Pergamon

PII: S0031-9422(96)00561-4

Phytochemistry, Vol. 44, No. 4, pp. 581–587, 1997

0031-9422/97 $17.00+0.00

ARBUSCULAR MYCORRHIZAL FUNGUS-INDUCED CHANGES IN THE ACCUMULATION OF SECONDARY COMPOUNDS IN BARLEY ROOTS

HEINRICH PEIPP, WALTER MAIER, JÜRGEN SCHMIDT, VICTOR WRAY* and DIETER STRACK†

Institut für Pflanzenbiochemie (IPB), Weinberg 3, D-06120 Halle (Saale), Germany; *Gesellschaft für Biotechnologische Forschung (GBF), Mascheroder Weg 1, D-38124 Braunschweig, Germany

(*Received* 16 *July* 1996)

Key Word Index—*Hordeum vulgare*; *Glomus intraradices*; arbuscular mycorrhiza; symbiosis; phenolics; hydroxycinnamic acid amides; polyamines; terpenoids; cyclohexenone glycosides; methyljasmonate.

Abstract—*Hordeum vulgare* (barley) was grown in a defined nutritional medium with and without the arbuscular mycorrhizal fungus *Glomus intraradices*. HPLC of methanolic extracts from the roots of mycorrhized and non-mycorrhized plants revealed fungus-induced accumulation of some secondary metabolites. These compounds were isolated and identified by spectroscopic methods (NMR, MS) to be the hydroxycinnamic acid amides *N*-(*E*)-4-coumaroylputrescine, *N*-(*E*)-feruloylputrescine, *N*-(*E*)-4-coumaroylagmatine and *N*-(*E*)-feruloylagmatine, exhibiting a transient accumulation, and the cyclohexenone derivatives 4-(3-*O*-β-glucopyranosyl-butyl)-3-(hydroxymethyl)-5,5-dimethyl-2-cyclohexen-1-one and 4-{3-*O*-[(2′-*O*-β-glucuronosyl)-β-glucopyranosyl]-butyl}-3,5,5-trimethyl-2-cyclohexen-1-one (blumenin), exhibiting a continuous accumulation. A third cyclohexenone derivative, 4-{3-*O*-[(2′-*O*-β-glucuronosyl)-β-glucopyranosyl]-1-butenyl}-3,5,5-trimethyl-2-cyclohexen-1-one, was detectable only in minute amounts. It is suggested that accumulation of the amides in early developmental stages of barley mycorrhization reflects initiation of a defence response. However, the continuous accumulation of the cyclohexenone derivatives, especially blumenin, seems to correlate with the establishment of a functional barley mycorrhiza. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The roots of most higher plants, including cereal roots, form arbuscular mycorrhizas with certain fungi. In addition to increased resistance against pathogen attack or stress, the mutual benefits of such a mycorrhizal symbiosis include supplies of mineral nutrients and water to the plants and carbohydrates to the fungi [1]. The arbuscular mycorrhiza is the most common symbiosis known to occur in higher plants. Factors that control the antagonistic balance of the usually mutualistic relationship between arbuscular mycorrhizal fungi and plant roots are virtually unknown.

There is increasing evidence that secondary compounds play a significant role in the various interactions occurring between plants and their natural environment [2]. In this respect, phenolics are known to be of major importance in pathogenic interactions between plants and fungi [3, 4]. It has been documented that certain flavonoids promote spore germination of arbuscular mycorrhizal fungi [5–8]. However, the role of flavonoids as necessary signal compounds in arbuscular mycorrhiza formation has been questioned [9]. Nevertheless, the accumulation of isoflavonoids seems to be correlated with arbuscule development in roots of *Medicago truncatula* [10, 11].

Recently, we have shown the *Glomus intraradices*-induced accumulation of a terpenoid glycoside (blumenin) in some cereal arbuscular mycorrhizas, including barley [12]. In the course of further studies on the metabolism of secondary compounds, possibly involved in the mycorrhization of barley, we detected by HPLC several other elevated UV light-absorbing compounds from the mycorrhizas, that were isolated and identified by spectroscopic methods (NMR, MS). We observed a transient increase in hydroxycinnamic acid amides, putrescine and agmatine conjugates, in the early stages of the root–fungus interaction, that is in contrast to some terpenoid glycosides (cyclohexenone derivatives), especially blumenin, which show an induced, continuous accumulation [12].

† Author to whom correspondence should be addressed.

