (m, 2H, H-6), 3.03 (m, 1H, H-5), 3.35 (m, 1H, H-5), 4.11 (m, 1H, H-3), 7.10 (m, 1H, H-11), 7.15 (m, 1H, H-10), 7.30 (d, J = 7.6, 1H, H-12), 7.49 ppm (d, J = 7.6, 1H, H-9); ^{13}C NMR (400 MHz, $CDCl_3$, δ): 21.7 (C-4′), 22.7 (C-6), 23.8 (C-3′), 24.6 (C-2′), 42.4 (C-5), 44.4 (C-1′), 50.5 (C-3), 108.7 (C-7), 110.6 (C-12), 118.0 (C-9), 119.3 (C-11), 121.4 (C-10), 127.6 (C-13), 135.6 (C-8), 136.6 ppm (C-2) (Fig. S5); ESI-MS ($C_{15}H_{20}N_2$): calculated m/z 229.1699 $[M+H]^+$, observed m/z 229.1707 $[M+H]^+$.

4.4.5. 1-Benzyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (19)

Yield 58% (94 mg, 0.36 mmol), mobile phase ratio 8:2 (DCM/MeOH), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.69–2.83 (m, 2H, H-6), 3.02 (m, 1H, H-5), 3.09 (d, J = 7.1 Hz, 2H, H-1′), 3.33 (m, 1H, H-5), 4.37 (m, 1H, H-3), 7.09 (m, 1H, H-11), 7.13 (m, 1H, H-10), 7.21 (d, J = 7.3 Hz, 1H, H-12), 7.28 (m, 2H, H-4′/6′), 7.31 (m, 1H, H-5′), 7.35 (m, 2H, H-3′/7′), 7.49 ppm (d, J = 7.3 Hz, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 22.5 (C-6), 41.5 (C-1′), 42.3 (C-5), 53.9 (C-3), 109.2 (C-7), 110.7 (C-12), 118.1 (C-9), 119.3 (C-11), 121.5 (C-10), 126.9 (C-5′), 127.1 (C-13), 128.8 (C-3′/7′), 129.3 (C-4′/6′), 135.3 (C-8), 135.5 (C-2), 138.0 ppm (C-2′) (Fig. S6); ESI-MS ($C_{18}H_{18}N_2$): calculated m/z 263.1542 $[M+H]^+$, observed m/z 263.1541 $[M+H]^+$.

4.4.6. 1-Hexyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (20)

Yield 21% (33 mg, 0.13 mmol), mobile phase ratio 9:1 (DCM/MeOH), ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.88 (t, J = 6.8 Hz, 3H, H-6′), 1.29 (m, 4H, H-4′/5′), 1.35 (m, 2H, H-3′), 1.45 (m, 1H, H-2′), 1.52 (m, 1H, H-2′), 1.71 (m, 1H, H-1′), 1.89 (m, 1H, H-1′), 2.77 (m, 2H, H-6), 3.06 (m, 1H, H-5), 3.37 (m, 1H, H-5), 4.12 (m, 1H, H-3), 7.08 (dd, J = 7.0, 7.4 Hz, 1H, H-11), 7.13 (dd, J = 7.0, 7.4 Hz, 1H, H-10), 7.32 (d, J = 7.4 Hz, 1H, H-12), 7.50 ppm (d, J = 7.4 Hz, 1H, H-

9); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 14.0 (C-6′), 22.2 (C-6), 22.6 (C-5′), 25.8 (C-2′), 29.5 (C-3′), 31.7 (C-4′), 34.8 (C-1′), 42.3 (C-5), 52.7 (C-3), 108.6 (C-7), 110.8 (C-12), 118.0 (C-9), 119.3 (C-11), 121.5 (C-10), 127.4 (C-13), 135.6 (C-2), 135.7 ppm (C-8) (Fig. S7); ESI-MS ($C_{17}H_{24}N_2$): calculated m/z 257.2012 $[M+H]^+$, observed m/z 257.2020 $[M+H]^+$.

