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Research Article

Biogenetic Conversion of Wyerone and Dihydrowyerone into Wyerone Epoxide in *Vicia faba* Cotyledons and Screening of Antibacterial Activity

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Background. *Vicia faba* is a plant that belongs to the family Fabaceae. Cotyledons of this plant produce compounds called phytoalexins as a result of fungal and bacterial infection or stress factors. The phytoalexins are furanoacetylenic compounds. They include wyerone, wyerol, wyerone acid, their dihydro derivatives, and wyerone epoxide. These compounds have antimicrobial activities mainly against fungi. *Objective*. The purpose of this study was to elucidate the biogenetic conversion of wyerone and dihydrowyerone to wyerone epoxide in *V. faba* and to investigate the antibacterial activities of some of these phytoalexins. *Materials and Methods*. Seeds of *Vicia faba* were used. Labelled wyerone and dihydrowyerone were obtained by treating $CuCl_2$-induced cotyledons with sodium (2-^{14}C) acetate and separated by TLC and HPLC. Labelled wyerone and dihydrowyerone were then applied to induced bean cotyledons to establish any possible interconversion to wyerone epoxide. Antibacterial activity of wyerone, wyerone acid, and wyerone epoxide was investigated by disc diffusion test against a panel of microorganisms. Zones of inhibition were reported. *Results*. The radiolabeling studies showed that 12.4% of ^{14}C-wyerone, and 6.01% of ^{14}C-dihydrowyerone were incorporated into wyerone epoxide. This indicates that wyerone epoxide was most probably derived from wyerone and dihydrowyerone. In addition, a new compound, 11-hydroxywyerone was isolated for the first time. Additionally, this study showed that wyerone, wyerone epoxide, and wyerone acid had no antibacterial activity against Gram-negative bacteria but were active against Gram-positive bacteria.

1. Introduction

Plants were found to have physical and biochemical responses towards pathogen infections. They evolve coordinate events by relying on their immune system to recognize and actively respond to these infections. One example of the defense mechanisms is the accumulation of antimicrobial secondary metabolites, which are called the phytoalexins [1, 2]. These phytoalexins are synthesized by plants as a result of infections (fungal, bacterial, or viral) [3, 4] or due to other forms of stress, which could be chemical elicitors (e.g., mercury salts, copper) or physical events like UV light [5]. Fabaceae plants usually synthesize certain isoflavonoid phytoalexins, but certain species accumulate nonisoflavanoid compounds instead of or in addition to isoflavanoid [6, 7].

Species of *Vicia* synthesize seven phytoalexins which are furanoacetylenes in structure as a result of fungal or bacterial infection [8, 9]. The major component is the furanoacetylene ester wyerone (I) accompanied by structurally similar derivatives. These include wyerone acid (II); the alcohol, wyerol (III); the 11, 12-dihydroanalogues, e.g., dihydrowyerone (IV), dihydrowyerol (V), and dihydrowyerone acid (VI); and the wyerone epoxide (VII)

