



Identification of 10-desoxyiridal as an intermediate in the biosynthesis of iridals

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Abstract

The metabolism of iridals, unusual triterpenoids found in sword lilies, was studied in a cell culture of *Iris pseudacorus* Linn. The cells contain mainly spiroiridals, esterified with capric acid, which are hydrolyzed by esterases upon cell disruption. In ether extracts of these cell homogenates 10-desoxyiridal, a hitherto unknown precursor of the iridals, was identified by comparison with a semisynthetic standard. The compound was isolated and its structure verified by spectroscopic means. Upon incubation of the cells with 3-amino-1,2,4-triazole, a bleaching agent, the amounts of iridal and 10-desoxyiridal in the cells increase, whereas the level of the conjugated iridotrienes is diminished. This proves that 10-desoxyiridal plays a central role in iridal biosynthesis and that the conjugated triene moiety of the iridotrienes is formed by the action of a desaturase on iridal. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The iridals (e.g. 1–3) and 10-desoxyiridals (e.g. 4) are unusual monocyclic, bicyclic or spirobicyclic triterpenoids, which are found in the lipid extracts of Iridaceae (Marner, 1997). Their biosynthesis undoubtedly is initiated by cyclization of 2,3-epoxysqualene to a bicyclic intermediate, which subsequently is rearranged to give the characteristic *seco*-ring A structure of these natural products (Marner, 1997). In the course of this reaction a CH₃ group is shifted from C6 to C11.¹ For stereochemical reasons we assume that 2,3-epoxysqualene is folded in a chair-boat form during this process and the first product to be expected is the aldehyde 5, which may be converted via several steps to 10-desoxyiridal 6 (Scheme 1). Both compounds have so far not been detected in nature, but the latter is the most plausible educt for all iridals and 10-desoxyiridals known today (Marner, 1997). Thus, the action of a monooxygenase should, with retention of the configuration at C10, give iridal 1, whereas oxidation of the side chain of 6 should result in the formation of 10-desoxy-17-hydroxyiridal 4 or its homologues. Iridal 1

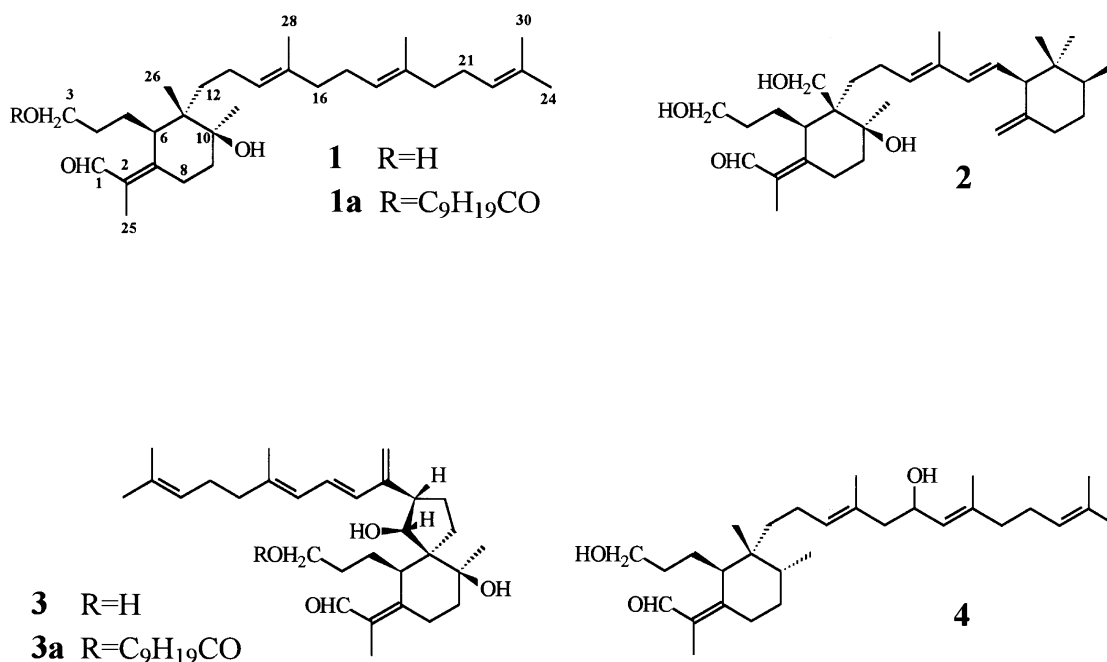
serves most likely as precursor of the other triterpenoids. Thus, it has been shown by incubation of an *Iris pallida* cell culture with various precursors that the conjugated triene moiety of the spiroiridals (e.g. 3) is formed by the action of a dehydrogenase on iridal 1 or 26-hydroxyiridal 7, respectively (Scheme 2) (Marner, Ritzdorf, & Johnen, 1993). Although compound 7 in this study undoubtedly was accepted by the dehydrogenase as a substrate, it was not possible to rule out that the formation of the 26-hydroxytriene 9 also may proceed via dehydrogenation of 1 and subsequent oxidation of the iridotriene 8 at C26 (Marner et al., 1993). We set out to get further insight into these reaction sequences by incubating a cell culture of *I. pseudacorus*, which synthesizes appreciable amounts of spiroiridals, with an appropriate enzyme inhibitor. The addition of a dehydrogenase inhibitor should, for instance, suppress the formation of the conjugated triene and lead to the accumulation of the respective precursors. We report here on the results of these studies.

2. Results and discussion

It seemed probable that the inhibition of the dehydrogenase as a key step in the iridotriene biosynthesis might not only lead to the accumulation of 26-hydroxyiridal 7

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¹The carbon skeleton of all compounds is numbered in analogy to squalene.



