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THE DISTRIBUTION OF HYDROXYCINNAMIC ACID AMIDES IN FLOWERING PLANTS

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Key Word Index—Monocotyledoneae; Dicotyledoneae; hydroxycinnamic acid amides; amine derivatives; identification; flower buds localization.

Abstract—Hydroxycinnamic acid amides have been identified as the main phenolic constituents in the reproductive organs of a range of flowering plants.

Amides of cinnamic acids have previously been observed in several higher plants. Thus, feruloylputrescine has been isolated and identified from citrus leaves and fruits [1] and caffeoylputrescine has been found in seeds of *Pentaclethra macrophylla* [2]. *p*-Coumaroyl and feruloyltryptamine were identified in kernels of *Zea mays* [3] and lunarine was found in the seeds of *Lunaria annua* and *L. rediviva* [4]. *p*-Coumaroyl-, feruloyl- and caffeoylputrescine have been reported in callus tissue cultures of *Nicotina tabacum* [5]. Recently we have identified *p*-coumaroyl-, feruloyl, di-*p*-coumaroyl- and di-feruloylputrescine in the leaves of virus-infected vegetative tobacco [6, 7]. Results obtained from our laboratory strongly suggest that aromatic amides have an antiviral effect [7] and are formed in tobacco after virus-infection as a protective mechanism. Caffeoylputrescine was also found in tobacco flowers, but it was absent from the fully-expanded leaves under normal growing conditions [8, 9] In a recent publication we have reported the presence of caffeoylputrescine, caffeoylspermidine, *p*-coumaroyl- and feruloylputrescine in the meristems of *Nicotiana* [10] and we have demonstrated [11] that there is an increase in the amount of these aromatic amides in the apical part of tobacco plants at the time of floral induction.

Table 1. The distribution of hydroxycinnamic acid amides in the angiosperms

Class, Family, genus, species	HYDROXYCINNAMIC ACID AMIDES																					
	Putrescine								Spermidine								Tyramine			Spermine		
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	3	5	6	7	8
Monocotyledoneae																						
Bromeliaceae																						
Ananas comosus Merrill	−	−	−	−	+	+	+	−	−	−	−	−	+	+	−	−	−	−	−	+	−	−
Gramineae																						
Pennisetum americanum (L.) K. Schum	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Triticum vulgare Vill.	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
*Zea Mays L.**	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liliaceae																						
Lilium sp	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	+
Dicotyledoneae																						
Amaranthaceae																						
Gomphrena globosa L	−	−	−	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−
Iresine herbstii Hoorer	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Caryophyllaceae																						
Dianthus caryophyllus L.	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−
Compositae																						
Helianthus annuus L.	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Cruciferae																						
Brassica oleracea L. var. *botryris* L.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Hippocastanaceae																						
Aesculus hippocastanum L	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	+	+	−	−	−	−
Lauraceae																						
Persea gratissima Gaertn. fil.	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Leguminosae																						
Vicia faba L	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−
Rosaceae																						
Pyrus communis L.	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Rubus idaeus L.	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Salicineae																						
Salix sp.	+	−	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−
Solanaceae																						
Lycopersicum esculentum Mill.	+	−	−	−	+	+	−	−	−	+	−	−	+	+	−	−	+	−	+	−	−	−
Nicotiana tabacum L.	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−	−	−
Petunia hybrid	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Key: 1 = *p*-coumaryl, 2 = di-*p*-coumaryl, 3 = caffeyl, 4 = di-caffeyl, 5 = feruloyl, 6 = di-feruloyl, 7 = sinapyl and 8 = di-sinapyl.
* Also contains *p*-coumaroyltryptamine and feruloyltryptamine.

Accumulation of hydroxycinnamic acid amides can also be induced in lower leaves by the topping of flowering plants [11], indicating that their production may be linked with floral induction. In *Nicotiana tabacum* cv Xanthi n.c. an increase in temperature which inhibits the flowering process suppresses the accumulation of aromatic amides [9, 10]. Aromatic amides were found in large amounts in the reproductive organs of tobacco; caffeylputrescine and caffeylspermidine were present in the ovaries but neutral compounds such as di-*p*-coumarylputrescine, di-*p*-coumarylspermidine and *p*-coumaryltyramine [11] occurred in the anthers.