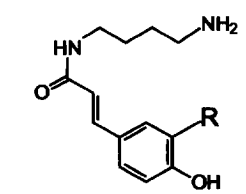
RESULTS AND DISCUSSION

Identification of root metabolites

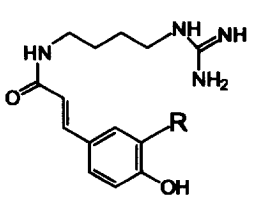
Four phenolic amides and two terpenoid glycosides, as possible components that are correlated with arbuscular mycorrhizal fungus colonization, were isolated from methanolic barley root extracts and identified by spectroscopic methods (NMR, MS) to be the hydroxycinnamic acid amides *N*-(*E*)-4-coumaroylputrescine (**1**), *N*-(*E*)-feruloylputrescine (**3**), *N*-(*E*)-coumaroylagmatine (**2**), and *N*-(*E*)-feruloylagmatine (**4**) and the cyclohexenone derivatives 4-(3-*O*-*β*-glucopyranosylbutyl)-3-(hydroxymethyl)-5,5-dimethyl-2-cyclohexen-1-one (**5**) and 4-{3-*O*-[(2′-*O*-*β*-glucuronosyl)-*β*-glucopyranosyl]-1-butenyl}-3,5,5-trimethyl-2-cyclohexen-1-one (**6**). A third, major cyclohexenone glycoside, named blumenin (**7**), was previously identified (12). In addition to these compounds we found tryptophan (identified by ^{1}H NMR, MS and chromatographic comparison with authentic compound).

The hydroxycinnamoylputrescines (**1** and **3**) are of widespread occurrence in higher plants. They have been identified as the main phenolic constituents in the reproductive organs of various flowering plants, including members of the Poaceae [13]. Compound **2** has previously been isolated from young barley shoots [14] and blumenin (**7**) was recently found to accumulate in mycorrhized roots of barley, oat, rye and wheat [12]. The aglycone of compound **6** [15] and a structure similar to compound **5**, bearing a butenyl instead of the butyl moiety [16], have also previously been described.

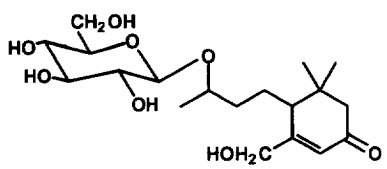
Since most of the spectroscopic data from structural identification of the isolated compounds are well known from other sources [17], only some essential points that substantiate the present structural elucidation will be mentioned below. Since compounds **5** and **6** are apparently described for the first time, their complete ^{1}H NMR data are listed in the Experimental section. The number of protons in each aromatic and aliphatic system was determined from the integrated 1D ^{1}H NMR spectra and the identity of each moiety was established from their characteristic ^{1}H shifts and coupling constants. The NMR spectra of **1** and **2** showed the presence of a second order AA′BB′ spin system with $J(AB)+J(AB')$ of 8.9 Hz of the aromatic system and two protons (δ 7.50, *d*, and 6.45, *d*) corresponding to the *E*-vinyl protons. An aromatic three-spin system for **3** and **4** was identified from the 8 Hz three-bond and 2 Hz four-bond couplings which are typical of the feruloyl system. The configuration of the olefinic bond in the coumaroyl and feruloyl conjugates was apparent from the magnitude of the three-bond coupling (15.7 Hz for *E*). The identity of the putrescine moiety in **1** and **3** and the agmatine moiety in **2** and **4** was established from 2D NMR spectra together with mass spectrometry data. The latter correspond to data obtained from **1** and **2** by Lee *et al.* [18]. Crosspeaks in the 2D ^{1}H correlation spectroscopy of **1**–**4** allowed the fragment $\cdot CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\cdot$ to be identified. The EI-mass spectra of **1** and **3** showed the $[M]^+$ peaks at *m/z* 234 and 264 and loss of the putrescine moieties (*m/z* 147 and 177, respectively). The agmatine conjugates **2** and **4**, however, failed to give $[M]^+$ in their EI-mass spectra. In the positive electrospray mass



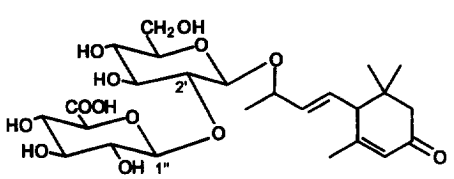
R
1 H
3 OMe



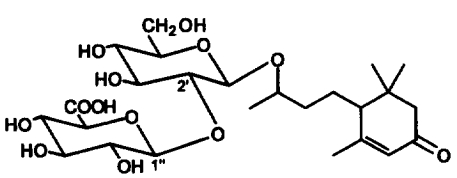
R
2 H
4 OMe



5



6



7

spectra the M_rs of **2** and **4** were indicated by $[M+H]^+$ peaks at *m/z* 277 and 307, respectively. In both cases, the fragment ion at *m/z* 131 and its daughter ions (*m/z* 114, 72, 60), obtained by collision induced dissociation MS, showed the pattern of the agmatine moieties.