4.4.7. 1-[(Tetrahydro-2*H*-pyran-4-yl)methyl]-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (21)

Yield 11% (18 mg, 0.07 mmol), mobile phase ratio 85:15 (DCM/MeOH), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.34 ppm (m, 1H, H-3′), 1.42 (m, 1H, H-6′), 1.60–1.64 (m, 1H, H-6′), 1.69 (m, 2H, H-1′), 1.85 (m, 1H, H-3′), 1.96 (m, 1H, H-2′), 2.75 (m, 2H, H-6), 3.03 (m, 1H, H-5), 3.35 (m, 1H, H-5), 3.45 (m, 2H, H-5′), 3.99 (m, 2H, H-4′), 4.16 (m, 1H, H-3), 7.10 (m, 1H, H-11), 7.15 (m, 1H, H-10), 7.32 (d, J = 7.8 Hz, 1H, H-12), 7.48 ppm (d, J = 7.6 Hz, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 22.8 (C-6), 31.3 (C-2′), 32.5 (C-3′), 34.2 (C-6′), 42.3 (C-5), 42.4 (C-1′), 49.4 (C-3), 67.9 (C-4′), 68.0 (C-5′), 109.1 (C-7), 110.7 (C-12), 118.0 (C-9), 119.5 (C-11), 121.6 (C-10), 127.6 (C-13), 135.6 (C-2), 136.3 ppm (C-8) (Fig. S8); ESI-MS ($C_{17}H_{22}N_2O$): calculated m/z 271.1804 $[M+H]^+$, observed m/z 271.1805 $[M+H]^+$.

4.4.8. Methyl-1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (22)

Yield 81% (115 mg, 0.38 mmol), mobile phase ratio 98:2 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.02 (m, 1H, H-6), 3.24 (m, 1H, H-6), 3.81/4.04 (s, 3H, H-15), 3.98 (dd, J = 4.3, 11.1 Hz, 1H, H-5), 5.23 (s, 1H, H-3), 7.09–7.13 (m, 1H, H-11), 7.13–7.17 (m, 1H, H-10), 7.18–7.21 (m, 1H, H-12), 7.34–7.41 (m, 5H, H-2′-6′), 7.51–7.56 ppm (m, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 25.6 (C-6), 52.2/52.6 (C-15), 56.9 (C-5), 58.6 (C-3), 108.8 (C-7), 110.9 (C-12), 118.2 (C-9), 119.6 (C-11), 121.9 (C-10), 127.1 (C-13), 128.6 (C-2′/6′), 128.9 (C-3′-5′), 134.5 (C-2), 136.1 (C-8), 140.6 (C-1′), 173.1 ppm (C-14) (Fig. S9); ESI-MS ($C_{19}H_{18}N_2O_2$): calculated m/z 307.1441 $[M+H]^+$, observed m/z 307.1438 $[M+H]^+$.

4.4.9. Methyl-1-cyclohexyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (23)

Yield 70% (101 mg, 0.32 mmol), mobile phase ratio 98:2 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.10–1.36 (m, 2H, H-2′/6′), 1.42 (m, 2H, H-2′/6′), 1.59–1.95 (m, 6H, H-3′/4′/5′), 1.99 (m, 1H, H-1′), 2.81 (m, 1H, H-6), 3.12 (m, 1H, H-6), 3.72/3.81 (s, 3H, H-15), 3.77 (m, 1H, H-5), 4.23 (m, 1H, H-3), 7.09/7.32 (m, 1H, H-11), 7.15/7.56 (m, 1H, H-10), 7.34/7.61 (m, 1H, H-12), 7.46/8.75 ppm (m, 1H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 25.6/25.8 (C-6), 26.2–31.0 (C-2′/3′/4′5′/6′), 42.2/43.2 (C-1′), 52.3/52.6 (C-15), 56.3 (C-5), 57.7 (C-3), 108.8 (C-7), 110.9/112.0 (C-12), 116.2/117.8 (C-9), 119.5/120.7 (C-11), 121.7/128.5 (C-10), 127.0 (C-13), 134.0 (C-2), 136.1 (C-8), 173.4 ppm (C-14) (Fig. S10); ESI-MS ($C_{19}H_{24}N_2O_2$): calculated m/z 313.1910 $[M+H]^+$, observed m/z 313.1905 $[M+H]^+$. Elemental analysis: $C_{19}H_{24}N_2O_2$ calcd C, 73.05; H, 7.74; N, 8.97; found C, 72.13; H, 7.68; N, 8.84.