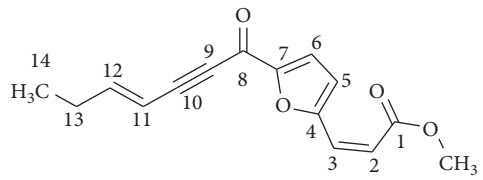
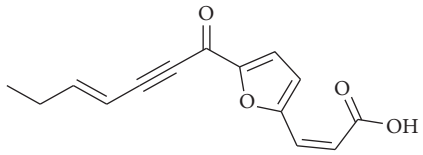
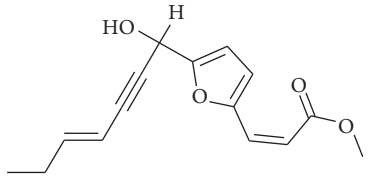
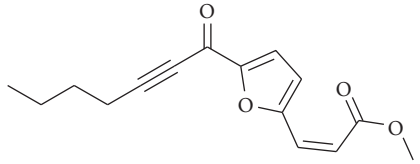
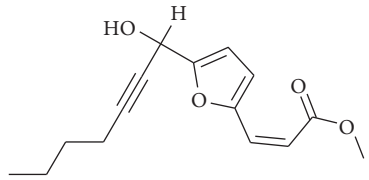
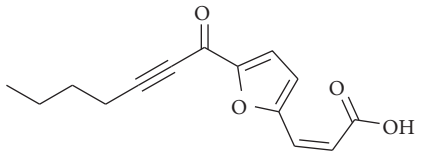
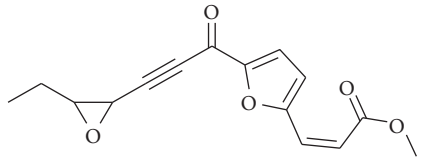
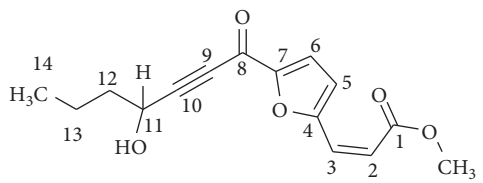
	Wyerone (I)
	Wyerone acid (II)
	Wyerol (III)
	Dihydrowyerone (IV)
	Dihydrowyerol (V)
	Dihydrowyerone acid (VI)
	Wyerone epoxide (VII)
	11-Hydroxywyerone (new metabolite)

Figure 1: Structures of phytoalexins of *Vicia faba* (I, II, III, IV, V, VI, and VII) and the new metabolite 11-hydroxywyerone.

(Figure 1) [10–13]. The accumulation of wyerone and its derivatives in cotyledon, pod, and leaves of *Vicia faba*, infected by Botrytis spp. fungi, were evaluated previously. Wyerone acid was observed to be the main phytoalexin in leaves and pods, while wyerone was predominant in cotyledons. The accumulation of wyerone and wyerone epoxide

in pod endocarp has led to the suggestion that wyerone may act as precursor for wyerone epoxide. This was supported by the observed conversion of wyerone to wyerone epoxide during fractionation of pod tissue [14–16]. The biosynthesis of wyerone in *Vicia faba* was elucidated previously and found to be derived from acetate malonate pathway via fatty acid derivatives [17], and the conversion of wyerone to wyerone acid had been elucidated earlier [18].

The aim of this study is to explore possible biogenetic conversions in *V. faba* cotyledons from wyerone and dihydrowyerone to wyerone epoxide. During the separation of phytoalexins, a new compound, 11-hydroxywyerone was isolated. This study excluded any fungal conversions, since such fungal transformation of wyerone derivatives has been studied extensively [14, 19, 20].

The antibacterial activity of phytoalexins has largely been confined to isoflavonoids from Fabaceae, with one report of this activity of wyerone in comparison with other isoflavonoid phytoalexins [21] was published. Moreover, most of the antimicrobial activity studies focused on antifungal rather than antibacterial activity [3, 4]. In the present study, we tried to explore more on this issue regarding three of these phytoalexins, wyerone, wyerone acid, and wyerone epoxide against five Gram-positive and two Gram-negative bacteria. The effect of wyerone acid and wyerone epoxide was not evaluated previously on these bacteria. These bacteria studied were human and plant pathogens and a saprophyte.

2. Materials and Methods

2.1. Materials. Seeds of *Vicia faba* (broad bean, cv. Aquadulce) were procured from Sinclair McGill. 2-^{14}C-sodium acetate, 58 mCi/mmol, was procured from New England Nuclear (NEN, Boston, MA), ^{14}C-Toluene (Internal liquid scintillation counting (LSC) standard) was procured from Perkin-Elmer, and Silica gel (0.5 mm, GF254 TLC plates) was procured from Merck. All other chemicals were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Bacterial Strains and Media. Human pathogens included *Bacillus cereus* ATCC 14579, *Micrococcus luteus* ATCC 49732, and *Klebsiella pneumoniae* ATCC 13883. Plant pathogens included *Rhodococcus fascians* ATCC 12974, *Curtobacterium flaccumfaciens pathovar oortii* ATCC 25283, and *Pseudomonas syringae pathovar phaseolicola* ATCC 19304, and the saprophyte used was *Bacillus megaterium* ATCC 14581. These microorganisms were procured from the American Type Culture Collection (ATCC). Media used were procured from Oxoid (UK). Amoxicillin was a kind gift from Hikma pharmaceuticals (Amman, Jordan).