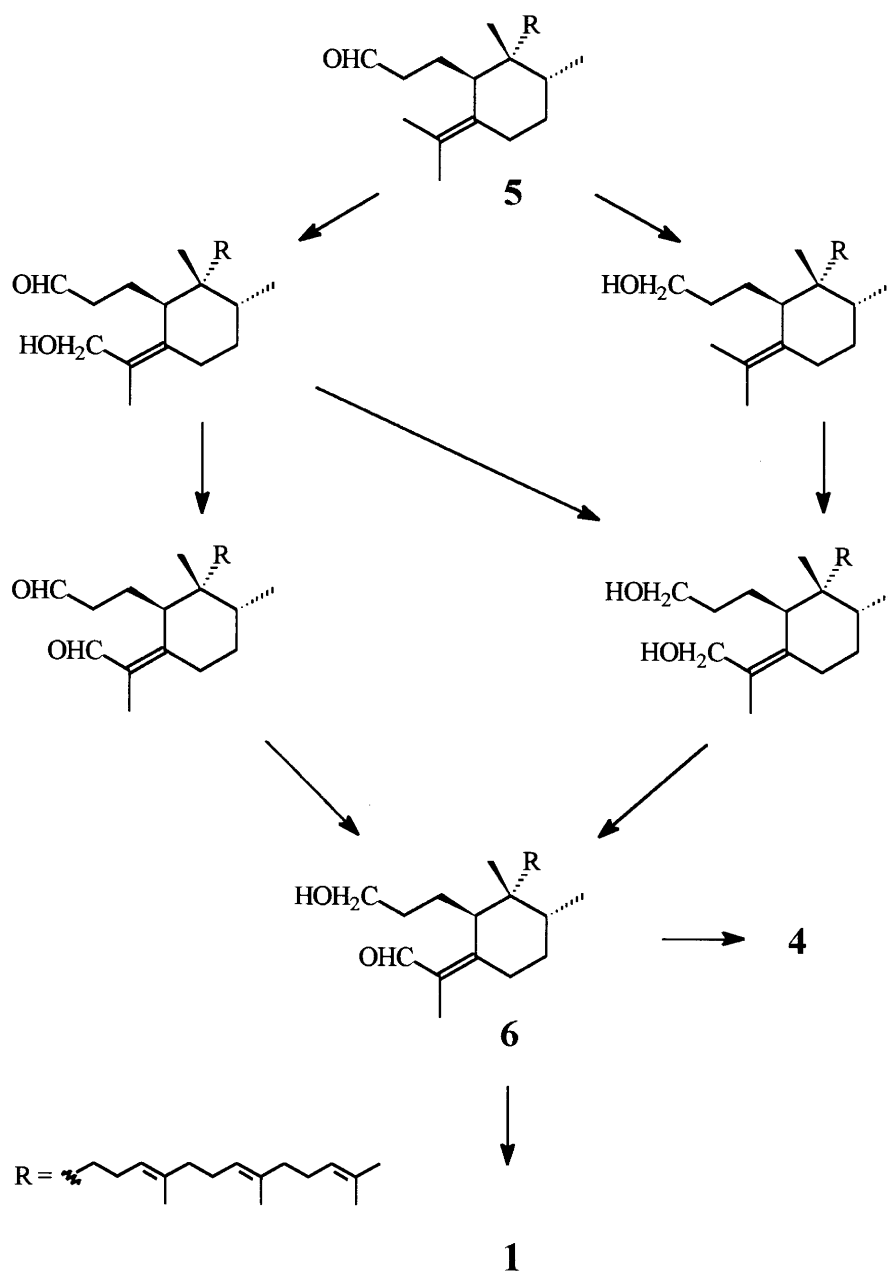
Structure 1.

or iridal **1** but also of one or more of its precursors, which are depicted in Scheme 1. Of special interest was 10-desoxyiridal **6** as a central intermediate in the proposed biosynthetic pathway. Since it was not at hand as a natural product, a semisynthetic access to this compound was worked out, which is outlined in Scheme 3. 10-Desoxy-17-hydroxyiridal **4**, which can be isolated from extracts of *I. sibirica* in reasonable amounts (Marner & Longerich, 1991), served as starting material. After reduction of the aldehyde function and protection of the two primary hydroxy groups as benzoates, the secondary OH in the terpenoid side chain was converted to the bromide. Subsequent reaction with LiAlH₄ lead in one step to removal of the protecting ester groups and reduction of the halide. Finally, the allylic alcohol was selectively oxidized with MnO₂ and 10-desoxyiridal **6** was obtained. With the help of this synthetic standard we tried to track down the substance in extracts of the cultured cells.

Upon HPLC analysis of total lipid extracts of the *I. pseudacorus* cells, prepared by soaking freshly harvested cells in CHCl₃/MeOH (1:2 v/v) according to Bligh and Dyer (1959), the three spiroiridals, esterified at C3 with capric acid, **3a** (56%), **12a** (11%) and **13a** (27%) were found as main products adding up to more than 90% of the iridal content. Side products are the capric acid esters of iridal **1a** (4%) and 16-hydroxyiridal **14** (2%). Isolation of a small amount of the compounds by semipreparative HPLC and enzymatic hydrolysis with Lipozym 10000L, as described elsewhere (Marner et al., 1993), gave the free spiroiridals and monocyclic iridals, which were identified by comparison with authentic standards (Marner,

Karimi-Nejad, Jaenicke, & Wray, 1990; Littek & Marner, 1991). After transesterification of the compounds with CH₃OH/HCl the fatty acid was identified by GC/MS. The retention behaviour of synthetic **6** was identical with that of the spirotriene ester **13a** and therefore no 10-desoxyiridal could be detected in these extracts. Also the search for this compound in essential oils of several *Iris* species was unsuccessful. When the cultured cells of *I. pseudacorus* are homogenated in a mortar or by ultrasound under addition of phosphate buffer prior to extraction, however, the fatty acid esters of the iridals are hydrolyzed by esterases present in the cell homogenate. Reversed phase chromatography of an ether extract now showed the unesterified iridals and spiroiridals, which elute much earlier than their corresponding esters. In addition, now small amounts of 10-desoxyiridal **6** were detected. To ensure its structure, the substance was isolated and characterized by NMR (¹H, ¹³C, DEPT, H,H-COSY and H,C long range), mass and UV spectroscopy. The absolute configuration was assured in analogy to 10-desoxy-17-hydroxyiridal **4** (Marner & Longerich, 1991) and by NOE measurements.

