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Jasmonates and related compounds from *Fusarium oxysporum*

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Abstract

The culture filtrate of *Fusarium oxysporum* f sp *matthiolae* was inspected on the occurrence of jasmonates and related compounds. Among compounds described for the first time of biological origin are 7-*iso*-cucurbic acid, (1*S*,2*S*)- and (1*S*,2*R*)-3-oxo-2-pentylcyclopentane-1-butyric acid, (1*S*,2*S*)- and (1*S*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-hexanoic acid, (1*S*,2*S*)- and (1*S*,2*R*)-3-oxo-2-pentylcyclopentane-1-hexanoic acid, (1*S*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-octanoic acid, (1*S*,2*S*)-3-oxo-2-pentylcyclopentane-1-octanoic acid and *N*-[9,10-dihydro-7-*iso*-jasmonoyl]-(*S*)-isoleucine. The following metabolites were identified for the first time for this fungus: (−)-Jasmonic acid, 9,10-dihydrojasmonic acid and *N*-[(−)-jasmonoyl-(*S*)]-isoleucine were major constituents of the culture filtrate, whereas as minor metabolites occurred *N*-[9,10-dihydrojasmonoyl]-(*S*)-isoleucine, cucurbic acid and 3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-butyric acid, 3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-octanoic acid and 3-oxo-2-pentylcyclopentane-1-octanoic acid. All cyclopentanones found carried a *cis*- or *trans*-attached side chain. Didehydrojasmonates, hydroxylated jasmonates or 12-oxophytodienoic acid could not be detected in the culture filtrate.

Keywords: *Fusarium oxysporum*; Deuteromycetes; Culture filtrate; (−)-Jasmonic acid; 9,10-Dihydro-jasmonic acid; Isoleucine conjugates; Octadecanoid-derived metabolites

1. Introduction

(−)-Jasmonic acid (**1**) and (+)-7-*iso*-jasmonic acid (**2**) are important members of the family of jasmonates which function as plant growth regulators (Meyer, Miersch, Büttner, Dathe & Sembdner, 1984; Koda, 1992; Creelman & Mullet, 1997; Wasternack & Parthier, 1997). They are widely distributed in plants including lower eukaryotes. Biogenetically they are derived from α-linolenic acid (Vick & Zimmerman, 1984) catalyzed by enzymes of the 'octadecanoid pathway' which contains 12-oxophytodienoic acid (**23**) as an intermediate compound. Reduction of the double bond of the cyclopentanone ring and β-oxidation of the carboxylic acid side chain lead finally to (+)-7-*iso*-jasmonic acid (**2**) (Hamberg & Gardner, 1992).

Jasmonates, their precursors, or their metabolites are known to accumulate in higher plants and in fungi (Creelman & Mullet, 1997). For fungi detailed analysis was performed on the occurrence of the various jasmonates in *Botryodiplodia theobromae* (Aldridge, Galt, Giles & Turner, 1971; Miersch, Preiss, Sembdner & Schreiber, 1987; Miersch, Schmidt, Sembdner & Schreiber, 1989; Miersch, 1991) and *Gibberella fujikuroi* (Cross & Webster, 1970; Miersch, Brückner, Schmidt & Sembdner, 1992). In *B. theobromae* (+)-7-*iso*-jasmonic acid (**2**) accumulates up to a level of 500 μg/ml culture filtrate. In addition, hydroxylated jasmonic acids, saturated or unsaturated derivatives and side-chain elongated compounds were found within this fungus. After long-term cultivation of the fungi or during isolation, jasmonates, carrying a *cis*-configurated side chain, may convert into compounds with a *trans*-attached side chain (Miersch et al., 1987; Vick, Zimmerman & Weisleder, 1979). Beside free jasmonates, *G. fujikuroi* forms isoleucine conjugates of the jasmonic acids **1** and **2**, or of the 9,10-dihydrojasmonic acids **11** and **12**. Inspection of 46 fungal strains revealed the occurrence of the jasmonic acids **1**, **2**, and of 9,10-dihydrojasmonic acid (**11**) as major constituents, whereas side chain modified jasmonates, includ-

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ing hydroxylated derivatives, were present in minor quantities in the fungi *Collybia confluens, Collybia dryophila, Coprinus alkalinus, Coprinus cinereus, Mycena tintinabulum, Phellinus laevigatus and Trametes versicolor* (Miersch, Günther, Fritsche & Sembdner, 1993). Recently, **1** was identified in the ectomyccorhizal fungi *Laccaria laccata* and *Pisolithus tinctorius* (Regvar, Miersch & Gogala, 1998).