2.3. Plant Material, Feeding Techniques, and Isolation of Phytoalexins. The phytoalexins were synthesized using the procedure described earlier by Al-Douri and Dewick [17, 18]. Seeds of *V. faba* were immersed in ethanol for 10 minutes, washed with water, soaked in dilute detergent (Quadralene, UK) for 5 minutes, sterilized by immersion with sodium hypochlorite (2%) for 5 minutes, and washed again. Damaged seeds distinguished by eye during sterilization were discarded. The healthy seeds were imbibed in distilled water for 24 hours. The seed coat was removed, and 100 g of the obtained cotyledons were separated and placed flat side up on a moist tissue paper in glass trays (Figure 2). The chemical elicitor, aqueous $CuCl_2$ (3 mM), was spotted onto the surface of these cotyledons using a syringe. Sufficient amount of elicitor solution was added to keep the whole surface moistened. The glass trays containing the cotyledons were covered by a foil paper and kept at 25°C for several days in a dark cabinet. Extra $CuCl_2$ was added when necessary to keep the surface moist. In our laboratory, a five-day time period was found the best to give the highest production of phytoalexins. When the five days elapsed, the inducer solution was removed by a syringe and replaced by drops of sodium (2-^{14}C) acetate solution (1 mCi) as a precursor for synthesis of the phytoalexins to be studied. The cotyledons were left for another two days adding drops of distilled water to keep the cotyledons moist. After this period, the cotyledons were homogenized in a blender with 200 ml dichloromethane. This was repeated ten times after filtering the extract. Dichloromethane extract was concentrated using a rotary evaporator.

2.4. Purification of the Different Compounds. The extract was applied on TLC chromatography (silica gel GF254; hexane-acetone, 2 : 1) to separate the zones representing the phytoalexins [17, 18]. ^{14}C-phytoalexin mixture was detected as a deep blue fluorescent band under UV254. The band was scratched and eluted by acetone. The resulting mixture was further purified by second TLC using chloroform-light petroleum (2 : 1) and then by chloroform: methanol (25 : 1). The resulting band, the purified phytoalexins, was scratched, eluted with acetone, and dried. A sample from this phytoalexins mixture was subjected to Proton NMR analysis (^{1}HNMR) to identify the structure of the separated phytoalexins. High-performance liquid chromatograph (HPLC) connected to a variable wavelength Perkin-Elmer LC55 UV detector was used to separate the labelled phytoalexins. Samples were dissolved in MeOH prior to injection. Reversed-phase columns (Spherisorb S5, ODS-2 hypersil), 25 cm × 8 mm (semipreparative), were employed with solvent methanol : acetonitrile : water (30 : 20 : 50), at flow rates of 1 ml/min or 2 ml/min [22]. Radioactivity of the compounds isolated was measured by SL 4000 liquid scintillation counter with dioxin-based liquid scintillator (BDH). ^{14}C-toluene internal standard was used for measurement of efficiencies.

