N,N′,N″-Triferuloylspermidine, a New UV Absorbing Polyamine Derivative from Pollen of *Hippeastrum* × hortorum

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A new hydroxycinnamoyl polyamine derivative, *N,N′,N″*-triferuloylspermidine (= *(E)*-N-*(4-aminobutyl)-3,3′,3″-tris(4-hydroxy-3-methoxyphenyl)*-N,N′,N″-*(butane-1,4-diyl)tris [prop-2-enamide])* (**1**) was detected in the H_2O/MeOH extract of pollen from *Hippeastrum* × hortorum. The compound was identified by on-line-coupled high-performance liquid chromatography and atmospheric-pressure chemical-ionization mass spectrometry (HPLC-UV(DAD)/APCI-MS and MS/MS). The structure was proven by comparing the HPLC/MS data after UV-induced (*E*) ⇆ (*Z*) photoisomerization and catalytic hydrogenation of the natural compound and the synthetic reference compound. This is the first report of a triferuloylspermidine in nature.

Introduction

In the last years the development of highly sensitive analytical techniques allows the search for new natural compounds in plant material of small amounts. Considering that secondary metabolites play in plants often a role as protectants we can expect that the reproductive organs of plants are rich sources of undiscovered active compounds.

Several studies report of hydroxycinnamoyl-polyamines and alkaloids in anthers and pollen from different plant families (Kretschmar and Baumann, 1999; Meurer *et al.*, 1988a; Meurer *et al.*, 1988b; Werner *et al.*, 1995). A striking example of chemical diversity was given recently from the pollen of *Hippeastrum* sp. (Amaryllidaceae) were a large amount of different disubstituted hydroxycinnamoyl-spermidines (at the most 84) were separated and identified by using on-line coupled HPLC/APCI-MS and MS/MS (Youhnovski *et al.*, 1998). Analyzing now a more unpolar fraction from the same pollen extract we could identify a new trisubstituted spermidine derivative. Its structure was identified as *N,N′,N″*-triferuloyl-spermidine (= *(*E*)*-N-*(4-aminobutyl)-3,3′,3″-tris(4-hydroxy-3-methoxyphenyl)*-N,N′,N″-*(butane-1,4-diyl) tris-[prop-2-enamide])* (**1**) by comparison the natural and the synthetic reference compound by HPLC-UV(DAD)/APCI-MS and MS/MS. The significance of such highly UV-absorbing compounds in pollen is discussed.

Materials and Methods

General

MeOH, CH_3CN, and $CHCl_3$ (HPLC-grade, *Scharlau*, Barcelona, Spain); TLC: *Merck* precoated plates *silica gel 60* F_{254}. Schlittler-reagent: $K_2[PtI_6]$ in aq. HCl for amines and amides TLC detection.

Plant material

The plants originate from one *Hippeastrum* x hortorum plant bought on a local market (Zurich) in 1991. All plants were subcultivated from this plant by vegetative propagation in the greenhouse of our institute. During the flowering period (Feb. 1999), the anthers were cut and the pollen collected and stored at −20°.

Analytical HPLC

Waters 626 LC system, *Waters 996* photodiode array detector and *Waters 600S* controller with *Millenium* chromatography manager 2010 v.2.15 (*Waters* Corp.). HPLC-APCI-MS: Triple stage quadrupole instrument *Finnigan TSQ 700*, equipped with a *Finnigan* atmospheric pressure chemical ionisation ion source. The UV absorption data are taken from the current on-line HPLC-UV detection (λ_{max} in nm); ESI-MS: *Finnigan TSQ 700*

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mass spectrometer, equipped with a *Finnigan* electrospray ionisation (ESI) ion source.

Extraction

The pollen (1.0 g) were extracted as described previously (Youhnovski *et al.*, 1998). After separation on TLC (silica gel F_{254},$CHCl_3$/MeOH/25% aq. NH_4OH 78:19:3) 2.1 mg of a compound (R_f-value 0.4) showing *Schlittler* positiv reaction was obtained. It was dissolved in MeOH (in concentrations 0.5–1 mg/ml) and analyzed with HPLC-UV-(DAD), HPLC-UV(DAD)-MS and HPLC-APCI-MS/MS.