Jasmonates are discussed to be an important signal in plant responses to various biotic and abiotic stresses (Creelman & Mullet, 1997; Wasternack & Parthier, 1997). Wounding, osmotic stress, touch of tendrils, and pathogen attack led to an accumulation of jasmonates or its precursor 12-oxophytodienoic acids (**23**), followed by the expression of specific genes coding for proteinase inhibitors, enzymes of phytoalexine synthesis or membrane degradation, and defense proteins such as thionins and defensins (Creelman & Mullet, 1997; Wasternack & Parthier, 1997; Weiler, 1997). Thionins are a family of usually basic cysteine-rich peptides with toxic and antimicrobial properties (Bohlmann, 1994).

In *Arabidopsis thaliana* thionins are expressed upon inoculation with the pathogenic fungus *Fusarium oxysporum* f sp *matthiolae* (Epple, Apel & Bohlmann, 1995). *Arabidopsis* plants overexpressing the thionin THI 2.1 exhibit enhanced resistance (Epple, Apel & Bohlmann, 1997). Thionin 2.1 gene expression occurs upon jasmonate treatment, and seedlings inoculated with a *F. oxysporum* f sp *matthiolae* spore suspension accumulated jasmonates (Bohlmann, Vignutelli, Hilpert, Miersch, Wasternack et al., 1998. This suggests that jasmonates may be a signal in plant pathogen interaction. The fungal culture medium contains jasmonates and is highly active in inducing the thionin *Thi 2.1* expression suggesting that the jasmonates are formed by the fungus (Bohlmann et al., 1998).

Here, we show that *F. oxysporum* f sp *matthiolae* is able to form numerous jasmonates and compounds originating from the octadecanoid pathway. Among 22 identified compounds ten were isolated from a plant source for the first time. (−)-Jasmonic acid, its isoleucine conjugate and 9,10-dihydrojasmonic acid were detected as the main constituents within the culture filtrate.

2. Results and discussion

The culture filtrate of *Fusarium oxysporum* f sp *matthiolae* was separated by chromatographic methods using DEAE-Sephadex A25, Lichrolut RP-18 and Eurospher 100-C18 followed by HPLC and GC-MS identification. Structural elucidation of the compounds **1**–**22** was done by comparing retention times for HPLC and GC and fragments of MS spectra with that of authentic substances.

In the culture filtrate 22 compounds could be identified, 10 of them described for the first time for plants (Fig. 1, Table 1). Among the major constituents of the medium (−)-jasmonic acid (**1**) and (+)-7-*iso*-jasmonic acid were detected (**2**). Their methyl esters show the typical mass fragments m/z 224 M^+, loss of side chains m/z 156 $[M-C_5H_8]$, m/z 151 $[M-CH_2-CO_2Me]^+$ and m/z 83 for the pentanone ring part $[C_5H_7O]^+$. The compounds **1** and **2** were found in a ratio of about 9:1 as determined by GC. The same ratio was found for all other compounds with *trans*- or *cis*-attached side chains (**1**–**20**). In quantifications by GC-MS, jasmonic acid (**1**, **2**) was found to occur at about 492 ng/ml (Bohlmann et al., 1998). Similar quantities were found using an enzyme linked immunosorbent assay (ELISA) in which only the (−)-enantiomer is detected.