2.5. Feeding Experiment of the Labelled (Wyerone and Dihydrowyerone) into the Induced Cotyledons to Produce Wyerone Epoxide. Approximately 1 mg samples of ^{14}C-wyerone and ^{14}C-dihydrowyerone obtained from the previous experiment (Section 2.4) were added as dispersions in 1-2 mL of water with the aid of few drops of Tween 20. Each was placed on 20 g of induced *V.* faba cotyledons. Extraction of the cotyledons was carried out as described above to

Figure 2: *V. faba* cotyledons treated with aqueous $CuCl_2$ (3 mM) and placed flat side up. Scale 2 cm.

isolate and purify the ^{14}C-wyerone epoxide. However, ^{14}C-Wyerone epoxide was detected as a small blue fluorescent band under UV254 below the band of ^{14}C-wyerone/^{14}C-dihydrowyerone mixture. The band was scratched and eluted with acetone. A sample was analysed by ^{1}HNMR to identify the compound. In this TLC, a new band was observed under UV254. It was scratched, eluted with acetone, and purified again according to the procedure mentioned in Section 2.4. The compound was identified by ^{1}HNMR and ^{13}CNMR. Radioactivity of the compounds isolated was measured by liquid scintillation counter as described above.

2.6. Screening for Antibacterial Activity. Gram-positive and Gram-negative bacteria were selected to evaluate the antibacterial activity of the separated phytoalexins. These bacteria were chosen to represent plant pathogens, human pathogens, and a saprophyte. Bacterial suspensions of 0.5 McFarland (*ca* 1×10^8 CFU/ml) were prepared and diluted to get 10^6 CFU/ml. These suspensions were each spread separately on Mueller–Hinton agar plates (9 cm diameter). The separated phytoalexins from cotyledons of *V. faba* were dissolved in methanol. Blank paper discs (9 mm) were loaded gradually with phytoalexin methanolic solutions to reach a final amount of 60 μg/disc. The discs were left to dry by air. The loaded discs were placed on the inoculated Mueller–Hinton agar plates. Discs loaded with the broad-spectrum antibiotic, amoxicillin (60 μg), were used as a control, and discs loaded with methanol alone were used as negative control. The plates were incubated at 30°C for 24 hour for all strains except *Pseudomonas syringae* (26°C for 24–36 hr). Zones of inhibition were measured and reported. The test was performed in duplicate.

3. Results and Discussion

3.1. Biosynthetic Conversion, Isolation, and Purification of Compounds. In the first feeding experiment using ^{14}C-sodium acetate, the phytoalexin mixture separated by TLC chromatography was detected as deep blue fluorescent band under UV254, with R_f value 0.31. ^{1}HNMR analysis revealed the presence of approximately 8% of ^{14}C-dihydrowyerone in the mixture of ^{14}C-wyerone and ^{14}C-dihydrowyerone. This is in agreement with previous results reported in our laboratory [17, 18]. However, the mixture purified by TLC was reported previously to contain about 13% of the dihydro derivative [20]. The resulting phytoalexin mixture was separated by semi-preperative HPLC. This system was previously used for quantitative analysis of seven wyerone derivatives isolated from *V. faba* cotyledons as reported in the literature [22]. The purified ^{14}C-wyerone obtained was quantified by UV spectroscopy (Perkin-Elmer 554 Spectrophotometer). It has UV absorbance λ_{max} in ethanol at 224, 290, and 350 nm compared with those reported in the literature at 226, 291, and 351 nm (€ = 27,000) [22]. The purified ^{14}C-dihydrowyerone was quantified by UV spectroscopy and produced a spectrum (λ_{max} ethanol, 340, 235 nm) entirely consistent with that reported [18, 22].

The quantitation of the isolated ^{14}C-wyerone and ^{14}C-dihydrowyerone was determined using the reported extinction coefficient (€ = 27,000) [22]. Accordingly, the yields of the mixture of the labelled wyerone/dihydrowyerone were 20–28 mg/100 g fresh tissue of cotyledons. The biosynthesis of wyerone was proven previously to be from acetate units via acetate pathway. This was done by a series of experiments applying radiolabeled ^{14}C- and ^{13}C-isotopes techniques [17]. In the present study, ^{14}C–sodium acetate was incorporated efficiently into wyerone (Table 1). This is in agreement with earlier reports [17, 18]. The radiolabel analysis of the isolated ^{14}C-wyerone/^{14}C-dihydrowyerone is shown in Table 2.