The ^1H NMR spectrum of **5** showed resonances that are similar to those of blumenin (**7**) [12]. The appearance of the AB signals of a hydroxymethyl group at the double bond at δ 4.15 and 4.31 (geminal coupling $J = 17.7$ Hz) indicated that the aromatic methyl group of blumenin has undergone oxygenation. In contrast to the blumenin spectrum, the 2D ^1H correlation spectrum of **5** only showed one sugar moiety (β-glucose). The mass spectrometry data supported the proposed structure. The M_r of **5** was indicated by a peak at *m/z* 411 ($[M+Na]^+$, 100% intensity) in the positive electrospray mass spectrum, corresponding to M_r of 388.

The ^1H NMR spectrum of **6** is very similar to that of blumenin (**7**) except for the extra signals at δ 5.68 and 5.80. From this result and the loss of signals for the aliphatic CH_2 groups at δ 1.6 it was inferred that compound **6** is blumenin with a double bond between C-7 and C-8. The mass spectrometry data of **6** corroborate the NMR analysis. The M_r of **6** was indicated by peaks at *m/z* 569 ($[M+Na]^+$, 100% relative intensity) in the positive electrospray mass spectrum as well as *m/z* 545 ($[M-H]^-$, 100%) in the negative mode. The loss of a glucuronic acid moiety resulted in a peak at *m/z* 393 ($[M+Na-C_6H_8O_6]^+$, 40%). The subsequent loss of a glucose unit resulted in a peak at *m/z* 209 ($[M+H-C_{12}H_{18}O_{11}]^+$, 19%] which corresponds to the protonated ion of the aglycone, 3-oxo-α-ionol.

Fungus-induced changes in the patterns of root metabolites

HPLC traces of methanolic extracts from control roots and arbuscular mycorrhizas from 6-week-old barley plants showed fungus-induced changes in the accumulation pattern of several UV light-absorbing peaks (λ_{max} below 320 nm) (Fig. 1). These changes are restricted to the roots. There were no obvious differences observed from extracts of the shoots of mycorrhized and non-mycorrhized plants. The putrescine conjugates **1** and **3** of uninoculated barley roots were among the major constituents of the soluble compounds, reaching concentrations between 20 and 40 nmol (g fr. wt)$^{-1}$. The accumulation of both compounds was significantly stimulated in the early stages of fungal colonization. Figure 2 illustrates the induced transient accumulation, starting at three weeks after sowing and reaching maximal values at four weeks: the putrescine conjugates **1** and **3** approx. 100 nmol (g fr. wt)$^{-1}$, the agmatine conjugates **2** and **4** between 20 and 25 nmol. Thereafter, the concentration of these amides decreased after seven to eight weeks to levels obtained from the control plants. Although the data are based on increasing root weight during barley development, recalculation of the data,