Other main constituents of the culture medium were 9,10-dihydrojasmonic acid (**11**) and its *cis*-isomer (**12**). Both of them represented 5% of the amount of **1** + **2** estimated from the GC curves. The mass spectra gave the M^+ value of 226 and fragments indicating loss of the

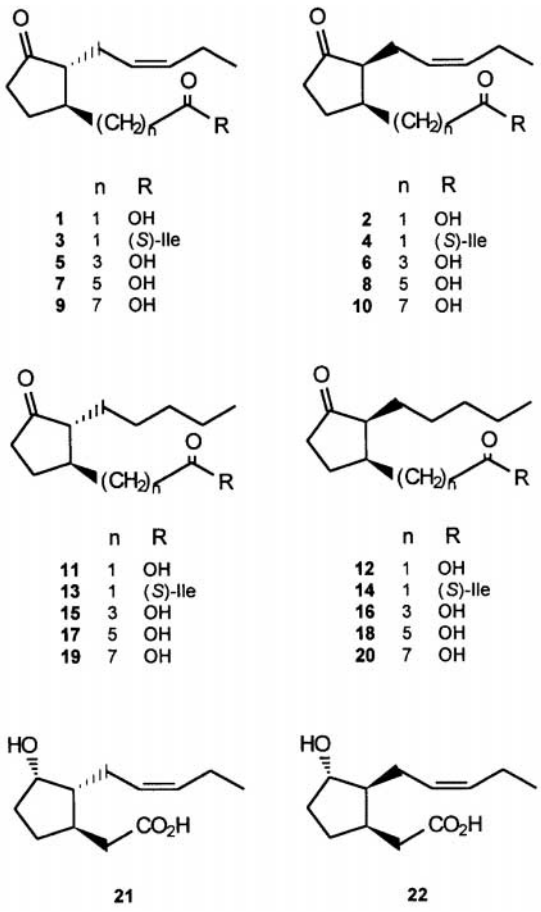

Fig. 1. Jasmonic acid and related compounds isolated from *Fusarium oxysporum*.

Table 1
Mass spectra of methyl esters of isolated compounds new for *F. oxysporum*

Compounds	1/2	3/4	5/6	7*/8*	9/10*	11/12	13/14*	15*/16*	17*/18*	19/20*	21*	22
$[M]^+$	224(32)	337(2)	252(4)	280(2)	308(1)	226(1)	339(1)	254(1)	282(1)	310(1)	226(1)	226(1)
$[M-H_2O]^+$	206(5)	319(2)	234(3)	262(5)	290(1)			236(1)	264(1)	292(1)	208(4)	208(3)
$[M-OMe]^+$	193(8)	306(1)	221(1)	249(3)	277(1)	195(1)		223(1)	251(2)	297(2)	195(7)	195(2)
$[M-C_5H_8]^+$	156(18)	269(2)	184(8)	212(11)	240(6)							
$[M-(C_5H_{10}]^+$							156(24)	269(4)	184(9)	212(8)	240(7)	
$[M-CO_2Me]^+$		278(4)					280(8)					
$[Ile + 2H]^+$		146(44)					146(46)					
$[CH_2COIle + H-C_4H_8]^+$		131(33)					131(31)					
$[CH_2COIle + H-CO_2Me]^+$		128(84)					128(87)					
$[Ile-CO_2Me]^+$		86(100)					86(100)					
$[M-(CH_2)CO_2Me-H_2O]^+$			133(14)								134(80)	134(38)
$[M-(CH_2)CO_2Me-H]^+$											152(39)	
$[M-(CH_2)CO_2Me]^+$	151(47)					153(27)						153(78)
$[M-(CH_2)_3CO_2Me]^+$			151(28)					153(24)				
$[M-(CH_2)_5CO_2Me]^+$					151(35)					153(14)		
$[M-(CH_2)_7CO_2Me]^+$						151(9)					153(66)	
$[C_5H_7O]^+$	83(100)		83(100)	83(100)	83(100)	83(100)		83(100)	83(100)	83(100)	83(41)	83(100)

(*) endogenous substances first described for plants given by ions, m/z and abundance in (%), Ile = *N*-isoleucine.