The dehydrogenation of iridal **1** or 26-hydroxyiridal **7**, resulting in an all-*E* conjugated triene moiety, closely resembles the reactions catalyzed by phytoene desaturase in the biosynthesis of carotenoids. The inhibition of these desaturase reactions in plants by 'chlorosis inducing herbicides' leads to the lack of cyclic carotenoids, which are used for the protection of chlorophylls against photo-oxygenation. Subsequently the chlorophylls are destroyed, as seen by the 'bleaching' of the plant, and finally the plants die. It has been shown that 3-amino-

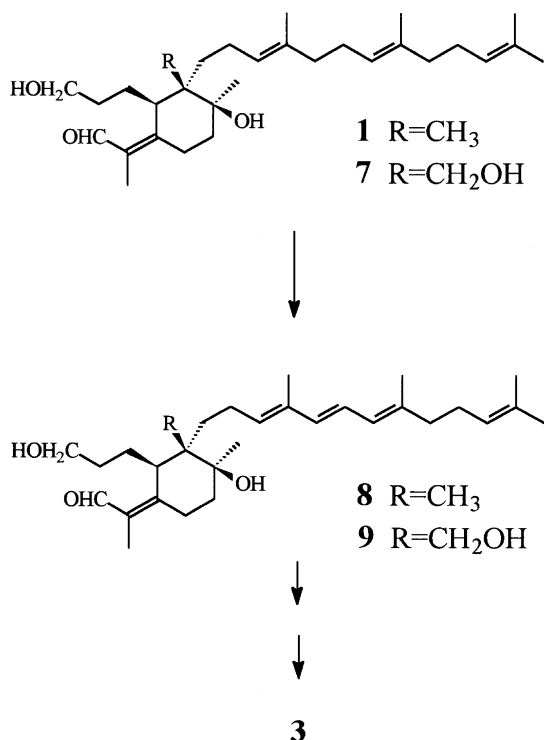


Scheme 1. Proposed biosynthesis of iridals and 10-desoxyiridals.

1,2,4-triazole (amitrole) belongs to this group of reagents. Thus, the treatment of cell suspension cultures or germinating seeds with this compound leads to the accumulation of carotenoids with low degree of unsaturation, e.g. phytoene and phytofluene (Burns, Buchanan, & Carter, 1971; Hassal, 1990). When 3-amino-1,2,4-triazole is added to the culture medium of the *I. pseudacorus* cells, a significant change in the iridal composition was observed after a few days. As shown in Fig. 1, in comparison to a blind, in the treated culture within two weeks a 2-, respectively, 4-fold increase in the relative amounts of iridal **1** and 10-desoxyiridal **6** was observed. The percentage of 28-hydroxyspiroiridal **12** and 28-acetoxy-

spiroiridal **13** remained nearly constant, whereas the relative amount of spiroiridal **3** decreased by a factor of about 1.5 to less than 35%. It is noteworthy to mention that 10-desoxyiridal **6** is, in contrary to all other iridals, not esterified, as seen in Bligh and Dyer extracts of the aminotriazole treated cultures. In course of the incubation period the growing HPLC peak of compound **6** can be observed, which previously was hidden underneath the signal of **13a**.

These results alone, however, are not significant enough for a sound statement, if iridal **1** and 10-desoxyiridal **6** really accumulate due to a desaturase inhibition. Thus, these changes in the relative composition of iridals



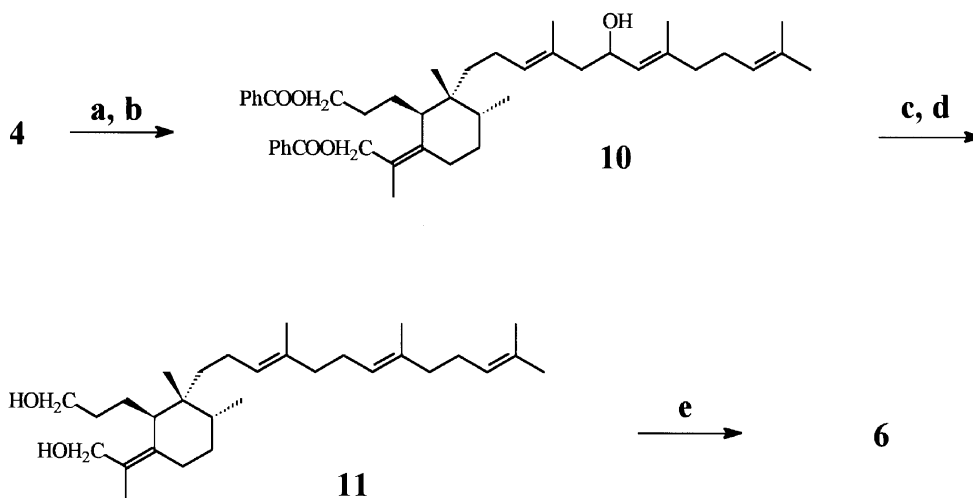
Scheme 2. Formation of the iridotrienes.

may also be observed, when the total of iridals in the cell culture decreases and when the spirotrienes **3**, **12** and **13** disappear at a faster rate than **1** and **6**. This may happen, for instance, when the cells die under the influence of aminotriazole and the iridal biosynthesis ceases. Since the conjugated trienes are much more susceptible to oxidative degradation than the less desaturated compounds **1** and **6** (Marner, 1997), the ratio of the latter to the former should increase in this situation. Therefore, it was essential to determine the cell growth and the absolute amount

of the compounds present in course of the incubation period.