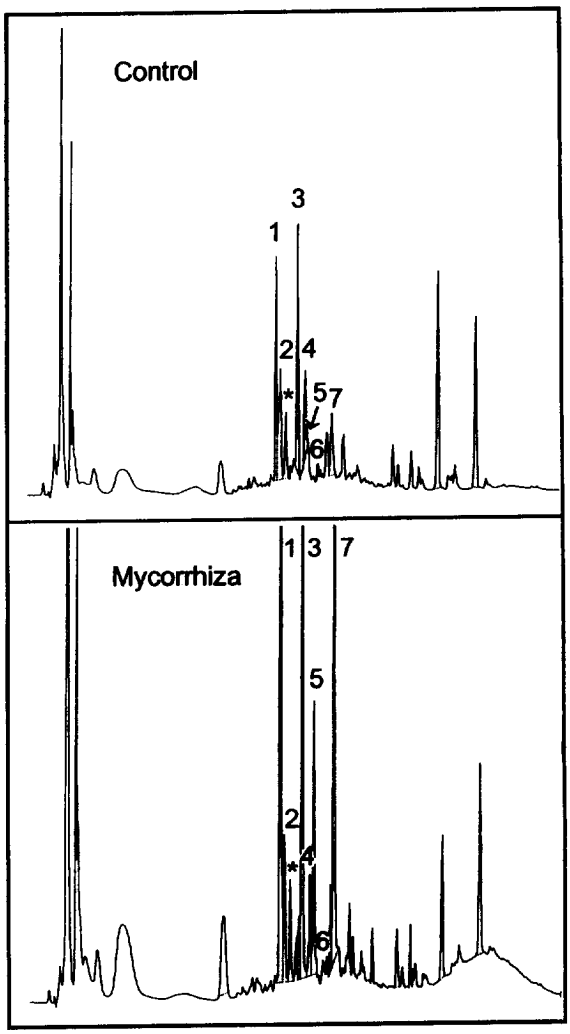


Fig. 1. HPLC traces (30 min) of methanolic extracts from 6-week-old uncolonized barley roots (control) and the respective roots colonized with *G. intraradices* (mycorrhiza): 20 µl from a 5 ml methanolic extraction volume from 1 g fr. wt of root material was injected. Components were traced by maxplot detection between 210 and 400 ml (0.05 full scale absorbance) {λ_{max} nm: **1**, 293 (305); **2**, 294 (305); **3**, 316 (292); **4**, 316 (292); **5**, 242; **6**, 241; **7**, 243}. For peak identification see structures. The asterisks mark the peaks corresponding to tryptophan (λ_{max} nm: 217, 278).

based on the individual plant (whole root system), showed the same trends. For example, between day 30 and day 58 the level of 4-coumaroylputrescine (**1**) decreases from 137 ± 39 to 34 ± 9 nmol (root system)$^{-1}$ in the control plants and 426 ± 98 to 90 ± 53 nmol (root system)$^{-1}$ in the mycorrhized plants. Thus the decrease in the concentrations of the amides, following the initial increase, is not merely due to dilution but rather indicates their further metabolism.

In contrast to the hydroxycinnamic acid amides, the cyclohexenone conjugates **5** and **7** exhibited a fungus-induced, continuous accumulation, reaching approximately 100 and 200 nmol (g fr. wt)$^{-1}$ in 5–6-week-old barley mycorrhizas (details not shown). The level of

