N-Feruloylputrescine in Infected Potato Tubers

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N-Feruloylputrescine \([N-(4'-aminobutyl)-3-(4'-hydroxy-3'-methoxyphenyl)-2-propenamide]\) 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 \([N-(4'-aminobutyl)-3-(4'-hydroxy-3'-methoxyphenyl)-2-propenamide]\) (1) was isolated for the first time as a precipitate from *Salsola subaphylla* in 1949.\(^1\) It was subsequently found in some citrus juices\(^2\) and in several flowering plants, as were also \(p\)-coumaroyl- (2) and caffeoylputrescines (3).\(^5\) The activity of enzymes involved in the biosynthesis of cinnamoyl putrescines in different tobacco cell lines\(^4\) 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-cleanup was used for the analysis.

![Chemical Structure](image)

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 \(^1\)H 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 \(^1\)H 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' (\(\delta 26.4\) ppm) was distinguished from carbon C-2' (27.0 ppm) by recording the \(^{13}\)C 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.

TLC\(^7\) 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$^b$ analysis of 1 in various parts of infected potato tubers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peeped tuber</th>
<th>Peel stress zone</th>
<th>Rotten part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bintje, 1977</td>
<td>&lt;0.05</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>HPLC</td>
<td>&lt;0.05</td>
<td>0.3</td>
<td>1.3</td>
</tr>
<tr>
<td>TLC</td>
<td>+$^c$</td>
<td>+++$^c$</td>
<td>+++$^c$</td>
</tr>
<tr>
<td>Bintje, 1978</td>
<td>&lt;0.05</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>HPLC</td>
<td>&lt;0.05</td>
<td>0.5</td>
<td>2.9</td>
</tr>
<tr>
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<td>+$^c$</td>
</tr>
</tbody>
</table>

$^a$ Amounts in µmol/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 2.27 (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 isolation$^8$ 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 ($^1$H NMR) and 22.5 MHz ($^{13}$C 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 H$_2$O (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 C$_{18}$ column (30 cm×0.4 mm i.d., Waters ass.). The mobile phase was a linear gradient of 17–50 % MeOH in 0.05 M KH$_2$PO$_4$ 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 Spectrometer 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 F$_{254}$ plates in (1) 2 % HOAc and (2) BuOH–HOAc–H$_2$O (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 acq. EtOH with increasing EtOH content from 0–95 %), cellulose powder (Whatman CF 11, eluent 0.5 % HOAc) and Dowex 50 W×8 ion-exchange resin in the ammonium form [washed with acetone, water and methanol and elution of 1 with conc. NH$_3$–MeOH (1:1)].$^9$ 14 mg of 1 was obtained as a mixture of cis-trans isomers in a 1:4 ratio according to $^1$H 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 furic acid chloride (500 mg) in 5 ml CHCl$_3$ was added to a solution of 10-fold excess of putrescine (2.2 g) in 250 ml of CHCl$_3$. 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 CHCl$_3$, acidified with HCl and extracted with EtOAc. After evaporation to dryness the acidic residue was extracted with MeOH and 1 was crystallized from H$_2$O 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). 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’). ¹C NMR: δ 169.4 (s, C-1), 149.9 and 149.4 (s, C-3’ and C-4’), 142.1 (d, C-5), 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, νKrprintln cm⁻¹: 1660, 1610, 1540, 1510.

UV, λmax nm: 220, 235, 296, 322.

Acknowledgements. I would like to thank Professor Olof Theander for his interest in this work and Mr. Rolf Andersson for recording the NMR spectra. I also wish to thank Karl-Erik Hellernäs, of the National Food Administration, Sweden, for the opportunity to test the fluorescence detector. This work was supported financially by the Swedish Board for Technical Development.

REFERENCES


Received April 6, 1983.

A Feruloylamide in Infected Potato Tubers