4.4.10. Methyl-1-ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (24)

Yield 40% (48 mg, 0.19 mmol), mobile phase ratio 9:1 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.07 (m, 3H, H-2′), 1.78 (m, 1H, H-1′), 2.02 (m, 1H, H-1′), 2.83 (m, 1H, H-6), 3.14 (m, 1H, H-6), 3.75/3.83 (s, 3H, H-15), 3.80/4.02 (m, 1H, H-5), 4.17/4.21 (m, 1H, H-3), 7.11 (m, 1H, H-11), 7.16 (m, 1H, H-10), 7.30/7.32 (d, J = 7.8 Hz, 1H, H-12), 7.48 ppm (d, J = 7.6 Hz, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 9.5/10.5 (C-2′), 24.8/25.9 (C-6), 27.3/28.3 (C-1′), 51.8/53.7 (C-3), 52.2 (C-15), 52.6/56.4 (C-5), 106.9/108.2 (C-7), 110.8 (C-12), 118.0 (C-9), 119.4/119.5 (C-11), 121.7 (C-10), 127.0/127.2 (C-13), 135.0/135.3 (C-2), 135.9/136.0 (C-8), 173.6/173.9 ppm (C-14) (Fig. S11); ESI-MS ($C_{15}H_{18}N_2O_2$): calculated m/z 259.1441 $[M+H]^+$, observed m/z 259.1433 $[M+H]^+$. Elemental analysis: $C_{15}H_{18}N_2O_2$ calcd C, 69.74; H, 7.02; N, 10.84; found C, 64.24; H, 6.61; N, 9.52.

4.4.11. Methyl-1-(2-methyl-propyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (25)

Yield 58% (76 mg, 0.27 mmol), mobile phase ratio 95:5 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.01 (d, J = 6.8 Hz, 3H, H-3′), 1.04 (d, J = 6.8 Hz, 3H, H-4′), 1.52/1.68 (m, 2H, H-1′), 2.03 (m, 1H, H-2′), 2.83/2.99 (m, 1H, H-6), 3.12/3.14 (m, 1H, H-6), 3.76/3.83 (s, 3H, H-15), 3.80/3.98 (m, 1H, H-5), 4.23/4.30 (m, 1H, H-3), 7.11 (m, 1H, H-11), 7.16 (m, 1H, H-10), 7.31 (d, J = 7.6 Hz, 1H, H-12), 7.48 ppm (d, J = 7.6 Hz, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 21.6/21.7 (C-4′), 23.6/23.8 (C-3′), 24.3/24.6 (C-2′), 25.0/26.0 (C-6), 44.3/44.5 (C-1′), 48.1/50.6 (C-3), 52.1 (C-15), 52.4/56.5 (C-5), 107.8 (C-7), 110.7 (C-12), 117.9 (C-9), 119.4/119.5 (C-11), 121.6 (C-10), 127.2 (C-13), 135.9 (C-2), 136.0 (C-8), 173.8/174.3 ppm (C-14) (Fig. S12); ESI-MS ($C_{17}H_{22}N_2O_2$): calculated m/z 287.1754 $[M+H]^+$, observed m/z 287.1756 $[M+H]^+$.

4.4.12. Methyl-1-benzyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (26)

Yield 51% (75 mg, 0.24 mmol), mobile phase ratio 95:5 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.86/3.04 (m, 1H, H-6), 3.05/3.09 (m, 1H, H-1′), 3.14/3.19 (m, 1H, H-6), 3.15/3.22 (m, 1H, H-1′), 3.74/3.81 (s, 3H, H-15), 3.79/4.53 (m, 1H, H-5), 4.07/4.64 (m, 1H, H-3), 7.09 (m, 1H, H-11), 7.13 (m, 1H, H-10), 7.18/7.20 (m, 1H, H-12), 7.25 (m, 1H, H-4′), 7.27 (m, 1H, H-6′), 7.34 (m, 2H, H-3′/5′), 7.37 (m, 1H, H-7′), 7.48 ppm (m, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 24.7/25.8 (C-6), 41.5/41.9 (C-1′), 52.1/52.7 (C-3), 52.2/52.6 (C-15), 54.1/56.4 (C-5), 107.0/108.3 (C-7), 110.8 (C-12), 118.0/118.1 (C-9), 119.4/119.5 (C-11), 121.8 (C-10), 126.6/126.7 (C-13), 127.0/127.2 (C-5′), 128.8/129.0 (C-3′/7′), 129.3/129.4 (C-4′/6′), 134.0/134.8 (C-2′), 135.7/135.8 (C-2), 137.5/137.7 (C-8), 173.2/173.4 ppm (C-14) (Fig. S13); ESI-MS ($C_{20}H_{20}N_2O_2$): calculated m/z 321.1604 $[M+H]^+$, observed m/z 321.1606 $[M+H]^+$.