The development of the fresh weight of the harvested cells is depicted in Fig. 2. It is obvious that within the first week of the aminotriazole incubation the treated and untreated cells grow at the same rate. From day 9 on the control cells grow significantly faster than the treated culture, which starts to die after 12 days. In untreated cultures of *I. pseudacorus* a slowdown of the cell growth is observed after two weeks (data not shown) due to an overpopulation in the culture vessel. It should be mentioned that the cells were transferred into fresh medium after 7 days, which in case of the treated culture again contained the inhibitor in the same concentration as before. This certainly sped up the inhibition of important enzymatic reactions, thus leading to the slower growth and finally the cell death observed. Fig. 3 shows the absolute amount of the triterpenoids within the cells in relation to their dry weight, which was determined after freeze drying the homogenated cells. Subsequently the cell powder was resuspended in water and after extraction with ether the unesterified compounds could be analyzed as before. The amount of iridals and spiroiridals present was determined by comparison with appropriate standards. As seen in Fig. 2 a steady decline in the contents of the spirotrienes **3**, **12** and **13** can be observed in the treated cell cultures, whereas the values in the control remain nearly constant. In contrast, the amount of the monocyclic precursors increases within the first week upon aminotriazole inhibition. From day 9 on also a drop in the quantity of these compounds is encountered which may be due to the increase in inhibitor as mentioned above. As for the trienes, also the amount of **1** and **6** is unchanged within the blind throughout the culture period.

These results undoubtedly point to an inhibition of the desaturase, which is responsible for the formation of the

Scheme 3. Synthesis of 10-desoxyiridal **6**. Reagents: (a) NaBH₄, (b) PhCOCl, pyridine, (c) Ph₃PBr₂, (d) LiAlH₄ and (e) MnO₂.

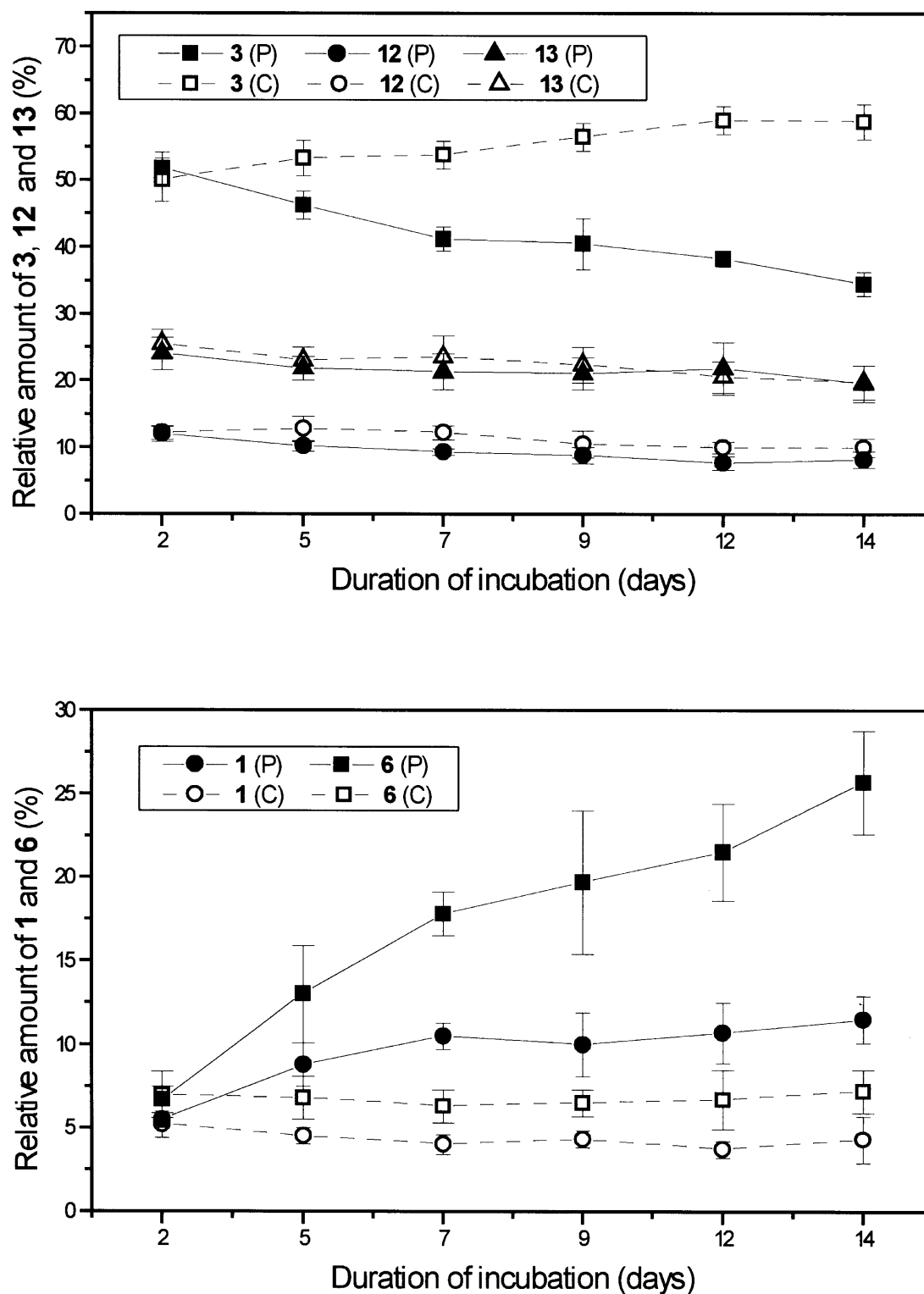
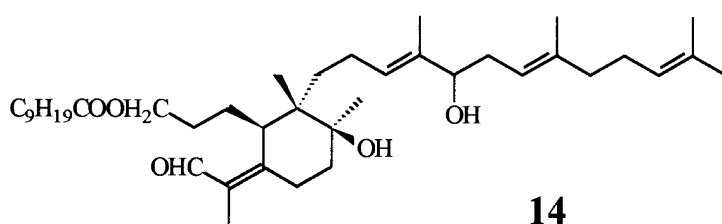
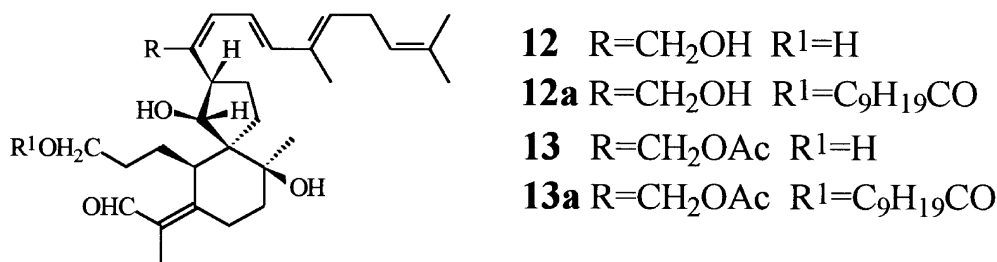