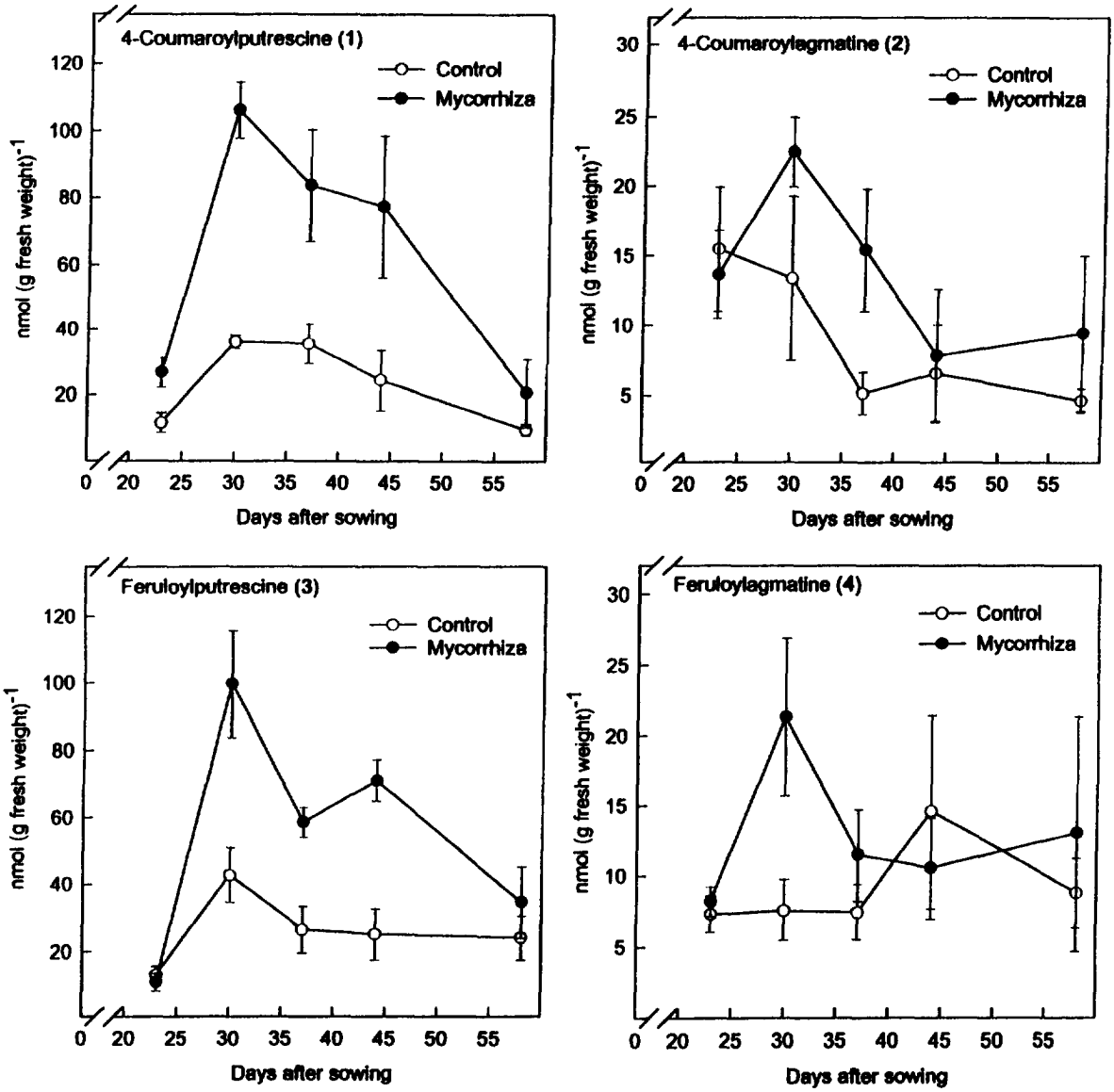


Fig. 2. Accumulation patterns of hydroxycinnamic acid amides based on fr. wt of uncolonized barley roots (control) and of barley roots colonized with *G. intraradices* (mycorrhiza). Note the different scale reading on the *Y* axes of the graphs for the putrescine and agmatine conjugates. The data points represent mean ± S.D. of three experiments with 10 plants each.

compound **7** (blumenin), shows a still further increase in older plants [12], reaching values around 350 nmol (g fr. wt)$^{-1}$. Occasionally, we observed more than 800 nmol blumenin. We observed in some of the control plants constitutive concentrations of these compounds together with **6** in the range 2–10 nmol. In a taxonomic survey of the occurrence of blumenin in roots of members of the Poaceae, we found a fungus-induced blumenin accumulation in mycorrhizas of some members of the tribes Aveneae, Poeae and Triticeae.

In conclusion, two arrays of secondary metabolites, arising from phenolic and terpenoid metabolism, obviously respond to mycorrhizal fungus colonization in barley roots in different ways. The transient accumulation of hydroxycinnamic acid amides (**1**–**4**) in early stages of mycorrhizal fungus colonization may reflect a component of a defence response of the plant against fungal penetration. However, the plant does not receive the respective elicitor-specific signals, nor seems to recognize the symbiotic fungus and turns down further defence reactions. On the other hand, the fungus might also actively suppress such reactions. In addition, there is no obvious cell wall reinforcement by increasing the level of bound phenolics [12] that might reject hyphal penetration. This is in agreement with the work of Codignola *et al.* [19], but in contrast to pathogenic plant–fungus interactions [20–22]. It has been discussed in a recent study that wall modifications including phenolic deposition are involved in

resistance reactions of a myc$^-$ mutant of *Pisum sativum* against arbuscular mycorrhizal fungi [23].

Concerning the transient defence response of mycorrhized barley, it is interesting to note that aromatic amides (hydroxycinnamic acid putrescine and tyramine amides) have been found to accumulate in potato cell cultures in response to elicitor preparations from *Phytophthora infestans* [24], an oomycete that causes late blight. Aromatic amides are also considered to exhibit antiviral effects and are formed, e.g. in tobacco after virus infection, as a protective mechanism [25]. In addition, it is interesting to note that the agmatine amides (**2** and **4**) are direct precursors of the so-called 'antifungal factors' in barley [26–28], hordatines A and B.

In a first approach to identify possible signal transducers in the mycorrhizal fungus-induced barley roots, we applied methyljasmonate as a potential candidate. Jasmonic acid and its methyl ester have been suggested to be involved in plant defence reactions [29–32]. Our preliminary results showed that methyljasmonate (0.5×10^{-6} M), applied to roots of young barley plants (1-week-old), induced the accumulation of putrescine and agmatine amides of 4-coumaric acid reaching *ca* 26 and 11 nmol (g fr. wt)$^{-1}$, respectively, and were not detected in the 1-week-old control plants. A detailed study on this phenomenon will be published elsewhere. It has been argued [33] that there are several indications against the hypothesis that jasmonate acts as a signal transducer in plant–pathogen interactions, at least in the case of barley leaves interacting with powdery mildew [34]. It has been shown in other studies [35] that methyljasmonate treatment results in growth suppression (albeit weak) of powdery mildew and that methyljasmonate also induces a massive increase in the levels of compounds **1** and **2** in barley leaves [18]. The additional increase in cell wall-bound ferulic acid in methyljasmonate-treated barley leaves [18] was not observed in our experiments with barley roots. In summary, our results corroborate the assumption that the fungus-induced accumulation of the hydroxycinnamic acid amides in barley roots reflects a transient defence response of the plant upon penetration of the mycorrhizal fungus.