4.4.13. Methyl-1-hexyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (27)

Yield 47% (68 mg, 0.22 mmol), mobile phase 95:5 (DCM/MeOH), diastereomeric mixture, ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.89 (m, 3H, H-6′), 1.29 (m, 2H, H-5′), 1.30 (m, 2H, H-4′), 1.35 (m, 2H, H-3′), 1.46/1.50 (m, 2H, H-2′), 1.69/1.77 (m, 1H, H-1′), 1.74/1.94 (m, 1H, H-1′), 2.82/3.00 (m, 1H, H-6), 3.13 (m, 1H, H-6), 3.75/3.82 (s, 3H, H-15), 3.79/4.01 (m, 1H, H-5), 4.18/4.24 (m, 1H, H-3), 7.09/7.11 (m, 1H, H-11), 7.15 (m, 1H, H-10), 7.29/7.31 (m, 1H, H-12), 7.48 ppm (m, 1H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 14.0 (C-6′), 22.5/22.6 (C-4′), 24.8/25.9 (C-6), 25.2/26.1 (C-2′), 29.3/29.5 (C-3′), 31.6/31.7 (C-5′), 34.7/35.5 (C-1′), 50.6/52.7 (C-3), 52.1/52.2 (C-15), 52.5/56.4 (C-5), 106.6/107.9 (C-7), 110.7/110.8 (C-12), 117.9 (C-9), 119.3/119.4 (C-11), 121.6 (C-10), 127.0/127.1 (C-13), 135.3/135.9 (C-2), 135.6/135.9 (C-8), 173.7/174.0 ppm (C-14) (Fig. S14); ESI-MS ($C_{19}H_{26}N_2O_2$): calculated m/z 315.2067 $[M+H]^+$, observed m/z 315.2069 $[M+H]^+$.

4.5. Preparation of the artificial diet

Each tested compound (**15**–**27**) was dissolved in acetone or MeOH, according to the desired final concentration (50–1000 ppm), and added to 367 mg freeze-dried feed powder.[16a,21] Afterwards the content was overlaid with the previously used

solvent (acetone or MeOH) and shaken for 15 min. The same routine was applied to the control. The mixture was dried at rt for 3 h (acetone) and for 14 h (MeOH) and then an aq chloramphenicol solution (725 μL; 52 mg/mL) was added to each sample. An aq agar-solution (70 mg/mL) was shortly boiled and 1.1 mL was quickly transferred to each sample. After cooling to rt each feeding sample was placed in a labelled petri dish.[16a,22]

4.6. Feeding bioassays

4.6.1. Short term tests

Feeding bioassays were conducted on neonate larvae of *S. littoralis* from a laboratory colony. Ten larvae were transferred on top of the artificial diet in each petri dish, and held in an incubator with >90% humidity at 26 °C under exclusion of light. After 4 d and 6 d the number of living insects was counted and their average mass was measured. Results were compared with those of a control group not being exposed to the investigated compounds. The frass was collected during several experiments for HPLC analysis of possible digestion products.

4.6.2. Long term tests

Substance **15** was used for the long term feeding bioassay with concentrations of 125 and 250 ppm, respectively. Again ten larvae were transferred on top of the artificial diet in each petri dish and held in an incubator with >90% humidity at 26 °C under exclusion of light. After 4, 6, 10, 12 and 14 d number of living insects was counted and their mass was measured. The insects were provided with a sufficient amount of freshly prepared artificial diet after each evaluation. The frass was collected for HPLC analysis of possible digestion products.

4.7. Antioxidant activity test

Freshly formulated methanolic DPPH solution (0.4 mM) and methanolic solutions of the substances **15**–**27** were used to prepare mixtures of multiple concentrations on Microtiter plates. The THBC concentration varied form 6.15 mg/mL to 1.52 μg/mL, while an equal quantity of a DPPH (0.08 mg/mL) was supplied in each sample. 5 min and 30 min after adding the DPPH to the dilution series the UV extinctions of all samples were measured at 550 nm (free radical DPPH) and 700 nm (reference), respectively and compared to those of sole, freshly prepared DPPH solution. The average extinction was used to calculate EC_{50} values, which was performed with Sigma Plot (Systat Software Inc.) version 12.5. The calculated antioxidant activity values were compared to the results of α-tocopherol, for which the investigated concentrations ranged from 4.11 mg/mL to 0.02 μg/mL.[19]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.12.028.