3.2. Separation of ^{14}C-Wyerone Epoxide, ^{14}C-Wyerone Acid, and a New Compound. After the feeding experiment of ^{14}C-Wyerone and ^{14}C-dihydrowyerone into the induced cotyledons (Section 2.5), ^{14}C-wyerone epoxide was detected as a small blue fluorescent band under UV254 below the labelled wyerone/dihydrowyerone mixture, with R_f value of 0.26. The purified ^{14}C-Wyerone epoxide was quantified by UV λ_{max} 350 using an extension coefficient (€ = 27,000) [15]. Radiolabel measurements of this compound are shown in Table 3. The results of this experiment indicated that wyerone was an effective precursor of wyerone epoxide.

^{14}C-Wyerone acid was separated using the same solvent with R_f value of 0.12. The compounds were isolated and identified using HPLC and ^{1}HNMR. The results of isolation and identification are consistent with previous reports [11, 13]. Nevertheless, Wyerone acid was isolated in order to test its antibacterial activity.

The new band which was observed under UV254 below the band of ^{14}C-wyerone and ^{14}C-wyerone epoxide on TLC had an R_f value of 0.21. This band was collected and purified using the same solvents used above. The resulting compound was identified as a new moiety, 11-hydroxywyerone, which is separated for the first time (Figure 1). ^{1}HNMR and ^{13}CNMR for this compound are the following: (250 MHz, $CDCl_3$): δ1.02 (3H, t, J = 7.1 Hz), 1.38–1.57 (2H,m), 1.58–1.70, (3H, q/br), 3.79 (3H, s), 4.16 (1H, t, J = 7.0 Hz), 6.69 (1H, d, J = 16.5 Hz), 7.34 (1H, d, J = 3.5 Hz), 7.45 (1H, d, J = 3.5 Hz), 7.69 (1H, d, J = 16.5 Hz).

^{13}CNMR (62.5 MHz, $CDCl_3$) δ (ppm): 13.90, 18.49, 31.03, 51.57, 62.01, 83.30, 85.97, 115.84, 119.16, 121.38, 129.80, 153.62, 154.83, 164.69, and 166.45.

The radioactivity of the new compound was measured and found to be 6.97×10^7 Sp.act. dpm/mM.

Table 1: Mean radioactivity measurement of ^{14}C-labelled wyerone and ^{14}C-dihydrowyerone mixture from feeding experiments with sodium (2-^{14}C) acetate. The results are average of two trials.

Compound	Yield (mg)	Sp. act dpm/mM	Dilution	Incorporation %
Mixture of ^{14}C-wyerone + ^{14}C-dihydrowyerone	16.91	6.66×10^6	1920	0.182

Table 2: Mean radioactivity measurement of separated ^{14}C-wyerone and ^{14}C-dihydrowyerone from first feeding experiment obtained after separation by HPLC. The results are average of two trials.

Compound	Yield (mg)	Sp. act dpm/mM	Dilution	Incorporation %
^{14}C-wyerone	10.3	7.99×10^7	1600	0.129
^{14}C-dihydrowyerone	1.014	9.56×10^7	1340	0.015

Table 3: Incorporation data of ^{14}C-labelled wyerone and ^{14}C-dihydrowyerone into ^{14}C-wyerone epoxide (according to Section 2.5).

Compound fed	Compound isolated	μg/g fr.wt.	Sp.act. dpm/mM	Dilution	Incorporation %
^{14}C-wyerone	^{14}C-wyerone epoxide	75	6.52×10^6	16	12.4
^{14}C-dihydrowyerone	^{14}C-wyerone epoxide	13.9	2.87×10^6	3.32	6.01

Incorporation data showed the highest conversion of ^{14}C-wyerone (12.4%) into ^{14}C-wyerone epoxide. The results in this study indicated that it is possible that a metabolic grid existed for these compounds in *V. faba*, in this case, hydroxylation of the ester followed by oxidation of the secondary alcohol. These might be controlled by relatively specific enzymes. Supporting this hypothesis would require further feeding experiment with this new labelled compound and isolation of wyerone epoxide. The incorporation value should be higher than that in the present study. Another aspect of this hypothesis is the involvement of the enzyme which catalyzes this first reaction in the biosynthetic pathway from 11-hydroxywyerone into the epoxide, which will need further elaboration as in other studies [23]. Experiments are still in progress.