Another type of arbuscular fungus–root interaction has been described for *M. truncatula* [10, 11] in which fungus-induced isoflavone biosynthesis may be causally related to arbuscule development. Elevated levels of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) transcripts were detected by *in situ* hybridization, specifically in the cells containing arbuscules. These studies also describe an induced transient increase in the level of the well-described phytoalexin medicarpin, an isoflavone-derived pterocarpan, in early stages of colonization that subsequently decreases below the levels in control plants. This is accompanied by a decrease in transcripts of the key enzyme isoflavone reductase (IFR). It has also been shown in a recent study [36], that root cells of *Phaseolus vulgaris* that contain arbuscules show a fungus-induced accumulation of PAL and chitinase transcripts. Induced defence-related hydrolytic enzyme activities (chitinase, glucanase) in arbuscular mycorrhizal fungus-colonized roots have repeatedly been described [37].

The induced, continuous accumulation of the cyclohexenone conjugates, however, might indicate a different correlation with the establishment of barley mycorrhizas. The terpenoid metabolism of barley, and most likely also of other gramineous plants, seems to be involved in mycorrhiza formation of the grass family. Related studies demonstrate a mycorrhizal fungus-induced accumulation of abscisic acid [38] and a C_{14} carotenoid in maize [39] and other members of the Poaceae [40].

Our future studies will focus on tissue and cell localization of the accumulating phenolic amides and the cyclohexenone conjugates as well as the enzymes involved in their formation. These enzymes include the hydroxycinnamoyl-CoA-dependent transferases involved in the formation of the putrescine [41, 42] and agmatine amides [43] as well as possible dioxygenase activities and glucosyl- and glucuronosyltransferases involved in the formation of the cyclohexenone glycosides. A dioxygenase is believed to catalyse the cleavage of a carotenoid as the first step on the biosynthetic route to the phytohormone abscisic acid [44], which is structurally related to the cyclohexenone aglycones of compounds **5**, **6** and **7**.

EXPERIMENTAL

Plant material and arbuscular mycorrhizal fungus application. Barley plants (*H. vulgare* L. cv. Salome) were cultivated in plastic pots filled with expanded clay and inoculated with *G. intraradices* Schenk & Smith by application of propagules (isolate 49) in expanded clay. Details of cultivation and control of mycorrhization were published previously [12].

Analytical extraction. Freshly harvested whole non-mycorrhized roots (controls), mycorrhized roots (mycorrhizas) or shoots from 10 plants were washed with H_2O, cut into small pieces and transferred (1 g fr. wt) into 5 ml of 80% aq. MeOH. The plant material was treated twice for about 1 min with an Ultra Turrax homogenizer, allowed to stand for 30 min with continuous stirring, and centrifuged. The supernatants were used for HPLC analysis (20 μl aliquots).

Preparative extraction and fractionation. Lyophilized barley roots (31 g) were homogenized in a mortar in the presence of dry ice. The resulting powder was stirred at 40° for 1 hr in 500 ml 80% aq. MeOH. The suspension was filtered and the residue reextracted ($\times 3$). The combined extracts were evaporated at 40° (*in vacuo*) to dryness, the residue redissolved in 50 ml 80% aq. MeOH, centrifuged and the clear supernatant fractionated on a polyamide column (perlon, CC 6, 250×4 mm i.d.; Macherey-Nagel Düren) using H_2O (fr. 1) (elution of blumenin) and 20% aq. MeOH (fr.

2) (elution of all other compounds). Fr. 2 was taken to dryness (*in vacuo*) and subjected to prep. HPLC.

HPLC. Analytical HPLC (Waters system; Nucleosil C_{18} column) was performed as described previously [12]. The liquid chromatograph (System Gold; Beckman Instruments, München) for prep. HPLC was equipped with a Nucleosil 100-10 C18 column (VarioPrep; 10 μm, 250 × 40 mm i.d.; Macherey-Nagel, Düren). The compounds were separated at a flow rate of 19 ml min^{-1} with a linear gradient within 40 min from solvent A (1% aq. HCO_2H) to 50% solvent B (MeOH) in A. The cyclohexenone glycosides and hydroxycinnamic acid amides were quantified by using abscisic acid, as described previously [12], and 4-coumaric and ferulic acids as external standards, respectively.

NMR and MS. Positive and negative electrospray mass spectrometry (TSQ 7000, Finnigan, Bremen) and ^1H and ^{13}C NMR spectroscopy (Bruker, Rheinstetten) were performed as described previously [12].

