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Absolute configuration of volicitin, an elicitor of plant volatile biosynthesis from lepidopteran larvae

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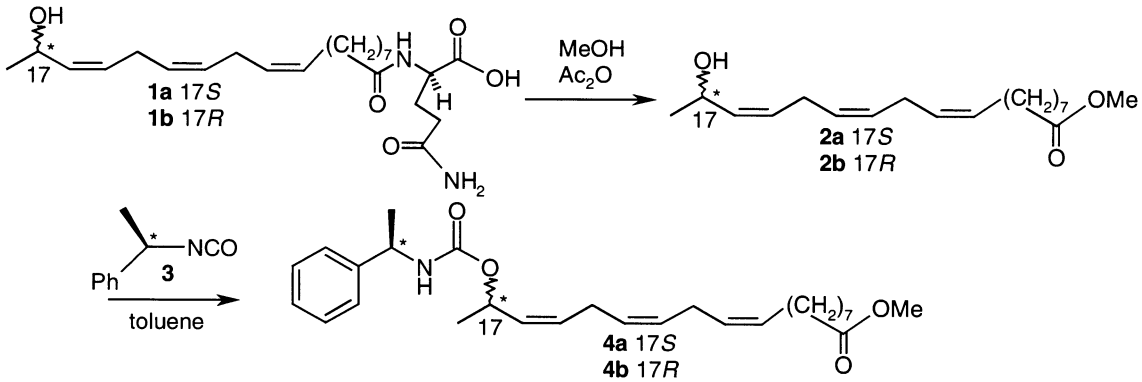
Abstract—The absolute configuration of the hydroxylinolenoyl moiety of volicitin (17-hydroxylinolenoyl-L-glutamine) **1a**/**b** from four lepidopteran larvae was determined by methanolysis and derivatisation of the resulting ester with (1*R*)-1-phenyl-ethylisocyanate **3**. The absolute configuration of the resulting (1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoates **4a**/**b** followed from GLC comparison with synthetic references. Natural volicitin **1** from caterpillars of *Spodoptera exigua*, *S. frugiperda*, *S. littoralis* and *Heliothis virescens* (Noctuidae) exhibits a high ee (92–96%) and has a 17*S* configuration. © 2001 Elsevier Science Ltd. All rights reserved.

Volicitin **1**, a conjugate of 17-hydroxylinolenic acid and L-glutamine, was first isolated from the oral secretion of herbivorous larvae of the beet armyworm (*Spodoptera exigua*). The compound received great interest since it was found to elicit the biosynthesis of volatiles in corn plants (*Zea mays*), which may attract the natural enemies of the herbivores.[1] Despite of intense work on the biosynthesis,[2,3] the mode of action,[1,2] and the stereoselective synthesis[4] of volicitin **1** and related amino acid conjugates, the enantiomeric excess and the absolute configuration of the 17-hydroxy group of natural **1** remained open. This is largely due to the fact that many derivatisation procedures with chiral reagents were low yielding because of the lability of the allylic 17-hydroxy group. Moreover, the low amount of **1** which can be isolated from the regurgitate of single larvae prevented the routine use of NMR methods.[5] Here, we describe the derivatisation of the labile 17-hydroxylinolenic acid methyl esters **2a**/**b** with chiral (1*R*)-1-phenyl-ethylisocyanate **3** under neutral conditions and the subsequent separation of the resulting diastereomers **4a** and **4b** by GLC.

Based on a previous report of Hamberg et al.,[6] who successfully separated the enantiomers of 17-hydroxymethyl stearate by GLC as diastereomeric carbamates, the catalytic hydrogenation of 17-hydroxy linolenic acid methyl esters **2a**/**b** and subsequent derivatisation with (1*R*)-1-phenyl-ethylisocyanate **3** appeared to be promising. However, owing to substantial hydrogenolytic



Scheme 1. Methanolysis and derivatisation of volicitins **1a**/**b** with (1*R*)-1-phenyl-ethylisocyanate **3**.

Keywords: fatty acid amides; *N*-17-hydroxy-linolenoyl-L-glutamine; 1-phenyl-ethylisocyanate; stereochemistry.