pentyl side chain (m/z 151 $[M-C_5H_{10}]^+$) and of the acetic acid side chain (m/z 153 $[M-CH_2CO_2Me]^+$). In addition, *N*-[(−)-jasmonoyl]-(*S*)-isoleucine (**3**) and *N*-[(+)-7-*iso*-jasmonoyl]-(*S*)-isoleucine (**4**) were detected at a level of up to 5% of the amount found for **1** + **2**. Both stereoisomeric forms clearly differed in their retention time in the GC. In elucidation of the structure by GC/MS, the isoleucine conjugates **3** and **4** exhibited characteristic fragmentation: m/z 337 for M^+ and fragments originating from the isoleucine moiety such as m/z 146 $[Ile + 2H]^+$, 131 $[CH_2COIle + H-C_4H_8]^+$, 128 $[CH_2COIle + H-CO_2Me]^+$ and the base peak 86 $[Ile-CO_2Me]^+$. The isoleucine conjugates **3** and **4** are formed by the fungus *Gibberella fujikuroi* (Cross & Webster, 1970; Miersch et al., 1992), and are commonly occurring compounds in higher plants (Schmidt, Kramell, Brückner, Schneider, Sembdner et al., 1990; Knöfel & Weiler, 1995). In barley leaves they are the main constituents of the JA amino acid conjugate fraction accumulating 10-fold upon stress like sorbitol treatment (Kramell, Atzorn, Schneider, Miersch, Brückner et al., 1995).

Biosynthetic precursors of **1** (Vick & Zimmerman, 1984) were also detected (**5**–**10**). Whereas **5**, **6** and **9** could be isolated before from fungi and higher plants (Miersch et al., 1989, 1993; Gundlach & Zenk, 1998), compounds **5**, **7** and **9** were also detected as metabolites formed after application of 12-oxophytodienoic acid (**23**) (Vick & Zimmerman, 1984). In *F. oxysporum*, compounds **5**–**10** occur in trace amounts (lower ng-range per ml culture filtrate). The prolonged carboxylic acid side chain of these compounds was indicated by the mass fragments $[M-C_5H_8]^+$ 184, 212 and 240 detected for compounds **5**/**6**, **7**/**8** and **9**/**10**, beside their M^+ fragments 252, 280 and 308, respectively, whereas the fragment 83 $[C_5H_7O]^+$ was characteristic for the cyclopentanone ring. Loss of the prolonged side chain always gave the fragment m/z 151 $[M-(CH_2)_nCO_2Me]^+$ as found in the mass spectrum of **1** (see Table 1).

Compounds **13** and **14** are the isoleucine conjugates of the 9,10-dihydrojasmonic acid (**9**) and 9,10-dihydro-7-*iso*-jasmonic acid (**10**), respectively, showing the typical fragments for methyl esters of isoleucine conjugates 278, 146, 131, 128 and 86 (compare with **3**/**4**) beside of the fragment $M^+ = 339$. Both conjugates (**13**, **14**) occur in lower amounts than their jasmonoyl derivatives **3**/**4**. So far, *N*-[9,10-dihydrojasmonoyl]-(*S*)-isoleucine (**13**) was detected only in the culture filtrate of *Gibberella fujikuroi* cultures (Cross & Webster, 1970).

The compounds **15**–**20** were found only in traces. Their methyl esters exhibited mass peaks of M^+ at m/z 254, 282 and 210. Their pentyl side chain was documented by the mass fragments m/z 153 $[M-(CH_2)_nCO_2Me]^+$ (loss of butanoic, hexanoic, octanoic moieties) and by the loss of the pentyl side chains $[M-C_5H_{10}]^+$: m/z 184, 212 and 240 in compounds **15**/**16**, **17**/**18** and **19**/**20**, respectively (cf. Table 1). The *trans*-configurated side chains of compounds **15**, **17** and **19** were also found as the *cis*-isomeric counterpart in compounds **16**, **18** and **20**, respectively.

7-*iso*-cucurbic acid (**21**), which was found recently as the (*S*)-isoleucine conjugate in *Pinus* pollen (Knöfel & Weiler, 1995) could be identified as the free acid. Mass fragments of **21**-Me ester were m/z 83 $[C_5H_7O]^+$ for a pentanol moiety, m/z 152 for the loss of an acetic acid side chain, m/z 134 for the loss of H_2O from fragment m/z 152, and 226 for M^+. The **21**-Me is clearly different from its isomer methyl cucurbate (**22**-Me) by the

