

N-Feruloylputrescine in Infected Potato Tubers

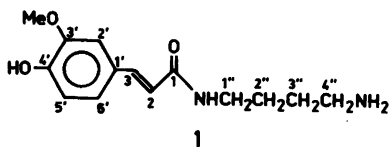
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N-Feruloylputrescine [*N*-(4''-aminobutyl)-3-(4'-hydroxy-3'-methoxyphenyl)-2-propenamamide] has been isolated from the blue fluorescent stress zone of potato tubers (cv. Bintje) infected by *Phoma exigua* var. *foveata*. Analysis of various parts of the infected tubers for this compound was performed by HPLC and TLC.

Amides of cinnamic acids and putrescine have been observed in some higher plants. *N*-Feruloylputrescine [*N*-(4''-aminobutyl)-3-(4'-hydroxy-3'-methoxyphenyl)-2-propenamamide] (1) was isolated for the first time as a picrate from *Salsola subaphylla* in 1949.¹ It was subsequently found in some citrus juices² and in several flowering plants, as were also *p*-coumaroyl- (2) and caffeoylputrescines (3).³ The activity of enzymes involved in the biosynthesis of cinnamoyl putrescines in different tobacco cell lines⁴ has also been reported. Compound 1 has now been isolated from the blue fluorescent stress zone of potato tubers (cv. Bintje) infected with *Phoma exigua* var. *foveata*, while 2 and 3 could not be detected. Analyses of compounds of this type have previously been performed with the use of ion-exchange chromatography and spectrophotometric measurements.² In this work an HPLC technique based on reversed phase column chromatography and with very simple pre-clean-up was used for the analysis.

Compound 1 was isolated from the stress zone



by column chromatography and was obtained as a mixture with its *cis* isomer (*cis-trans* ratio 1:4 according to ¹H NMR spectrum). The identification of 1 was performed by spectroscopic methods. All spectral data were in agreement with the data for a synthetically prepared specimen. The *cis* isomer was not isolated but was easily distinguished from the *trans* isomer by ¹H NMR; the olefinic protons H-2 and H-3 with a coupling constant of 11.8 Hz, clearly identify the *cis* isomer. Carbon C-3'' (δ 26.4 ppm) was distinguished from carbon C-2'' (27.0 ppm) by recording the ¹³C NMR spectrum of an acidified sample. The protonated amino group then causes a larger upfield shift of C-3'' than of C-2'' or C-4''.⁵

We were unable to reproduce the yield reported for an earlier synthesis of compound 1.⁶ According to HPLC, the yield was only 19 % before purification and considerable amounts of *N,N'*-diferuloylputrescine (4) and *N*-acetyl-*N'*-feruloylputrescine (5) were produced. By a 10-fold increase of putrescine and solvent the yield of 1 was raised to 55 %. Corresponding decreases in the yields of 4 from 25 to 11 % and of 5 from 18 % to only traces were noted.

TLC⁷ and HPLC analysis of extracts from various parts of the infected tuber (Table 1) showed that a significant amount of 1 is found in the stress zone and in the rotten part. Small amounts are also found in the peel, but 1 was not present in detectable amounts in peeled healthy tubers. These results indicate that 1 may be produced in the tuber when exposed to stress, but the substance does not seem to be a normal metabolite in the healthy tuber. The *cis* isomer was not present in detectable amounts in the extracts for HPLC analysis; and treatment of

Table 1. HPLC^a and TLC^{b,7} analysis of *1* in various parts of infected potato tubers.

Sample	Peeled tuber	Peel	Fluorescent stress zone	Rotten part
Bintje, 1977				
HPLC	<0.05	0.3	0.6	1.3
TLC		+ ^c	+++ ^c	+++ ^c
Bintje, 1978				
HPLC	<0.05	0.5	1.0	2.9
TLC		+ ^c	+ ^c	++ ^c

^a Amounts in $\mu\text{mol/g}$ extract. Retention time at pH 3.5 was 12.7 and 17.3 min (1.91 and 2.60 rel. I.S.) for the *trans* and *cis* isomers, respectively. Response factor 0.27 (calc. on the pure *trans* isomer). ^b Visual examination in UV light. ^c Relative amounts.

pure *trans* isomer according to the extraction process for the isolation⁸ of *1* produced only 0.3 % *cis* isomer. Accordingly the *cis* isomer may have been produced by isomerization during the latter part of the isolation procedure and does not seem to occur naturally in the plant. The quantification of *1* in the HPLC analysis of the rotten part was difficult because of the interference of UV-absorbing components with *1*. Since *1* is a highly fluorescent compound, this problem can be solved by the use of a fluorescence detector with suitable excitation and emission wavelengths.