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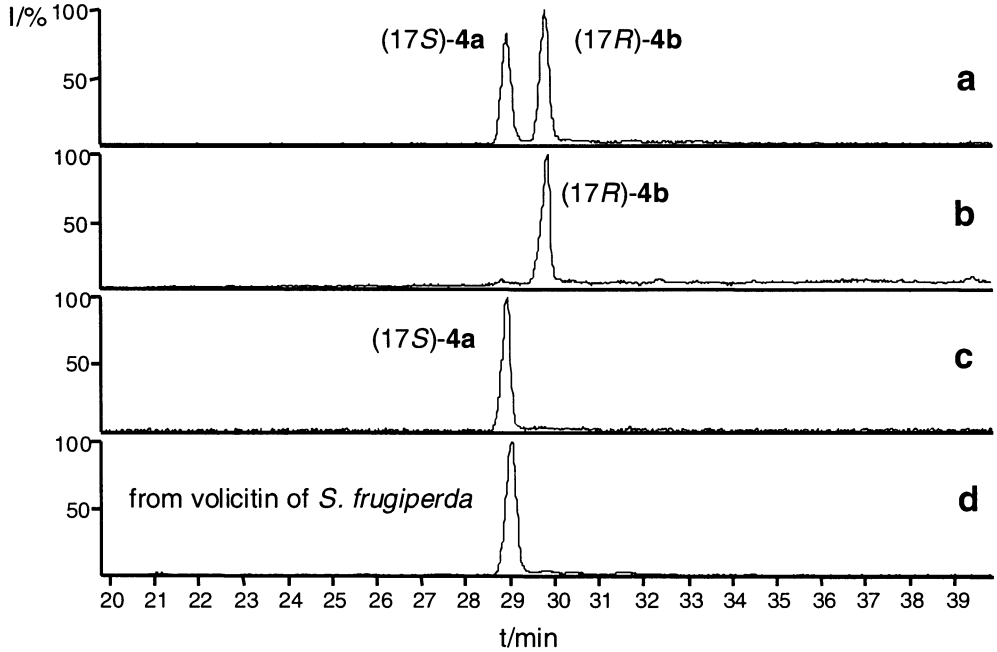

Figure 1. Ion trace $m/z=290$ of: (a) (17*RS*,1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoates **4a**/**b**; (b) (17*R*,1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoate **4b**; (c) (17*S*,1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoate **4a**; and (d) (17*S*,1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoate from volicitin of *S. frugiperda* larvae.

fission of the allylic alcohol, only moderate yields of the saturated methyl ester of 17-hydroxy stearic acid were obtained. On the other hand, the unsaturated esters **2a**/**b** were easily and efficiently converted into 17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoates **4a**/**b**. Thus, after methanolysis of volicitins **1a**/**b**,[1] derivatisation of the esters **2a**/**b** with chiral (1*R*)-1-phenyl-ethylisocyanate **3** at 120°C under neutral conditions[6] afforded the diastereomeric carbamates **4a**/**b** (Scheme 1).[7]

The derivatives **4a**/**b** proved to be thermally stable and could be baseline separated by GLC (Fig. 1a). Detection limits in the lower nanogram range were attained by single-ion monitoring (SIM) mass spectrometry. This procedure allowed the stereochemical analysis of volicitins **1a**/**b** from oral secretions of even individual larvae from different caterpillar species.

For method development ca. 200 μl of regurgitant from *S. littoralis* were collected as described[2] and volicitins **1a**/**b** were isolated by reversed-phase HPLC (RP-18). After derivatisation with (1*R*)-1-phenyl-ethylisocyanate **3** the pair of diastereomers **4a**/**b** could be baseline separated by GLC and the natural product showed 94% ee. The major diastereomer proved to be identical with the synthetic reference compound **4a** prepared from (1*R*)-1-phenyl-ethylisocyanate **3** and (17*S*)-17-hydroxylinolenic acid methyl ester **2a**. Optimisation of the procedure by monitoring only characteristic mass fragments of the diastereomers (SIM $m/z=105$, 290) allowed us to evaluate the configuration and ee of volicitin in samples of only 50–100 μl regurgitant without the need for initial purification. In all examined species natural volicitin **1a** showed a 17*S*-configuration and was of high ee (Table 1, Fig. 1).