base peak m/z 79 (structure unknown). Cucurbic acid Me ester (**22**-Me) differs in its fragmentation pattern by the base peak m/z 83 for $[C_5H_7O]^+$ and loss of CH_2CO_2Me with m/z 151. Additionally, the substance eluates later in GC analysis. Compound **22** was detected in *Cucurbita pepo*, three *Equisetum* sp. (Fukui, Koshimizu, Yamazaki & Usuda, 1977; Dathe, Miersch & Schmidt, 1989) and in culture filtrates of fungi *B. theobromae, Coprinus alkalinus* and *Collybia dryophila* (Miersch et al., 1987, 1993). The methyl ester of **22** was also identified in the absolute of *Boronia megastigma* (Weyerstahl, Marschall, Bork & Rilk, 1994). Here, both compounds (**21** and **22**) were detected in the culture filtrate only in the ng-range per ml culture filtrate.

There is a remarkable diversity among all these cyclopentane-containing compounds occurring in the culture filtrate of *F. oxysporum* f sp *matthiolae*. It is tempting to speculate on a biosynthetic pathway for jasmonates found in the culture filtrate (Fig. 2).

The common occurrence of **9**, **10**, **19** and **20** suggest a biosynthetic route similar to that proposed for higher plants (Vick & Zimmerman, 1984). This route originates from α-linolenic acid (18:3) and leads *via* 12-oxophytodienoic acid (**23**) to JA (**1**). A parallel pathway, originating from hexadeca-(7*Z*,10*Z*,13*Z*)-trienoic acid (16:3) and leading to dinor-12-oxophytodienoic acid (**24**), was described recently for *Arabidopsis* (Weber, Vick & Farmer, 1997).

All compounds with a pentyl-side chain (**11**-**20**) might be a substrate of a reductase (Vick & Zimmerman, 1984; Schaller & Weiler, 1997a). As shown recently, the PDA reductase of *Arabidopsis* exhibits sequence homology to WARBURG's yeast yellow enzyme, and the overexpressed protein as well as the enzyme purified from *Corydalis sempervirens* cell cultures were able to convert alkene structures (Schaller & Weiler, 1997a; Schaller & Weiler, 1997b).

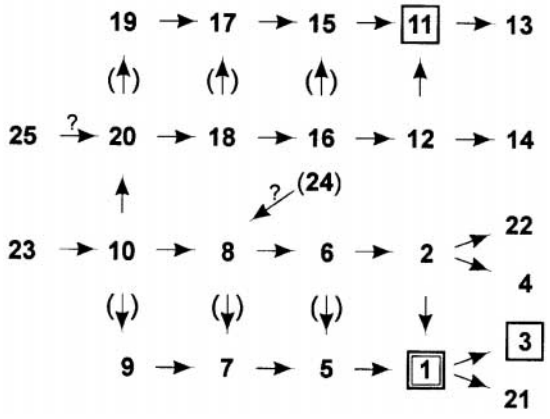

Fig. 2. Possible biosynthetic pathways for jasmonates detected in the culture filtrate of *Fusarium oxysporum*.

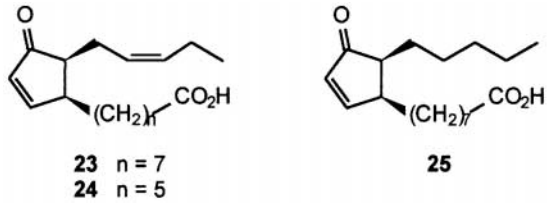

Fig. 3. Possible biogenetic precursors of jasmonates occurring in higher plants, but lacking in *Fusarium oxysporum* and other fungi studied so far.