HPLC and MS conditions

HPLC: ET 250/4 *Nucleosil* 100–5 C_8 column (*Macherey-Nagel*); injection volume 2 µl in concentration 1–2 mg/ml; flow rate 0.8 ml/min; mobile phase: H_2O/ MeCN/ 2% aq. soln of CF_3COOH. Gradient: 0 min – (65:25:10 *v/v*) → 30 min – (40:50:10), detection at 254 and 280 nm. MS operating conditions: APCI-MS, positive mode, vaporizer temperature 440–450°, corona voltage 4.5–5 kV, heated capillary temperature 200–220°, sheath gas N_2 with a pressure 60 PSI, conversion dynode: –15 kV. ESI-MS: heated capillary temp. 200 – 220°; sheath gas N_2, with an inlet pressure of 40 PSI; conversion dynode 15 kV; spray voltage 4.5 kV. Prep. HPLC: *LiChrosorb* RP-18, 250 × 25, 7 µm prep. column (*Merck*, Darmstadt, Germany); mobile phase: H_2O/ MeCN/ 2% aq. soln of CF_3COOH (50/40/10); flow 20 ml/min, detection at 280 nm.

N,N′,N″-*Triferuloylspermidine (= (*E*)-*N*-(4-aminobutyl)-3,3′,3″-tris(4-hydroxy-3-methoxyphenyl)-*N,N′,N″*-(butane-1,4-diyl)tris[prop-2-enamide])* (**1**)

To the cooled (ice bath) soln. of 0.2 g (1.4 mmol) spermidine in 50 ml THF 1.7 g (8.3 mmol) ferulic acid (4-hydroxy-3-methoxycinnamic acid) were added by stirring. The reaction mixture was constantly stirred during 5 h (ambient temp.) and then left without stirring for 24 h in the dark. The precipitation was filtered and the filtrate evaporated. The residue was dissolved in 100 ml $CHCl_3$, washed 3 × with 50 ml H_2O at pH 9 (adjusted with 10% aq. NaOH), once with 50 ml saturated aq. soln. of $NaHCO_3$ and once with 50 ml dest. H_2O. The org. layer was concentrated and the residue (1.2 g) was separated by CC (silica gel, mobile phase: $CHCl_3$/MeOH/25% aq. NH_4OH 78/19/3 *v/v*). The isolated fraction was additionally purified by prep. HPLC (*See HPLC and MS conditions*). All operations were made by avoiding the light exposition of the products.

Photoisomerization procedures

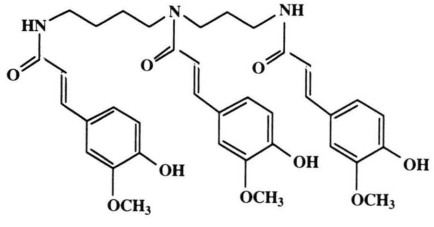
These were performed in a quartz cell, using a standard TLC UV-detection lamp *Camag* 365 nm placed at 10 cm above the cuvette, no filter.

Catalytic hydrogenation of the synthetic N,N′,N″-*triferuloylspermidine (= (*E*)-*N*-(aminobutyl)-3,3′,3″-tris(4-hydroxy-3-methoxyphenyl)-*N,N′,N″*-(butane-1,4-diyl)tris[prop-2-enamide]* (**1**)*, and the natural compound*

0.5 mg of each sample was dissolved in 1.5 ml MeOH containing 0.2 mg Pd/C (10%) and 10 µl HOAc. The reaction mixture was stirred for 6 h under hydrogen at atmospheric pressure, 25°. After filtration it was analyzed by ESI-MS. Yield (hexahydro conjugates): 99% (determined by ESI).