To assess whether or not *cis*/*trans* isomers in the fatty acid moiety (resulting from cleavage of the amide bond) of the natural sample interfere with the chromatographic approach, the linolenoates **4a**/**b** were hydrogenated using Pt/C (10%) to the corresponding diastereomeric (1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl stearates. However, since the ee of the stearates was only insignificantly higher (up to 1–2% for all investigated species) the additional hydrogenation is not essential for an unambiguous assignment of the stereochemistry and ee of natural volicitin **1a**.

To test for their biological activity, both enantiomers of synthetic volicitin **1a** and **1b**, respectively, were applied as described[1,8] to freshly detached plantlets of the lima bean (*Phaseolus lunatus*) and cotton (*Gossypium hirsutum*).

However, both enantiomers failed to induce volatile biosynthesis in the plants, even at high concentrations (500 μM), while feeding caterpillars induced a significant level of volatiles. Considering the high ee of natural and synthetic volicitin (*N*-(17*S*)-17-hydroxylino-

Table 1.

Caterpillar species	Optical purity of (17*S*)-17-hydroxymethyl linolenoate **2a**[a]
S. exigua	94% ee
S. littoralis	94% ee
S. frugiperda	96% ee
H. virescens	92% ee

[a] Determined as (1′*R*)-17-(1′-phenyl-ethylcarbamoyloxy)-methyl linolenoate.

nolenoyl-L-glutamine) **1a**, antagonistic effects from enantiomeric mixtures cannot account for the lack of bioactivity in our assays. Instead, the above findings support the view that the insect's oral secretions and regurgitants comprise more than a single compound endowed with the capability to induce plant defence reactions. Volicitin **1a** should not, therefore, be considered as a 'general elicitor' of plant volatile biosynthesis but appears to be active in certain plants or plant cultivars only. Detailed comparative studies with other plants and other elicitor-active compounds, such as coronatine[9] and indanoyl conjugates with L-isoleucine,[10] will be reported in due course.

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7. 100 μl MeOH were added to 100 μl oral secretion from lepidopteran larvae and centrifuged (5 min, 13 000 U/min). The concentrated supernatant was treated with 100 μl MeOH and 100 μl acetanhydride (60°C, 30 min). After removal of the solvents, 100 μl toluene and 0.5 μl (1*R*)-1-phenyl-ethylisocyanate were added (120°C, 2 h). After concentration to 10 μl, 1 μl was injected and analysed by GLC/MS (fused silica EC5: 15 m×0.25 mm; carrier gas: He at 1 ml/min) under programmed conditions: 210°C (2 min), at 1°C/min to 255°C, then at 10°C/min to 300°C (5 min). For accurate analysis of the diastereomers of **4a**/**b** the linolenoates were hydrogenated using Pt/C (10%) as the catalyst. The resulting stearates were re-analysed by GLC/MS on the same column. Selected spectroscopic data for **4b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.19 (3 H, d, $J=6.3$), 1.2–1.3 (8 H, m), 1.39 (3 H, d, $J=6.8$), 1.55 (2 H, m), 2.23 (2 H, t, $J=7.6$), 2.72 (2 H, m), 2.85 (2 H, s, br), 1.94–2.00 (2 H, m), 4.71–4.86 (2 H, m), 5.20–5.42 (6 H, m), 5.49 (1 H, dq, $J=6.3$, $J=7.3$), 7.15–7.3 (5 H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 20.09, 21.59, 23.94, 24.61, 25.09, 26.19, 28.07, 28.10, 28.14, 28.54, 33.10, 49.56, 50.41, 66.41, 124.88, 126.18, 126.23, 126.30, 126.55, 127.60, 128.02, 129.18, 129.42, 129.52, 142.76, 154.13, 173.29. EI-MS (m/z): 59, 79, 91, 105, 147, 259, 261, 290, 455. HR-MS calcd: 455.3033; found: 455.3036.
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