A biosynthetic route for 9,10-dihydrojasmonic acid (**11**/**12**), originating from α-linoleic acid (18:2) *via* compound **25** was proposed for higher plants (Gundlach & Zenk, 1998). It remains to be elucidated whether fungi can catalyze similar reactions. In the culture filtrate of *F. oxysporum* no 12-oxophytodienoic acid (**23**), dinor-12-oxophytodienoic acid (**24**) or 15,16-dihydro-12-oxophytodienoic acid (**25**) could be found (Fig. 3). Since these compounds are intermediates in the formation of jasmonoyl-containing compounds from polyunsaturated fatty acids, the fungi seem to turnover octadecanoids very rapidly. Interestingly, *F. oxysporum* f sp *matthiolae* is unable to accumulate any side-chain-hydroxylated jasmonates as shown for *B. theobromae* or several other fungi (Miersch, 1991; Miersch et al., 1993). Furthermore, any ring or side-chain desaturated jasmonates like (−)-4,5-didehydrojasmonic acid and (+)-3,4-didehydro-7-*iso*-jasmonic acid (Miersch, 1991; Kaiser & Lamparsky, 1974), 11,12-didehydrojasmonic acid and (+)-11,12-didehydro-7-*iso*-jasmonic acid (Miersch et al., 1987), or 4,5-didehydro-9,10-dihydrojasmonic acid (Vick et al., 1979) could not be detected in the culture filtrate of *F. oxysporum*. 7-*iso*-cucurbic acid (**21**) and cucurbic acid (**22**) seem to be formed from **1** and **2**, respectively, by a specific reduction of the keto group into the (*S*)-configurated hydroxyl groups. The exclusive occurrence of isoleucine-conjugated jasmonoyl- and dihydrojasmonoyl derivatives (**3**, **4**, **13** and **14**) suggests, that conjugation needs a two-carbon carboxylic side chain. So far, jasmonoyl isoleucine conjugates were detected only in *Fusarium* which includes *G. fujikuroi*, a form of *Fusarium moniliforme* (Cross & Webster, 1970; Miersch et al., 1992). In higher plants jasmonic acid amino acid conjugates are regular constituents (Sembdner, Atzorn & Schneider, 1994) which accumulate like JA (**1**/**2**) upon sorbitol treatment (Kramell et al., 1995) or wounding (Dorans, Boland, Krumm, Atzorn, Kramell et al., 1998). In tomato leaves, the JA conjugates occur up to 10% of the JA level and are effective inducers of the JA/ethylene dependent proteinase inhibitor 2 gene expression (Dorans et al., 1998). In barley leaves, JA amino acid conjugates induce specific genes without being cleaved (Kramell, Miersch, Hause, Ortel, Parthier et al., 1997).

The diversity of octadecanoid and jasmonoyl compounds found in the culture filtrate of *F. oxysporum* f sp *matthiolae* rises the question whether these compounds are also formed in the interaction with plants such as *Arabidopsis* and, if it is so, what the function of these compounds might be. Recently, it could be shown by inhibition and mutant studies, that the octadecanoid pathway and the formation of jasmonates are ultimate steps in the expression of the thionin *Thi 2.1* occurring upon infection with *F. oxypsorum* f sp *matthiolae*. In this plant-pathogen interaction, the signal jasmonate and many other jasmonoyl compounds presented in this paper are generated by the fungus (Bohlmann et al., 1998).

In contrast, the fungal root pathogen *Pythium mastophorum* induces jasmonate responsive gene expression in *Arabidopsis*, and a mutant of *Arabidopsis* which is unable to form jasmonate is extremely susceptible to this pathogens (Vijayan, Shockey, Levesque, Cook & Browse, 1998), indicating that in this interaction jasmonate is formed by the plant.

3. Experimental

3.1. Source and synthesis of compounds tested

(±)-**1** and (±)-**11** were prepared from their methyl esters (Firmenich, Geneva, Switzerland) (Miersch et al., 1987) and used for the preparation of further jasmonates. Amino acid conjugates were synthesized and separated into the enantiomers as described (Kramell, Schmidt, Schneider, Sembdner & Schreiber, 1988; Schneider, Kramell & Brückner, 1989). (+)-7-*iso*-JA/(−)-JA(**2**/**1**) were isolated from *B. theobromae* culture filtrates (Miersch et al., 1987). Cucurbic acid (**22**) and 7-*iso*-cucurbic acid (**21**) were prepared from a mixture of (+)-7-*iso*-JA/(−)-JA by reduction with $NaBH_4$ (Dathe, Schindler, Schneider, Schmidt, Porzel et al., 1991). 3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-butyric acid [(±)-**5**/(±)-**6**], 3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-hexanoic acid [(±)-**7**/(±)-**8**], 3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-octanoic acid [(±)-**9**/(±)-**10**], were synthesized from (±)-**1** and succinic acid mono Et ester, adipinic acid mono Me ester and suberinic acid mono Me ester, followed by Kolbe-synthesis (Hamberg, Miersch & Sembdner, 1988) and subsequent saponification of the corresponding esters with sodium hydroxide. Starting from (±)-**11** and using the same procedure 3-oxo-2-pentylcyclopentane-1-butyric acid [(±)-**15**/(±)-**16**], 3-oxo-2-pentylcyclopentane-1-hexanoic acid [(±)-**17**/(±)-**18**], and 3-oxo-2-pentylcyclopentane-1-octanoic acid [(±)-**19**/(±)-**20**] were synthesized. All compounds were purified by column chromatography on silicagel (Merck) and HPLC and checked on purity by GC-MS.