References and notes

1. (a) Michal, G.; Schomburg, D. *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*, 2nd ed.; Wiley: Oxford, 2013; (b) do Nascimento, N. C.; Fragoso, V.; Moura, D. J.; e Silva, A. C.; Fett-Neto, A. G.; Saffi, J. *J. Environ. Mol. Mutagen* **2007**, *48*, 728.
2. Panjikar, S.; Stoeckigt, J.; O'Connor, S.; Warzecha, H. *Nat. Prod. Rep.* **2012**, *29*, 1176.
3. O'Connor, S. E.; Maresh, J. J. *Nat. Prod. Rep.* **2006**, *23*, 532.
4. (a) Faßhuber, H.K. Master Thesis, University of Vienna, Vienna, 2011.; (b) Berger, A.; Fasshuber, H.; Schinnerl, J.; Brecker, L.; Greger, H. *Phytochem. Lett.* **2012**, *5*, 558; (c) Plagg, M. Master Thesis, University of Vienna, Vienna, 2012.
5. Stoeckigt, J.; Antonchick, A. P.; Wu, F.-R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 8538.
6. (a) Schinnerl, J.; Orlowska, E. A.; Lorbeer, E.; Berger, A.; Brecker, L. *Phytochem. Lett.* **2012**, *5*, 586; (b) Bernhardt, P.; O'Connor, S. E. *Tetrahedron Lett.* **2009**, *50*, 7118.
7. Verpoorte, R.; Van der Heijden, R.; Moreno, P. R. H. In *Chemistry and Pharmacology*; Cordell, G. A., Ed.; Elsevier Science, 1997; pp 221–299.
8. Guirimand, G.; Courdavault, V.; Lanoue, A.; Mahroug, S.; Guihur, A.; Blanc, N.; Giglioli-Guivarc'h, N.; St-Pierre, B.; Burlat, V. *BMC Plant Biol.* **2010**, *10*, 182.
9. Guirimand, G.; Guihur, A.; Ginis, O.; Poutrain, P.; Hericourt, F.; Oudin, A.; Lanoue, A.; St-Pierre, B.; Burlat, V.; Courdavault, V. *FEBS J.* **2011**, *278*, 749.
10. Konno, K.; Hirayama, C.; Yasui, H.; Nakamura, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9159.
11. Paul, J. H. A.; Maxwell, A. R.; Reynolds, W. F. *J. Nat. Prod.* **2003**, *66*, 752.
12. Pictet, A.; Spengler, T. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 2030.
13. Hadjaz, F.; Yous, S.; Lebegue, N.; Berthelot, P.; Carato, P. *Tetrahedron* **2008**, *64*, 10004.
14. Tang, J.; Wang, Y.; Wang, R.; Dong, Z.; Yang, L.; Zheng, Y.; Liu, J. *Chem. Biodivers.* **2008**, *5*, 447.
15. Bailey, P. D.; Hollinshead, S. P.; McLay, N. R.; Morgan, K.; Palmer, S. J.; Prince, S. N.; Reynolds, C. D.; Wood, S. D. *J. Chem. Soc., Perkin Trans. 1* **1993**, 431.
16. (a) Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *J. Agric. Food Chem.* **2002**, *50*, 6383; (b) Gelbic, I.; Adel, M.; Hussein, H. *Cent. Eur. J. Biol.* **2011**, *6*, 861; (c) EFSA PLH Panel (EFSA Panel on Plant Health) *EFSA J.* **2015**, *13*, 3987.
17. Burow, M.; Müller, R.; Gershenzon, J.; Wittstock, U. *J. Chem. Ecol.* **2006**, *32*, 2333.
18. Meissle, M.; Vojtech, E.; Poppy, G. M. *IOBC WPRS Bull.* **2004**, *27*, 117.
19. Blois, M. S. *Nature* **1958**, *181*, 1199.
20. Finney, D. J. *J. Am. Pharm. Assoc.* **1952**, *41*, 627.
21. Srivastava, R. P.; Proksch, P. *Entomol. Gen.* **1990**, *15*, 265.
22. Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kählig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803.