^1H *NMR data of compound* **5** *in* CD_3OD: δ 6.06 (*s*, H-4), 4.30 (*d*, H-1′, $J_{1',2'} = 7.8$), 4.15 (*d*, H-1′, $J_{1',2'} = 7.8$), 4.15 (*d*, H-13B), 3.83 (*dd*, H-6′A, $J_{5',6'A} \sim 2$), 3.82 (*m*, H-9), 3.66 (*dd*, H-6′B, $J_{6'A\text{-}6'B} \sim 12$), 3.36 (*dd*, H-3′, $J_{3'\text{-}4'} \sim 9$), 3.34 (*dd*, H-4′, $J_{4'\text{-}5'} \sim 9$), 3.26 (*ddd*, H-5′, $J_{5'\text{-}6'B} \sim 5$), 3.14 (*dd*, H-2′, $J_{2'\text{-}3'} = 8.9$), 2.58 (*d*, H-2B, $J_{2A\text{-}2B} = 17.4$), 2.02 (*d*, H-2A, $J = 17.7$), 1.92 (*m*, H-6), 1.55–1.80 (*m*, H-7A, H-7B, H-8A, H-8B), 1.24 (*d*, 10-CH_3, $J = 6.3$), 1.11 (*s*, 12-CH_3), 1.02 (*s*, 11-CH_3).

^1H *NMR data of compound* **6** *in* CD_3OD: δ 5.91 (*s*, H-4), 5.80 (*dd*, H-8, $J_{8\text{-}9} = 6.7$), 5.68 (*dd*, H-7, $J_{7\text{-}8} = 14.9$), 4.59 (*d*, H-1″, $J_{1''\text{-}2''} = 7.2$), 4.55 (*d*, H-1′, $J_{1'\text{-}2'} = 7.5$), 4.51 (*m*, H-9), 3.89 (*d*, H-5″), 3.84 (*dd*, H-6′A, $J_{5'\text{-}6'A} = 2.1$), 3.71 (*dd*, H-4″, $J_{4''\text{-}5''} = 10.8$), 3.70 (*dd*, H-6′B, $J_{6'A\text{-}6'B} = 12.1$), 3.58 (*dd*, H-3′, $J_{3'\text{-}4'} \sim 9$), 3.44 (*dd*, H-3″, $J_{3''\text{-}4''} = 8.8$), 3.42 (*dd*, H-2′, $J_{2'\text{-}3'} \sim 9$), 3.39 (*dd*, H-4′, $J_{4'\text{-}5'} \sim 9$), 3.32 (*dd*, H-2″, $J_{2''\text{-}3''} = 9.0$), 3.23 (*ddd*, H-5′, $J_{5'\text{-}6'B} = 5.0$), 2.72 (*d*, H-6, $J_{6\text{-}7} = 9.0$), 2.49 (*d*, H-2B, $J_{2A\text{-}2B} = 16.7$), 2.09 (*d*, H-2A), 1.97 (*s*, 13-CH_3), 1.33 (*s*, 10-CH_3) 1.07 (*s*, 12-CH_3), 1.05 (*s*, 11-CH_3).

Acknowledgements—We thank Gerlinde Waiblinger (IPB) for skillful technical assistance and H. von Alten (Hanover, Germany) for a generous gift of fungal inoculum (isolate 49). This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REFERENCES