3.2. Preparation of the culture filtrate

The fungus *F. oxysporum* f sp *matthiolae* (strain 247.61) was from the Centraalbureau voor Schimmelcultures, Baarn-Delft, The Netherlands. Microspores were produced by growing the fungus on PDA plates at ambient temp. on the lab bench for 3 weeks. Spores were separated of the plate and used for inoculation of 4×500 ml liquid potato-dextrose medium (Difco) in 2000 ml bottles. After 8 weeks incubation without shaking at ambient temp, the mycelium was filtered from the culture medium.

3.3. Extraction and isolation

50 ml culture filtrate was passed through a column (100×14 mm) filled with DEAE-Sephadex A25 (Pharmacia fine Chemicals, Stockholm, Sweden) (Ac^-form in MeOH). The gel was washed with 15 ml MeOH and 15 ml 0.1 N HOAc in MeOH. Acidic jasmonates were eluted with 1 N HOAc in MeOH (5 ml) and the solvent was evaporated. The residue was dissolved in 500 µl MeOH and separated in parallel on 5 cartridges of LiChrolut RP-18 (500 mg) (Merck, Darmstadt, Germany). After application of 100 µl MeOH-solution onto the cartridges, each column was eluted as follows (S = 0.2% HOAc in MeOH): 2 ml (20% S +80% H_2O) = fr. A; 2 ml (30% S +70% H_2O) +2 ml (40% S +60% H_2O) = fr. B; 2 ml (50% S +50% H_2O) +2 ml (60% S +40% H_2O) = fr. C; 2 ml (70% S +30% H_2O) +2 ml (80% S +20% H_2O) = fr. D; 2 ml (90% S +10% E) = fr. E. Fractions were evaporated, and aliquots were methylated with ethereal CH_2N_2, and prepared for GC-MS analysis. Subsequently, HPLC of frs. B-E was used for the separation of dihydro-compounds **13–20** from compounds **3–10**.

All compounds were compared with authentic samples by HPLC and GC-MS. Typical fragments of mass spectra are summarised in Table 1 for the detected compounds:

(1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [(−)-jasmonic acid, (**1**)]: in fr. B and C, (Dathe, Rönsch, Preiss, Schade, Sembdner et al., 1981), enantiomeric form defined by immunoassay (Bohlmann et al., 1998);

(1*R*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [(+)-7-*iso*-jasmonic acid (**2**)]: in frs. B and C, **1** + **2** 492 ng ml^{-1}, (Miersch et al., 1987);

N-[(1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetyl]-(*S*)-isoleucine (*N*-[(−)-jasmonoyl-(*S*)]-isoleucine, **3**);

N-[(1*R*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetyl]-(*S*)-isoleucine (*N*-[(+)-7-*iso*-jasmonoyl-(*S*)]-

isoleucine, **4**): in frs. C and D, **3** + **4** 25 ng ml^{-1}, (Miersch et al., 1992);

(1*S*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-butyric acid (**5**);

(1*S*,2*S*)-3-(1S,2S)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-butyric acid (**6**): **5** + **6** traces in fr. C, (Miersch et al., 1989);

(1*S*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-hexanoic acid (**7**): fr. D, traces, identical with synthetic compound (Vick & Zimmerman, 1984);

(1*S*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-hexanoic acid (**8**): fr. D, traces, identical with synthetic compound;