The results also indicated a high incorporation (6.01%) of ^{14}C-dihydrowyerone into ^{14}C-wyerone epoxide. In each experiment, recovery of the precursor fed was considerably less than that of wyerone indicating further metabolism or degradation of the applied compound. In this case, dihydrowyerone may be transformed into wyerone epoxide quite efficiently by dehydrogenation to produce the 11, 12-alkene first, followed by the same pathway as for wyerone itself.

3.3. Antimicrobial Activity. The antimicrobial activity of the three phytoalexins against five Gram-positive and two Gram-negative bacteria were evaluated. These bacteria represent human pathogens, plant pathogens, and a saprophyte (Table 4). The 60 μg amount of phytoalexin per disc was chosen to be tested based on an amount of compound versus zone of inhibition experiments performed to determine the minimum amount of phytoalexins that produced the largest zone of inhibition (data not shown). For most bacteria-phytoalexins combination, 50–60 μg/disc was the lowest amount to produce the largest zone of inhibition. Above this amount, a plateau was reached. Therefore, 60 μg/disc was selected to be tested for the three compounds against the bacteria. Amoxicillin showed the highest zone of inhibition against all the tested microorganisms. The negative control (methanol on disc) showed no inhibition zone. The three phytoalexins showed no antimicrobial activity against the Gram-negative bacteria but had noticeable activity against the Gram-positive bacteria. Assuming that the three phytoalexins have similar diffusion coefficients, then, these phytoalexins have comparable activity against Gram-positive bacteria, since at the same dose (60 μg/disc), the zones of inhibition against plant pathogens and human pathogens are very close (range between 14 and 16 mm). The saprophyte *Bacillus megaterium* showed the least susceptibility to the three phytoalexins with zones of inhibition (12-13 mm). These results are in line with the previous report. Gnanamanickam and Mansfield [21] had shown that wyerone and some flavonoid phytoalexins were more toxic against Gram-positive than Gram-negative bacteria. Several other researches were conducted to investigate the antifungal activity of phytoalexins and only one studied their antibacterial effect [4, 5, 14]. To the best of our knowledge, this is the first study to report antibacterial activity of wyerone acid and wyerone epoxide against the microorganisms selected in this research. These results could open the door for developing new atypical antimicrobial agents.

4. Conclusion

The current study tried to elucidate the biogenetic conversion of some of the furanoacetyene phytoalexins of *V. faba,* particularly the biogenetic conversion among wyerone, dihydrowyerone, and wyerone epoxide. The conversions were studied through feeding experiments with labelled precursors to yield the phytoalexins. The results from the incorporation and dilution data showed that wyerone epoxide is most probably derived from wyerone. Moreover, dihydrowyerone could be a precursor to wyerone epoxide through more sequence of events,

Table 4: Mean zones of inhibition in (mm), $n = 2$, for the three phytoalexins and amoxicillin (positive control) tested against different bacterial genera.