Fig. 1. Iridal composition (%) of an *I. pseudacorus* cell culture upon incubation with 3-amino-1,2,4-triazole. Top: spiroiridal **3**, 28-hydroxyspiroiridal **12** and 28-acetoxyspiroiridal **13**. Bottom: iridal **1** and 10-desoxyiridal **6**. Straight lines: probe and dashed lines: control.

conjugated triene. Certainly, iridal **1** is its natural substrate and not 26-hydroxyiridal **7**, at least in the cell strain of *I. pseudacorus* used in these experiments, since otherwise the latter compound should have accumulated.

Also, neither iridotriene **8** nor 26-hydroxytriene **9** could be found in the treated cells. Therefore, it can be excluded that aminotriazole prevents only the formation of the spirobicyclic ring system, which presumably is the prod-



Structure 2.

uct of an intramolecular Prins reaction after oxidation of C26 to an aldehyde (Märner, 1997). It can be concluded from the data shown in Figs 1 and 3 that the drop in the levels of 28-hydroxyspiroiridal **12** and its acetate **13** is less pronounced than that of their immediate precursor **3**. Therefore the oxidation of C28 and its acetylation are not affected in the same way by the presence of aminotriazole as the desaturation and formation of the spi-

robicyclic triene moiety. Since most of the other iridal precursors, outlined in Scheme 1, lack a chromophore, it was not possible to detect them with the analytical methods used. It should, however, be possible to establish their presence with other detectors, e.g. LC/MS. Corresponding studies are underway and it will be interesting to find out if it is possible to trace the biosynthetic pathway of the iridals this way further back to epoxysqualene.

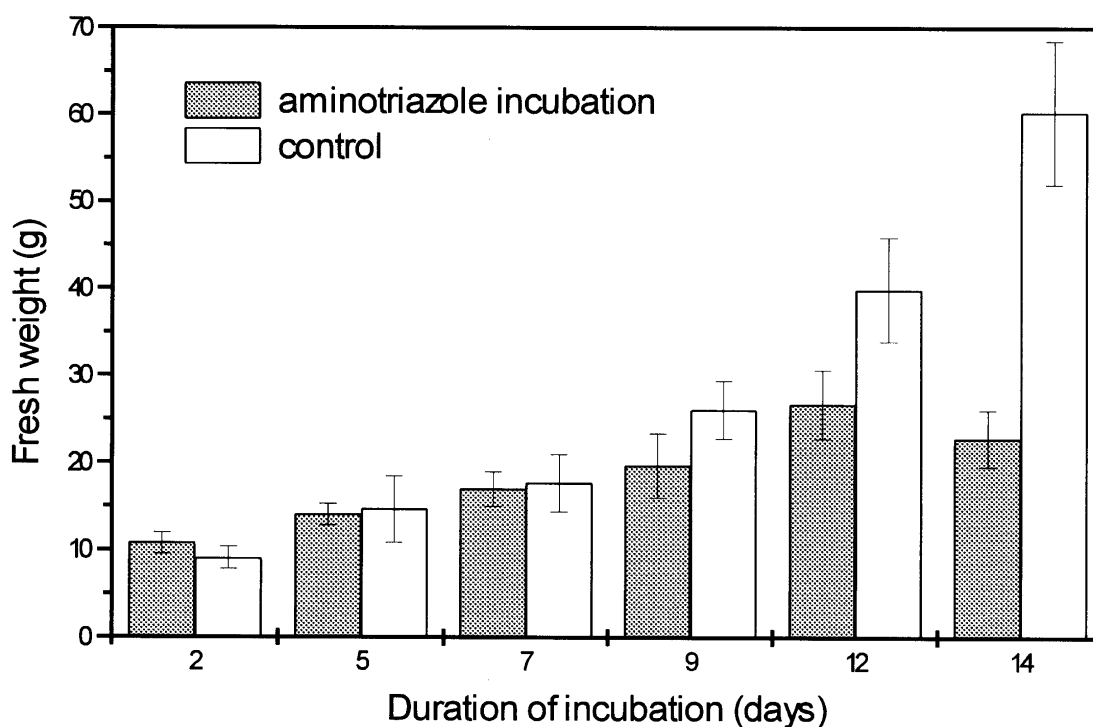


Fig. 2. Growth of an *I. pseudacorus* cell culture (fresh weight of cells) upon incubation with 3-amino-1,2,4-triazole.