(1*S*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-octanoic acid (**9**): fr. E, traces, (Vick & Zimmerman, 1984);

(1*S*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-octanoic acid (**10**): fr. E, traces, identical with synthetic substance;

(1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [9,10-dihydrojasmonic acid] (**11**);

(1*R*,2*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [9,10-dihydro-7-*iso*-jasmonic acid (**12**)]: **11** + **12** 30 ng ml^{-1} in frs. B an C, (Miersch et al., 1987);

N-[(1*R*,2*R*)-3-oxo-2-pentylcyclopentane-1-acetyl]-(*S*)-isoleucine (*N*-[9,10-dihydrojasmonoyl]-(*S*)-isoleucine, **13**);

N-[(1*R*,2*S*)-3-oxo-2-pentylcyclopentane-1-acetyl]-(*S*)-isoleucine [(*N*-[9,10-dihydro-7-*iso*-jasmonoyl]-(*S*)-isoleucine, (**14**)]: **13** and **14** traces in fr. D, (Cross & Webster, 1970);

(1*S*,2*R*)-3-oxo-2-pentylcyclopentane-1-butyric acid (**15**);

(1*S*,2*S*)-3-oxo-2-pentylcyclopentane-1-butyric acid (**16**): **15** and **16** traces in frs. C and D, identical with synthetic substance;

(1*S*,2*R*)-3-oxo-2-pentylcyclopentane-1-hexanoic acid (**17**);

(1*S*,2*S*)-3-oxo-2-pentylcyclopentane-1-hexanoic acid (**18**): traces of **17** and **18** in fr. D, identical with synthetic substance;

(1*S*,2*R*)-3-oxo-2-pentylcyclopentane-1-octanoic acid (**19**);

(1*S*,2*S*)-3-oxo-2-pentylcyclopentane-1-octanoic acid (**20**): traces of **19** and **20** in fr. E, identical with synthetic substance;

(1*R*,2*R*,3*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [7-*iso*-cucurbic acid (**21**)]: traces in frs. B and C, identical with synthetic **21** (Dathe et al., 1991).

(1*R*,2*S*,3*S*)-3-oxo-2-(2*Z*-pentenyl)cyclopentane-1-acetic acid [cucurbic acid (**22**)]: traces in frs. B and C, (Miersch et al., 1987).

3.4. Chromatographic methods

HPLC: Eurospher 100-C18 (5 μm, 250×4 mm), elution with MeOH (A): 0.2% HOAc in H_2O (B), method a: using the following gradient: 60% A; 0–35 min, to 90% a, 1 ml min^{-1}, UV-detector 210 nm, R_t (min): **1**/**2** 6.5, **5**/**6** 10.8, **7** 17.8, **8** 18.1, **9** 25.6, **10** 26.0, **11**/**12** 8.8, **15**/**16** 14.8, **17** 22.5, **18** 22.9, **19** 30.8, **20** 31.5; method b: 70% A: 30% B, 1 ml min^{-1}, R_t (min): **1** 4.4, **3**/**4** 5.8, **13**/**14** 7.1, method c: 60% A: 40% B, 1 ml min^{-1}, R_t (min): **1** 7.1, **21** 6.5, **22** 7.9.

GC-MS: (MD-800, Fisons Instruments): 70 eV, EI, source temp. 200°, column DP5MS (15 m×0.32 mm, 25 μm film thickness), inj. temp. 250°, interface temp. 300°; He 1.3 ml min^{-1}; splitless inj.; column temp. Program: 1 min 60°, 25° min^{-1} to 110°, 10° min^{-1} to 270°, 10° min^{-1} to 290°, 15 min 290°; R_t (min) of methyl esters: **1** 6.8, **2** 7.05, **3** 14.17, **4** 15.04, **5** 9.28, **6** 9.55, **7** 11.33, **8** 11.6, **9** 13.15, **10** 13.35, **11** 7.0, **12** 7.28, **13** 14.22, **14** 15.09, **15** 9.32, **16** 9.57, **17** 11.29, **18** 11.52, **19** 13.27, **20** 13.47, **21**, 7.18, **22** 7.53.

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