	Gram stain	Pathogenicity	Wyerone	Wyerone acid	Wyerone epoxide	Amoxicillin
Bacillus cereus ATCC 14579	Positive	Human	16	16	15	25
Bacillus megaterium ATCC 14581	Positive	Saprophyte	13	12	12	25
Curtobacterium flaccumfaciens pathovar oortii (Corynebacterium oorti) ATCC 25283	Positive	Plant	16	15	16	34
Klebsiella pneumoniae ATCC 13883	Negative	Human	0	0	0	23
Micrococcus luteus ATCC 49732	Positive	Human	15	14	15	33
Pseudomonas syringae pathovar phaseolicola (Pseudomonas phaseolicola) ATCC 19304	Negative	Plant	0	0	0	20
Rhodococcus fascians ATCC 12974	Positive	Plant	16	15	15	33

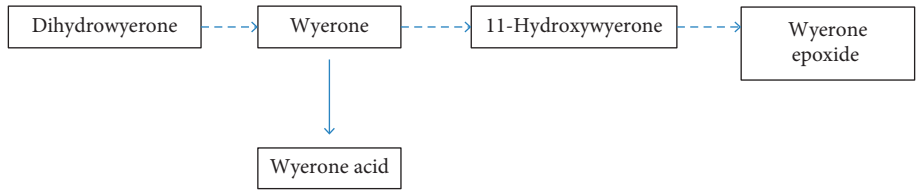


Figure 3: Isolation of a new compound, 11-hydroxywyerone, during the biosynthetic conversion of wyerone into wyerone epoxide. Dotted lines: the proposed pathway in the present study. Solid line: the previously reported conversion of wyerone into wyerone acid.

which is explained by less incorporation value. It is not possible at this stage to surmise about the natural sequence leading to wyerone epoxide. Nevertheless, we may suggest that the epoxidation is a late process of the biogenesis starting from wyerone and dihydrowyerone, which could be passing through the new compound 11-hydroxywyerone (Figure 3). However, further work is required to elicit these steps by feeding of this new compound to the cotyledons and measuring the incorporation data to wyerone epoxide. On the contrary, the three phytoalexins showed noticeable activity against the Gram-positive bacteria with no activity against the Gram-negative bacteria chosen. This activity could be promising to explore new sources for antimicrobial agents.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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References

[1] P. Jeandet, C. Clement, E. Courot, and S. Cordelier, "Modulation of phytoalexins biosynthesis in engineered plant for disease resistance," *International Journal of Molecular Sciences*, vol. 14, no. 7, pp. 14136–14170, 2013.

[2] R. L. Arruda, A. T. Santana Paz, M. T. Freitas Bara, M. V. de Carvalho Barros Côrtes, M. C. Corsi de Filippi, and E. C. Cardoso da Conceição, "An approach on phytoalexins: function, characterization and biosynthesis in plants of the family Poaceae," *Ciência Rural*, vol. 46, no. 7, pp. 1206–1216, 2016.

[3] P. Jeandet, "Structure, chemical analysis, biosynthesis, metabolism, molecular engineering, and biological functions of phytoalexins," *Molecules*, vol. 23, no. 1, p. 61, 2018.

[4] I. Ahuja, R. Kissen, and A. M. Bones, "Phytoalexins in defense against pathogens," *Trends in Plant Science*, vol. 17, no. 2, pp. 73–90, 2012.

[5] J. Kuc and W. Currier, "Phytoalexins, plants, and human health," *Advances in Chemistry*, Chapter 16, vol. 149, pp. 356–368, American Chemical Society, Washington, DC, USA, 1976.

[6] M. Wink, "Evolution of secondary metabolites in legumes (Fabaceae)," *South African Journal of Botany*, vol. 89, pp. 164–175, 2013.

[7] S. Soylu and M. Kemal, "*Induction of phytoalexin accumulation in Broad Bean* (*Vicia faba* L.) cotyledons following treatments with biotic and abiotic elicitors," *Turkish Journal of Agriculture and Forestry*, vol. 26, pp. 343–348, 2002.

[8] D. J. Robenson, "Furanoacetylene and isoflavonoid phytoalexins in Lens cullinaris," *Phytochemistry*, vol. 17, no. 4, pp. 807-808, 1978.

[9] D. J. Robenson and J. B. Harborne, "A chemical dichotomy in phytoalexins induction within the tribe Vicieae of the Leguminosea," *Phytochemistry*, vol. 19, no. 11, pp. 2359–2365, 1980.