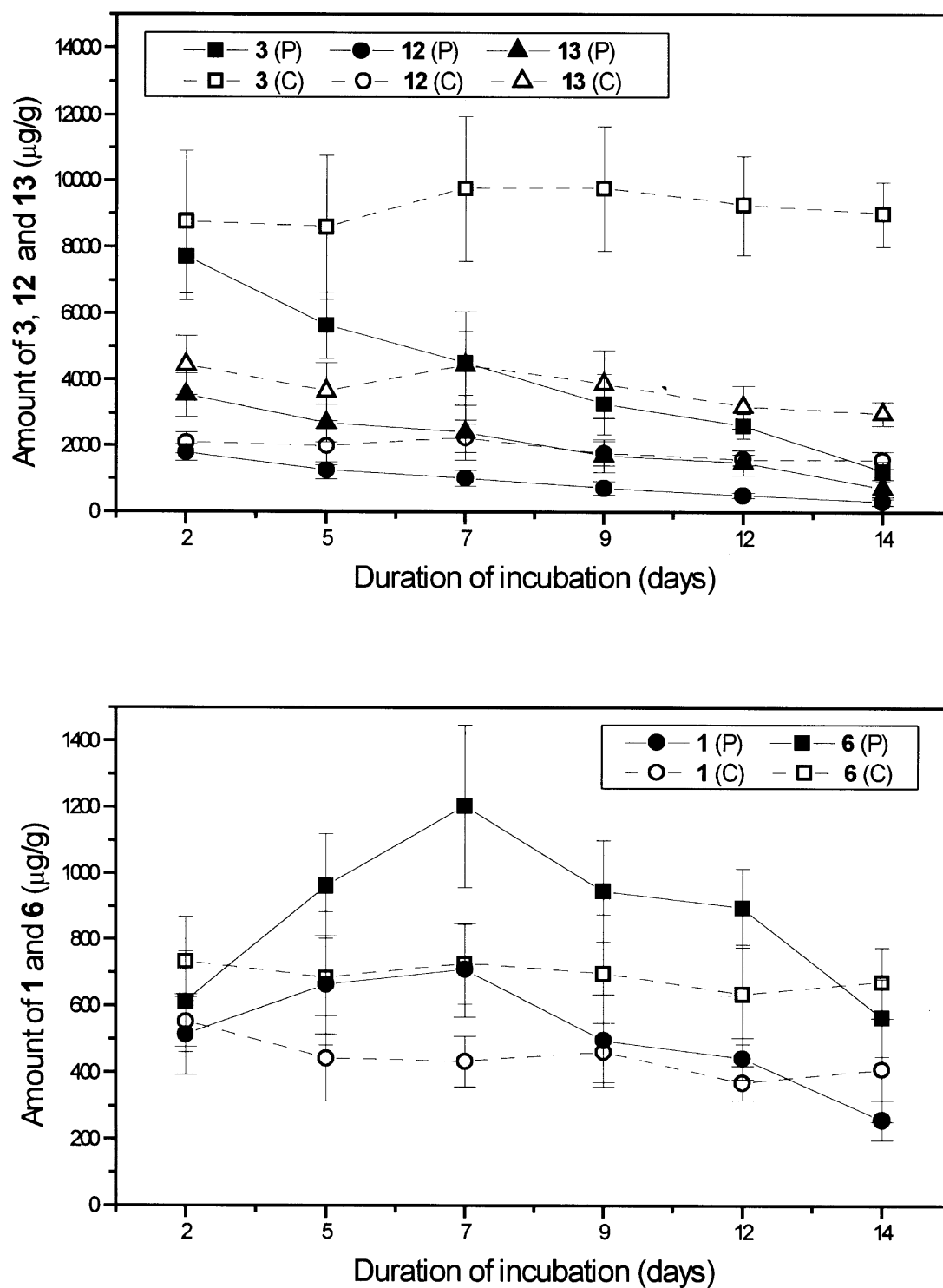


Fig. 3. Absolute amount of iridals (μg/g dry weight) in *I. pseudacorus* cells upon incubation with 3-amino-1,2,4-triazole. Top: spiroiridal 3, 28-hydroxyspiroiridal 12 and 28-acetoxyspiroiridal 13. Bottom: iridal 1 and 10-desoxyiridal 6. Straight lines: probe and dashed lines: control.

3. Experimental

3.1. General

HPLC: Kontron model 200, column: LiChrocart RP 18 (125 mm, Merck); solvent: MeOH/H₂O 7:3 (5 min), linear gradient to 100% MeOH (15 min), 100% MeOH (20 min); Hewlett-Packard 1040A diode-array detector. Flash CC: Merck Kieselgel 60 (40–63 μ m). MS: Finnigan-MAT 4510 GC/MS (EI: 70 eV), inlets: capillary column OV1 (15 m, 0.25 mm i.d.) and solid probe. NMR: Bruker AM 300 (¹H: 300 MHz, ¹³C: 75 MHz), solvent CDCl₃. UV: Hitachi U 2000, solvent MeOH. Ultrasound processor: Dr. Hieschler UP 400S. Freeze drying: Leybold Lyovac GT2.

3.2. Compounds

The iridals used in this study as standards were isolated from extracts of *I. foetidissima* as described elsewhere (Marner et al., 1990; Littek & Marner, 1991). 6*S*,10*R*,11*R*-10-Desoxyiridal **6** was synthesized as follows.