Excitation at 318 nm and emission at 425 nm according to the fluorescence spectrum in the solution used in HPLC analysis decreased the detection limit 10 times, compared with UV detection, to 0.5 ng and no interfering peaks were obtained in the chromatogram.

Analysis of *1* in a number of potato varieties was performed and the results will be published elsewhere.

EXPERIMENTAL

General. The NMR spectra were recorded at 90 MHz (¹H NMR) and 22.5 MHz (¹³C NMR) in MeOD solutions. The mass spectra were obtained at 70 eV on a Finnigan 4021 mass spectrometer. The fluorescence spectrum was measured on an Aminco SP F-500 Model Corrected Spectra with a 5 nm band-pass. The MeOH extracts for HPLC and TLC analysis from

various parts of infected potato tubers were a gift from Dr. G. von Rosen. The extracts were prepared by boiling the potato sample in H₂O (15 ml/10 g potato), homogenizing, diluting with 0.1 % HCl in MeOH (3 ml/10 g potato), shaking thoroughly and filtering. HPLC analysis was performed on a μ -Bondapak C₁₈ column (30 cm \times 0.4 mm i.d., Waters ass.). The mobile phase was a linear gradient of 17–50 % MeOH in 0.05 M KH₂PO₄ buffer (pH 3.50) (A). The flow rate was 1.0 ml/min and a UV detector measuring at 313 nm was used. Tests with a fluorescence detector (Schimadzu, Fluorescence Spectromonitor RF-530) with excitation and emission wavelengths at 318 and 425 nm were also performed. Two-dimensional TLC was performed on Merck DC-Alufolien Cellulose F₂₅₄ plates in (1) 2 % HOAc and (2) BuOH–HOAc–H₂O (4:1:5, upper layer). The plates were inspected in UV light (360 nm).⁷

Isolation. The extraction of the blue fluorescent stress zone of potato tubers (cv. Bintje) infected with *Phoma exigua* var. *foveata* has been described previously.⁸ Compound *1* was isolated from the aqueous fraction from the extract by column chromatography on Sephadex LH-20 (eluent aq. EtOH with increasing EtOH content from 0–95 %), cellulose powder (Whatman CF 11, eluent 0.5 % HOAc) and Dowex 50 W \times 8 ion-exchange resin in the ammonium form [washed with acetone, water and methanol and elution of *1* with conc. NH₃–MeOH (1:1)].⁹ 14 mg of *1* was obtained as a mixture of *cis-trans* isomers in a 1:4 ratio according to ¹H NMR spectra.

HPLC analysis. A MeOH extract containing 10–40 mg dry matter was mixed, without further separation, with a solution of 10 mg 2,5-dihydroxybenzoic acid, which was used as internal standard (I.S.). The mixture was evaporated and dissolved in 0.5 ml of 17 % MeOH in buffer A. The sample was analyzed after filtration through a 0.5 μm Millipore filter.

Synthesis. Compound *1* was prepared by a modified method by Mizusaki *et al.*⁶ The acetylated ferulic acid chloride (500 mg) in 5 ml CHCl₃ was added to a solution of 10-fold excess of putrescine (2.2 g) in 250 ml of CHCl₃. After 2 h at room temperature the mixture was evaporated to dryness and treated with 5 ml 2 M NaOH for 2 h. The basic solution was extracted with CHCl₃, acidified with HCl and extracted with EtOAc. After evaporation to dryness the acidic residue was extracted with MeOH and *1* was crystallized from H₂O as pure *trans*-isomer. Yield (according to HPLC): 55 % of *1* and 11 % of *4*.

Compounds *4* and *5* were isolated from a synthesis according to the method by Mizusaki *et al.*⁶ The yields of *1*, *4* and *5* obtained by this

method were 19, 25 and 18 %, respectively.