Results and Discussion

In the electrospray-ionization mass spectrometry (ESI-MS) experiment the isolated compound showed a quasi-molecular ion $[M + H]^+$ at *m/z* 674 which indicates a three-substituted derivative. The extracted-ion chromatogram for the quasi-molecular ion *m/z* 674 in the HPLC-MS analysis is shown in Fig. 1a and the HPLC-UV(280 nm) chromatogram is given in Fig. 1b. Five distinct peaks with retention time between 18 and 25 min are identical in both chromatograms with an UV absorption at 280 nm and $[M+H]^+$ peaks at *m/z* 674. The ob-



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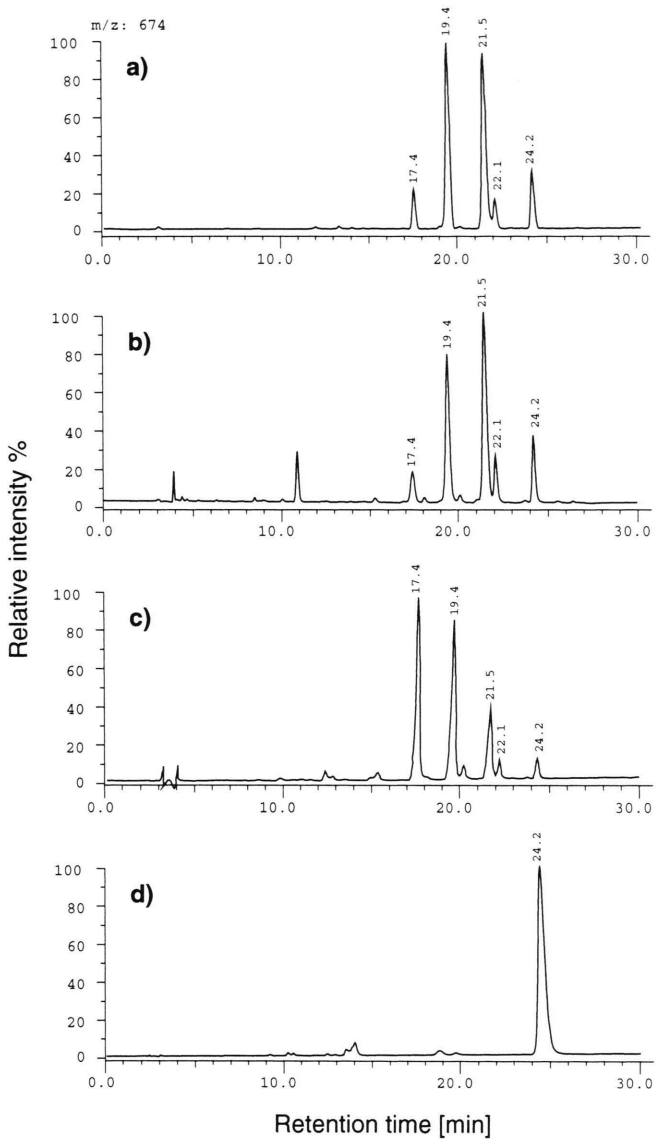

Fig. 1. Summary of the HPLC-UV(DAD)/APCI-MS experiment of natural and synthetic *N,N′,N″*-triferuloylspermidine: a) extracted ion-chromatogram of quasi-molecular ion *m/z* 674 of the natural product. b) UV-chromatogram registered at 280 nm of the natural product. d) UV-chromatogram registered at 280 nm of synthetic tri-feruloylspermidine before and c) after UV photoizomerization.

served fragment-ion signal of all five peaks showed *m/z* 177 as the sole hydroxycinnamoyl fragment, which corresponds to ferulic acid (data not shown). This lead to suppose that the unknown compound is a *N,N′,N″*-triferuloylspermidine and the peak-pattern in the chromatograms corresponds to different (*E,Z*)-isomers.