3.2.1. 6*S*,10*R*,11*R*,17 ξ -1,3-Dibenzoyl-1-dihydro-17-hydroxy-10-desoxyiridal, **10.** 460 mg (1 mmol) 6*S*,10*R*,11*R*,17 ξ -10-desoxy-17-hydroxyiridal, **4**, obtained from extracts of *I. sibirica* (Marner & Longerich, 1991), were dissolved in 50 ml MeOH. 60 mg (1.6 mmol) NaBH₄ were added and the soln was stirred for 2 h at RT. After concentration in vacuo, 50 ml satd NH₄Cl soln were added and the cloudy soln was extracted twice with 50 ml Et₂O. The organic phase was washed with H₂O, dried (Na₂SO₄) and evaporated to dryness. The product (425 mg, 0.9 mmol), dissolved in 25 ml CH₂Cl₂ was cooled to –40°C. Pyridine (600 mg, 7.6 mmol) and benzoyl chloride (320 mg, 2.3 mmol) were added and the soln was stirred for 8 h. After warming to RT the reaction mixture was diluted with 20 ml CH₂Cl₂, washed with H₂O and dried (Na₂SO₄). After evapn of the solvent the product was purified by flash CC on silica gel with pentane/Et₂O (80:20, v/v) to yield 220 mg (36%) of the diester. EI-MS *m/z* (rel. int.): 650 [M⁺-H₂O] (0.1), 528 (1.5), 459 (1), 404 (2), 394 (3), 105 (100), 69 (50); ¹H NMR: δ 8.02–7.92 (4H, AB, Phe-H), 7.52 (2H, m, Phe-H), 7.44–7.30 (4H, AB, Phe-H), 5.2–5.05 (3H, m, H-14, H18 and H-22), 4.90 (2H, d, H-1), 4.42 (1H, m, H-17), 4.25 (2H, m, H-3), 2.68 (1H, m, H-6), 2.15–1.85 (xH), CH₂, CH), 1.82 (3H, s, H-25), 1.66 (3H, s, CH₃), 1.62 (3H, s, CH₃), 1.60 (3H, s, CH₃), 1.54 (3H, s, CH₃), 0.90 (3H, s, H-26), 0.80 (3H, d, H-27).

3.2.2. 6*S*,10*R*,11*R*-1-Dihydro-10-desoxyiridal, **11.** 260 mg (1 mmol) triphenylphosphine were dissolved in 2.5 ml benzene and a soln of 160 mg (1 mmol) Br₂ in 1.5 ml benzene was added dropwise. After 15 min at RT the light yellow suspension of PPh₃Br₂ was cooled to 5°C and

the diester **10** (220 mg, 0.32 mmol), dissolved in 1 ml benzene, was added. The reaction mixture was stirred for 2.5 h, filtrated and evaporated at RT. The crude product was dissolved in 5 ml THF and a soln of 800 mg LiAlH₄ in 7 ml THF was added at –20°C within 4 h. Subsequently H₂O (1 ml) and Et₂O (15 ml) were stirred in and the mixture was allowed to warm to RT. The organic phase was washed with satd NH₄Cl soln, dried (Na₂SO₄) and evaptd. The product was purified by flash CC on silica gel (pentane/Et₂O 30:70 v/v) to give 25 mg (20%) **11**. EI-MS *m/z* (rel. int.): 444 [M⁺] (0.5), 426 [M⁺-H₂O] (4), 287 (3.5), 274 (2), 134 (39), 95 (58), 81 (54), 69 (100); ¹H NMR: δ 5.15–4.95 (3H, m, H-14, H18 and H-22), 4.12 (2H, AB, *J*_{AB}=8.5 Hz, H-1), 3.6 (2H, t, *J*=7 Hz, H-3), 2.60 (1H, m, H-6), 2.15–1.85 (xH), CH₂, CH), 1.82 (3H, s, H-25), 1.70 (3H, s, CH₃), 1.58 (9H, s, 3 CH₃), 0.90 (3H, s, H-26), 0.78 (3H, d, H-27). The compound contained 22% of the Δ 17,18 isomer, formed by allylic shift of the Δ 18,19 double bond during the LiAlH₄ reduction. This is seen by signals at δ 5.28 (2H, m, H17 and H18) and 2.59 (2H, d, H-16).

3.2.3. 6*S*,10*R*,11*R*-10-Desoxyiridal, **6.** 25 mg (0.056 mmol) of the diol **11** and 185 mg MnO₂ (2.13 mmol) in 3 ml CH₂Cl₂ were shaken at RT for 16 h. The soln was filtrated over 100 mg silica gel and evaptd in vacuo. The crude product was purified by flash CC on silica gel (30% Et₂O) to yield 12 mg (48%) **6**, containing 22% of the Δ 17,18 isomer. The spectral characterization can be seen in Section 3.5.

3.3. Cell culture

Callus cultures of *I. pseudacorus* were prepared by placing sterile cuts of a seedling on agar plates (0.8% agar) containing the medium described below. After several weeks of growth a suspension culture was established from the calli. The cells were raised in a *Linsmaier-Skoog* medium (Linsmaier & Skoog, 1965) pH 6.0 with 3% sucrose, 1.2 μ M thiamine hydrochloride, 0.56 μ M myo-inositol, 1 μ M 2,4-dichlorophenoxyacetic acid and 1 μ M 1-naphthaleneacetic acid. The cultures were cultivated at continuous light (300 lx) and 23°C on a gyratory shaker (100 rpm). They were subcultured every 7 days by transferring 10 g of the old cells to 250 ml of fresh medium in a 1 l Erlenmeyer flask. During this time the fresh weight of the cells doubled.

3.4. Extraction and analysis of the iridals

For the extraction of the lipids according to Bligh and Dyer (1959) the cells were filtrated through a sieve and extracted twice with CHCl₃/MeOH (1:2, v/v, 50 ml). After evaporation of the solvent the residue was partitioned between Et₂O (2 $\times$ 100 ml) and H₂O (50 ml), the organic phase was separated, dried (Na₂SO₄) and the solvent was

evaporated in vacuo. A soln of the crude oil in MeOH was analyzed by HPLC.