1. Varma, A. and Hock, B. (eds), *Mycorrhiza. Structure, Function, Molecular Biology and Biotechnology*. Springer, Berlin, 1995.
2. Harborne, J. B., *Introduction to Ecological Biochemistry*, 4th edn. Academic Press, London, 1993.
3. Matern, U. and Kneusel, R. E., *Phytoparasitica*, 1988, **16**, 153.
4. Dixon, R. A., Harrison, M. J. and Lamb, C. J., *Annual Review of Phytopathology*, 1994, **32**, 479.
5. Gianinazzi-Pearson, V., Branzanti, B. and Gianinazzi, S., *Symbiosis*, 1989, **7**, 243.
6. Tsai, S. M. and Phillips, D. A., *Applied and Environmental Microbiology*, 1991, **57**, 1485.
7. Bécard, G., Douds, D. D. and Pfeffer, P. E., *Applied and Environmental Microbiology*, 1992, **58**, 821.
8. Chabot, S., Bel-Rhlid, R., Chênevert, R. and Piché, Y., *New Phytologist*, 1992, **122**, 461.
9. Bécard, G., Taylor, L. P., Douds, Jr, D. D., Pfeffer, P. E. and Doner, L. W., *Molecular Plant–Microbe Interactions*, 1995, **8**, 252.
10. Harrison, M. J. and Dixon, R. A., *Molecular Plant–Microbe Interactions*, 1993, **6**, 643.
11. Harrison, M. J. and Dixon, R. A., *Plant Journal*, 1994, **6**, 9.
12. Maier, W., Peipp, H., Schmidt, J., Wray, V. and Strack, D., *Plant Physiology*, 1995, **109**, 465.
13. Martin-Tanguy, J., Cabanne, F., Perdrizet, E. and Martin, C., *Phytochemistry*, 1978, **17**, 1927.
14. Stoessl, A., *Phytochemistry*, 1965, **4**, 973.
15. Kodama, H., Fujimori, T. and Kato, K., *Phytochemistry*, 1984, **23**, 583.
16. Aimi, N., Hoshino, H., Nishimura, M., Sakai, S.-I. and Haginiwa, J., *Tetrahedron Letters*, 1990, **31**, 5169.
17. Buckingham, J. (exec. ed.), *Dictionary of Natural Products*. Chapman & Hall, London, 1994.
18. Lee, J., Vogt, T., Schmidt, J., Parthier, B. and Löbler, M., *Phytochemistry*, 1997, **44**, 589.
19. Codignola, A., Verotta, L., Spanu, P., Maffei, M., Scannerini, S. and Bonfante-Fasolo, P., *New Phytologist*, 1989, **112**, 221.
20. Graham, M. Y. and Graham, T. L., *Plant Physiology*, 1991, **97**, 1445.
21. Beimen, A., Witte, L. and Barz, W., *Botanica Acta*, 1992, **105**, 152.
22. Kauss, H., Franke, R., Krause, K., Conrath, U., Jeblick, W., Grimmig, B. and Matern, U., *Plant Physiology*, 1993, **102**, 459.
23. Gollotte, A., Gianinazzi-Pearson, V., Giovannetti, M., Sbrana, C., Avio, L. and Gianinazzi, S., *Planta*, 1993, **191**, 112.
24. Keller, H., Hohlfed, H., Wray, V., Hahlbrock, K., Scheel, D. and Strack, D., *Phytochemistry*, 1996, **42**, 389.
25. Martin-Tanguy, J., Martin, C., Gallet, M. and Vernoy, R., *Comptes Rendus de L'Academie Des Sciences (Serie D)*, 1976, **282**, 2231.
26. Stoessl, A., *Tetrahedron Letters*, 1966, **21**, 2287.
27. Stoessl, A., *Canadian Journal of Chemistry*, 1967, **45**, 1745.
28. Stoessl, A. and Unwin, C. H., *Canadian Journal of Botany*, 1970, **48**, 465.
29. Farmer, E. E. and Ryan, C. A., *Proceedings of*

the National Academy of Sciences (*U.S.A.*), 1994, **87**, 7713.
30. Gundlach, H., Müller, M. J., Kutchan, T. M. and Zenk, M. H., *Proceedings of the National Academy of Sciences* (*U.S.A.*), 1992, **89**, 2389.
31. Kauss, H., Krause, K. and Jeblick, W., *Biochemical and Biophysical Research Communications*, 1992, **189**, 304.
32. Cohen, Y., Gisi, U. and Niderman, T., *Phytopathology*, 1993, **83**, 1054.
33. Wasternack, C., *Trends in Comparative Biochemistry and Physiology*, 1994, **1**, 1255.
34. Kogel, K. H., Jarosch, B., Atzorn, R. and Wasternack, C., *European Journal of Plant Pathology*, 1995, **101**, 319.
35. Schweizer, P., Green, R. and Mösinger, E., *Plant Physiology*, 1993, **102**, 503.
36. Blee, K. A. and Anderson, A. J., *Plant Physiology*, 1996, **110**, 675.
37. Gianinazzi-Pearson, V. and Gianinazzi, S., in *Mycorrhiza. Structure, Function, Molecular Biology and Biotechnology*, eds A. Varma and B. Hock. Springer, Berlin, 1995, p. 251.
38. Danneberg, G., Latus, C., Zimmer, W., Hundeshagen, B., Schneider-Poetsch, H. and Bothe, H., *Plant Physiology*, 1992, **141**, 33.
39. Klingner, A., Bothe, H., Wray, V. and Marner, F.-J., *Phytochemistry*, 1995, **38**, 53.
40. Klingner, A., Hundeshagen, B., Kernebeck, H. and Bothe, H., *Protoplasma*, 1995, **185**, 50.
41. Meurer-Grimes, B., Berlin, J. and Strack, D., *Plant Physiology*, 1989, **89**, 488.
42. Negrel, J., Paynot, M. and Javelle, F., *Plant Physiology*, 1992, **98**, 1264.
43. Bird, C. R. and Smith, A., *Phytochemistry*, 1983, **22**, 2401.
44. Parry, A. D. and Horgan, R., *Physiologia Plantarum*, 1991, **82**, 320.