For proving this hypothesis we synthesized (*E,E,E*)-*N,N′,N″*-triferuloylspermidine (**1**) and perfomed an UV-photoisomerization experiment (see *Exper. Part*). The HPLC chromatogram of synthetic (*E,E,E*)-*N,N′,N″*-triferuloylspemidine is shown in Fig. 1d. After UV-photoisomerization the HPLC chromatogram (Fig. 1c) shows in the aerea of 18 to 25 min exactly the same peak pattern as the natural compound (Fig. 1b) with $[M+H]^+$ at *m/z* 674 for all peaks (data not shown).

After catalytic hydrogenation of the synthetic and the natural compound both showed the molecular ion at *m/z* 680 (674 +6 H) proving three double bonds in the hydroxycinnamic-acid parts of the molecule.

The variety and richness of hydroxycinnamoyl polyamines in pollen of *Hippeastrum* is striking.

Besides the high amount of different diacylated spermidine derivative we now identified a new triacylated compound namely, *N,N′,N″*-triferuloylspermidine (**1**). Until now three examples of trihydroxycinnamoyl-spermidines isolated from pollen or anthers, are reported. Tricoumaroylspermidines were found in different Rosaceae (Strack *et al.*, 1990) and in the genus *Aphelandra* (Werner *et al.*, 1995). In the pollen *of Quercus dentata* several triacylated spermidines containing coumaric-, caffeic- and ferulic acids in different combinations were identified (Bokern *et al.*, 1995; Nimtz *et al.*, 1996).

The effect of UV B radiation on plants has been reviewed by Jordan (1996). According to him one of the most profound influences of increased UV B radiation is upon the reproductive organs of plants. The hydroxycinnamoyl-spermidines show a high absorption in the range of 270 to 330 nm as is demonstrated in Fig. 2. The UV-spectra of the synthesized (*E,E,E*)-*N,N′,N″*-triferuloylspermidine has a maximum at 320 nm (peak 24.2 min), this is shifted to lower wavelengths by isomerization (270 nm; peak 17.4 min).

Recently we postulated that the disubstituted hydroxycinnamoyl-spermidines could have an UV protecting function for the DNA in the pollen grains (Youhnovski *et al.*, 1998). The presence of high UV absorbing trisubstituted hydroxycinnamoyl-spermidines in pollen gives an additional support to this hypothesis.

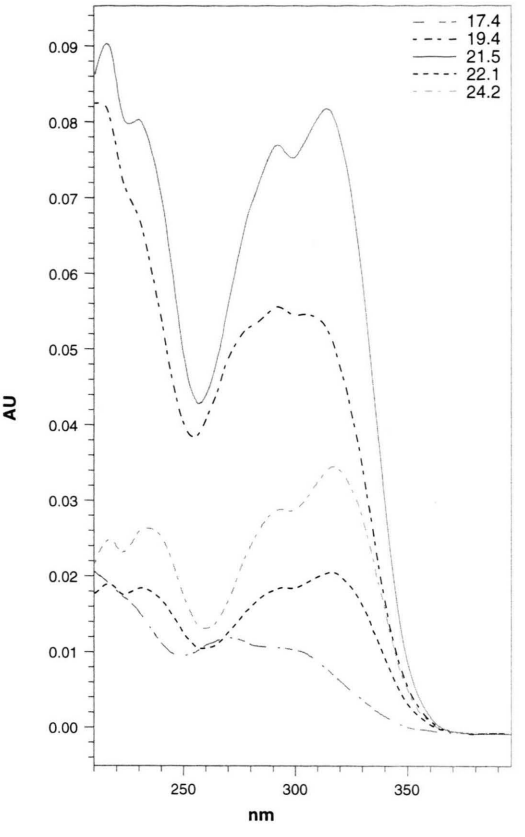

Fig. 2. UV-spectra of the different isomers of *N,N′,N″*-triferuloylspermidine extracted from the HPLC-UV-(DAD) experiment. The numbers correspond to the retention time of the peaks shown in the HPLC-chromatograms in Fig. 1. AU, absorption units.

Acknowledgements

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