3.5. Isolation of 6*S*,10*R*,11*R*-10-desoxyiridal, **6**

50 g of freshly harvested cells were mixed with 50 ml buffer solution (0.1 M KH_2PO_4 , 1 mM EDTA, 1 mM glutathione, pH 6.2) and 10 g quartz sand and ground in a mortar. The homogenate was pressed through a nylon net (50 μm), allowing also the sand particles to pass. The filtrate was discarded and the residue was stirred 4–6 h with 100 ml Et_2O , transferred into a Waring blender and mixed for several minutes. The organic phase was separated and the aqueous phase mixed two more times with Et_2O in the blender. The combined ether phases were dried and evaptd to give 185 mg of crude extract. 400 mg of the oil were separated on silica gel (10 g) using a pentane/ Et_2O gradient. The fraction eluting with pentane/ Et_2O (20:80 v/v) was rechromatographed on silica gel with pentane/ Et_2O (25:75 v/v) to give 6 mg of pure **6**. UV λ_{max} nm (ϵ): 254 (12500); EI-MS m/z (rel. int.): 442 [M^+] (3), 424 [$\text{M}^+ - \text{H}_2\text{O}$] (2), 205 (7.5), 109 (47), 95 (48), 81 (53), 69 (100), 55 (45); ^1H NMR: δ 10.18 (1H, s, H-1), 5.07 (1H, m, H-22), 5.05 (1H, m, H-18), 4.96 (1H, t, $J=6$ Hz, H-14), 3.6 (2H, t, $J=6.3$ Hz, H-3), 3.35 (1H, dd, $J=7.7/6.9$ Hz, H-6), 2.63/2.15 (2H, m, H-8), 2.02 (2H, m, H-17), 1.93 (4H, m, H-16, H-20), 1.88 (1H, m, H-10), 1.79 (2H, m, H-13), 1.78 (3H, s, H-25), 1.65 (3H, s, H-24), 1.63 (2H, m, H-5), 1.60/1.38 (2H, m, H-9), 1.57 (3H, s, H-30), 1.55 (3H, s, H-29), 1.49 (3H, s, H-28), 1.3 (2H, m, H-4), 1.18/1.09 (2H, m, H-12), 0.96 (3H, s, H-26), 0.80 (3H, d, $J=6.8$ Hz, H-27); ^{13}C NMR: 190.0 (d, C-1), 163.3 (s, C-7), 135.2 (s, C-15), 134.9 (s, C-19), 133.3 (s, C-2), 131.2 (s, C-23), 124.4 (d, C-14), 124.4 (d, C-22), 124.2 (d, C-18), 63.0 (t, C-3), 43.3 (d, C-6), 40.1 (s, C-11), 39.7 (t, C-16), 39.7 (t, C-20), 35.7 (d, C-10), 31.8 (t, C-12), 31.5 (t, C-4), 30.5 (t, C-9), 27.4 (t, C-8), 26.8 (t, C-21), 26.6 (t, C-17), 25.7 (q, C-24), 24.2 (q, C-26), 24.0 (t, C-5), 21.1 (t, C-13), 17.7 (q, C-30), 16.0 (q, C-29), 15.9 (q, C-28), 15.2 (q, C-27), 10.2 (q, C-25).

3.6. Incubations

10.5 mg 3-amino-1,2,4-triazole (0.125 mmol) (Sigma) dissolved in 200 μl ethanol were given to 250 ml freshly

autoclaved medium in a 1 l Erlenmeyer flask and the solution was added to 10 g of the cells. The medium of the control culture was mixed with 200 μl ethanol only. For incubation periods longer than a week, after 7 days the cells were transferred into 500 ml fresh medium containing 0.5 mM aminotriazole, respectively, the appropriate amount of ethanol. After the appropriate incubation time 10 g of the cell material was mixed with 10 ml buffer soln (0.1 M KH_2PO_4 , 1 mM EDTA, 1 mM glutathione, pH 6.2), treated with ultrasound and the homogenate was freeze dried. After determination of the dry weight (the weight of the buffer salts was subtracted) the material was resuspended in 10 ml H_2O and extracted once with 70 ml and two times with 50 ml Et_2O . The combined organic phases were dried (Na_2SO_4) and evaptd in vacuo. A 1% soln of the extract in MeOH was used for the quantitative analysis of the iridals by RP HPLC. For the determination of the relative composition the response of the compounds at 254 nm was used, since it is known that the molar extinction coefficient of the iridals at this wavelength is equal (Marner & Kerp, 1992). 10-Desoxyiridal **6** and spiroiridal **3** served as standards for the calculation of the absolute amount present.

Acknowledgements

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References

- Bligh, E. G., & Dyer, W. J. (1959). *Can. J. Biochem. Physiol.*, 37, 911.
- Burns, E. R., Buchanan, G. A., & Carter, M. C. (1971). *Plant Physiol.*, 47, 144.
- Hassal, K. A. (1990). *The biochemistry and use of pesticides*. Weinheim/New York/Basel/Cambridge: Verlag Chemie.
- Linsmaier, E. M., & Skoog, F. (1965). *Physiol. Plant.*, 18, 100.
- Littek, A., & Marner, F.-J. (1991). *Helv. Chim. Acta*, 74, 2035.
- Marner, F.-J. (1997). *Curr. Org. Chem.*, 1, 153.
- Marner, F.-J., & Kerp, B. (1992). *Z. Naturforsch.*, 47c, 21.
- Marner, F.-J., & Longerich, I. (1991). *Liebigs Ann. Chem.*, 269.
- Marner, F.-J., Karimi-Nejad, Y., Jaenicke, L., & Wray, V. (1990). *Helv. Chim. Acta*, 73, 433.
- Marner, F.-J., & Ritzdorf, I., Johnen, G. (1993). *Phytochemistry*, 33, 573.