N-Feruloylputrescine (trans). Cryst. from H₂O. M.p. 97–98 °C (lit. ~97 °C).⁹ ¹H NMR: δ 7.48 (d, *J* 15.7 Hz, H-3), 7.15 (d, *J* 1.8 Hz, H-2'), 7.07 (dd, *J* 8.1 and 1.8 Hz, H-6'), 6.85 (d, *J* 8.1 Hz, H-5'), 6.57 (d, *J* 15.6 Hz, H-2), 3.89 (s, MeO-3'), 3.3 (2H, m, H-1''), 3.0 (2H, m, H-4''), 1.7 (4H, m, H-2'' and H-3''). ¹³C NMR: δ 169.4 (s, C-1), 149.9 and 149.4 (s, C-3' and C-4'), 142.1 (d, C-3), 128.3 (s, C-1'), 123.3 (d, C-6'), 118.9 (d, C-2), 116.6 (d, C-5'), 111.8 (d, C-2'), 56.6 (q, MeO-3'), 40.4 and 39.6 (t, C-1'' and C-4''), 27.5 (t, C-2''), 25.9 (t, C-3''). MS [*m/z* (% of rel. int.)]: 264 [M]⁺ (5), 192 (8), 177 (45), 150 (5), 149 (7), 145 (20), 70 (100). IR, ν_{\max}^{KBr} cm⁻¹: 1640, 1570, 1540. UV, $\lambda_{\max}^{\text{MeOH}}$ nm: 220, 235, 296, 322 (lit.: 230, 292, 314).⁹ Fluorescence spectrum (corrected E) on a 10 ppm solution: solvent MeOH, $\lambda_{\text{ex.max}}$ 325 nm and $\lambda_{\text{em.max}}$ 410 nm, solvent 30 % MeOH in buff. A, $\lambda_{\text{ex.max}}$ 318 nm and $\lambda_{\text{em.max}}$ 425 nm.

N-Feruloylputrescine (cis). Not isolated. Signals different from the *trans* isomer in the NMR spectra of the isolated *cis-trans* mixture. ¹H NMR: δ 6.62 (d, *J* 11.8 Hz, H-3), 5.34 (d, *J* 11.8 Hz, H-2), 6.72 (d, *J* 7.9, H-5'), 3.83 (s, Ar-OCH₃). ¹³C NMR (D₂O): δ 138.8 (d, C-3), 120.4 and 118.8 (d, C-2 and C-6'), 114.4 (d, C-2').

N,N'-Diferuloylputrescine (trans.). Cryst. from H₂O-MeOH. M.p. 198–200 °C. ¹H NMR: δ 7.44 (d, *J* 15.7 Hz, H-3), 7.10 (d, 1.9 Hz, H-2'), 7.03 (dd, *J* 8.2 and 1.9 Hz, H-6'), 6.78 (d, *J* 8.2 Hz, H-5'), 6.42 (d, *J* 15.7 Hz, H-2), 3.86 (6H, s, MeO-3'), ~3.3 (4H, m, H-1'' and H-4''), ~1.6 (4H, m, H-2'' and H-3''). ¹³C NMR: δ 196.3 (s, C-1), 150.4 and 149.5 (s, C-3' and C-4'), 142.1 (d, C-3), 128.1 (s, C-1'), 123.3 (d, C-6'), 118.8 (d, C-2), 116.7 (d, C-5'), 111.8 (d, C-2'), 56.5 (q, MeO-3'), 40.3 (t, C-1'' and C-4''), 28.0 (t, C-2'' and C-3''). MS [*m/z* (% of rel. int.)]: 306 (5), 247 (5), 234 (3), 192 (15), 177 (100), 149 (78), 145 (60), 70 (41). IR, ν_{\max}^{KBr} cm⁻¹: 1650, 1610, 1540, 1510. UV, $\lambda_{\max}^{\text{MeOH}}$ nm: 220, 235, 295, 321 (lit.: 240, 284–318).¹⁰

N-Acetyl-N'-feruloylputrescine (trans). Cryst. from H₂O. M.p. 69–70 °C. ¹H NMR: δ 7.44 (d, 15.7 Hz, H-2'), 7.11 (d, 1.8 Hz, H-2'), 7.03 (dd, 8.2 and 1.8 Hz, H-6'), 6.79 (d, 8.1 Hz, H-5'), 6.41 (d, 15.7 Hz, H-2), 3.88 (3H, s, MeO-3'), ~3.2 (4H, m, H-1'' and H-4''), 1.92 (3H, s, Me-C=O), ~1.6 (4H, m, H-2'' and H-3''). ¹³C NMR: δ 173.3 (s, Me-C=O), 169.2 (s, C-1), 149.9 and 149.4 (s, C-3' and C-4'), 142.0 (d, C-3), 128.4 (s, C-1'), 123.2 (d, C-6'), 118.9 (d, C-2), 116.6 (d, C-5'), 111.8 (d, C-2'), 56.5 (q, MeO-3'), 40.2 (t, C-1'' and C-4''), 27.9 (t, C-2'' and C-3''), 22.6 (q, Me-C=O). MS [*m/z* (% of rel. int.)]: 236 (1), 222 (36), 194 (8), 177 (38), 150 (28), 145 (25), 101 (61), 86 (31), 73 (64), 70 (47),

43 (100). IR, ν_{\max}^{KBr} cm⁻¹: 1660, 1610, 1540, 1510. UV, $\lambda_{\max}^{\text{MeOH}}$ nm: 220, 235, 296, 322.

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