The results of the present survey (Table 1) show the presence of hydroxycinnamic acid amides in a number of other species of flowering plants, representing 13 different families. Some 24 hydroxycinnamic acid derivatives of the 5 amines: putrescine, spermidine, tryptamine, tyramine and spermine were characterized. Thus aromatic amides appear to be common constituents of flowering plants. However, within the plant these compounds were found only in the reproductive organs, where they appear to be the main phenolic compounds and were absent from the green parts (leaves, stems), petals and sepals. Earlier findings, which were obtained in a continuing screening program at this laboratory, indicated the presence of large amounts of aromatic amides in the seeds of a number of plants (unpublished).

EXPERIMENTAL

Plants were grown in a greenhouse or in controlled environment chambers or collected in the vicinity of Dijon. Green parts (leaves, stems) and different floral parts were examined separately.

Plant tissue (50 g fr. wt) was extracted in a blender with 80% aq. MeOH containing 0.1% HCl (100 ml × 2) [11]. The soln, after centrifugation was evapd at 40° to *ca* 10 ml, diluted with H_2O (40 ml) and then treated with: (1) petrol (100 ml × 3) (to remove cinnamic acid tryptamine conjugates), (2) C_6H_6 (100 ml × 3) (to remove cinnamic acid tyramine conjugates), (3) $CHCl_3$ (100 ml × 3) (to remove di-cinnamic acid putrescine conjugates), (4) EtOAc (100 ml × 3) to remove di-cinnamic acid spermidine and spermine conjugates). Each fraction was taken to dryness *in vacuo*, the residue dissolved in 20 ml MeOH, and 1 g of cellulose powder (microcrystalline, Macherey–Nagel) added. The resulting mixture, concd to dryness, was applied on a column (20 × 3 cm) packed with the same kind of cellulose [6, 11]. The elution was made successively with H_2O (500 ml) and MeOH (500 ml) [11]. The aq. fraction contained mainly cinnamic acid tyramine and tryptamine conjugates and the MeOH fraction furnished di-cinnamic putrescine, spermidine and spermine conjugates. These fractions, concd *in vacuo*, dissolved in 20 ml of MeOH, were further purified on a Polyclar AT (Serva) column (15 × 3 cm) employing MeOH as developing solvent [11]. Fractions (25 × 20 ml) were collected and analysed by 2D PC (Whatman No. 2) using BuOH–EtOH–H_2O (4:1:2) and H_2O [6]. Appropriate fractions were combined on the basis of their PC similarities and rechromatographed in the same system. The various compounds were cut from the chromatogram and eluted with 70% MeOH [11]. It was interesting to note that neutral compounds (di-cinnamic acid putrescine, spermidine and spermine conjugates, *trans* cinnamic acid tyramine and tryptamine conjugates) could be crystallized from H_2O–MeOH (4:1).

The remaining H_2O phase (40 ml) left after removal of neutral compounds was concd under red. pres. to 10 ml. This aq. soln was passed through a column (7 × 4 cm) of Amberlite resin Serva CG 50 (H^+ form) and the column washed with H_2O (400 ml) and EtOH 40% (200 ml) [11]. The combined inactive eluates were discarded. Further elution with 800 ml of 3N HOAc furnished basic aromatic amides [11]. The eluate was taken to dryness and the residue dissolved in 10 ml of MeOH and applied to a Polyclar AT column (15 × 3 cm). The column was eluted with MeOH. Fractions (10 × 20 ml) were collected and analysed [11]. The elution pattern form Polyclar AT in MeOH followed the order: (1) *p*-coumarylputrescine, (2) ferulylputrescine, (3) caffeylputrescine, (4) caffeylspermidine. Appropriate fractions based on their PC similarities in BuOH–EtOH–H_2O and H_2O were combined, concd, and further purified by 2D PC as before.

Methods used in identification of substances included spectral data (IR, UV and MS), fluorescence in UV light alone, and with NH_3, R_f values, colour reaction with ninhydrin, identification of phenolic acid and the amine produced by hydrolysis [6, 10, 11]. Amines were determined using a Technicon Auto Analyser [12, 13]. Final confirmation of the structure was obtained by comparison with compounds synthesized as in refs. [5, 6, 10]. For amine analysis, the hydrolysis conditions were 4N HCl, 6 hr and 100° [11]. For phenolic acid analysis, hydrolysis was made with 2N NaOH for 4 hr at 100° [11]. Phenolic acid extracted by Et_2O from the aq. phase (adjusted to pH 1) was identified by methods previously described [14].

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