[10] A. M. Tarrad, Y. Y. El-Hyatemy, and S. A. Omer, "Wyerone derivatives and activities of peroxidase and polyphenooxidase in faba bean leaves as induced by chocolate spot disease," *Plant Science*, vol. 89, no. 2, pp. 161–165, 1993.

[11] R. M. Letcher, D. A. Widdoswson, B. J. Deverall, and J. W. Mansfield, "Identification and activity of wyerone acid as a phytoalexins in broad bean after infection by botrytis," *Phytochemistry*, vol. 9, no. 2, pp. 249–252, 1970.

[12] G. P. Chapman "Systems for cytogenetic analysis in Vicia *faba* L," in *Proceedings of a Seminar in the EEC Programme of Coordination of Research on Plant Productivity,* G. P. Chapman, and S. Tarawali, Eds., Ashford, UK, April 1984.

[13] J. W. Mannsfield and D. A. Widdowson, "The metabolism of wyerone acid (a phytoalexins from Vicia faba L.) by Botrytis faba and *B. cinerea*," *Physiological Plant Pathology*, vol. 3, no. 3, pp. 393–404, 1973.

[14] S. Rossall, J. W. Mansfield, and R. A. Hutson, "Death of Botrytis cinerea and B. faba following exposure to wyerone derivatives in vitro and during infection development in broad bean leaves," *Physiological Plant Pathology*, vol. 16, no. 1, pp. 135–138, 1980.

[15] J. A. Hargreaves, J. W. Mansfield, D. T. Coxon, and K. R. Price, "Wyerone epoxide as a phytoalexins in Vicia faba and its metabolism by Botrytis cinerea and B. faba in vitro," *Phytochemistry*, vol. 15, no. 7, pp. 1119–1121, 1976.

[16] J. A. Hargreaves, J. W. Mansfield, and S. Rossal, "Changes in phytoalexin concentration in tissues of the broad bean plant (Vicia faba) following inoculation with species of Botrytis," *Physiological Plant Pathology*, vol. 11, no. 3, pp. 227–242, 1977.

[17] N. A. Al-Douri and P. M. Dewick, "Biosynthesis of the furanoacetylene phytoalexin wyerone in Vicia faba," *Zeitschrift für Naturforschung C*, vol. 41, no. 1-2, pp. 34–38, 1986.

[18] N. A. Al-Douri, "Biosynthetic conversion of antifungal phytoalexins from broad beans cotyledons," *Bothalia*, vol. 44, no. 5, pp. 161–166, 2014.

[19] S. Rossall and J. W. Mansfield, "Relationships between germ-tube growth by Botrytis cinerea and B. faba and their metabolism of the phytoalexins wyerone acid and wyerone epoxide," *Transactions of the British Mycological Society*, vol. 82, no. 3, pp. 571–576, 1984.

[20] J. W. Mansfield, A. E. A. Porter, and R. V. Smallman, "Dihydrowyerone derivatives as component of furanoacetylenic phytoalexinic phytoalexin response of tissues of Vicia faba," *Phytochemistry*, vol. 19, no. 6, pp. 1057–1061, 1980.

[21] S. Gnanamanickam and J. Mansfield, "Selective toxicity of wyerone and other phytoalexins to Gram positive bacteria," *Phytochemistry*, vol. 20, no. 5, pp. 997–1000, 1981.

[22] A. E. A. Porter, R. V. Smallman, and J. W. Mansfield, "Analysis of furanoacetylenic phytoalexins from the broad bean plant by high performance liquid chromatography," *Journal of Chromatography A*, vol. 172, no. 1, pp. 498–504, 1979.

[23] T. Hashimoto, A. Hayashi, Y. Amano et al., "Hyoscyamine 6 beta-hydroxylase, an enzyme involved in tropane alkaloid biosynthesis, is localized at the pericycle of the root," *Journal of Biological Chemistry*, vol. 5, no. 7, pp. 4648–4653, 1991.