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HYDROXYCINNAMOYL ACID AMIDES AND AROMATIC AMINES IN THE INFLORESCENCES OF SOME ARACEAE SPECIES

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Key Word Index—Monocotyledoneae; Araceae; hydroxycinnamic acid amides; amine derivatives; aromatic amines; flowering.

Abstract—Hydroxycinnamoyl acid amides (HCA's) were found to be important components in the inflorescences of different Araceae species. HCA's occurred in large amount in spathes and in the male and female flowers, and were totally absent from the sterile flowers, commonly found on Araceae spadices. Differences in the distribution of HCA's were noted between male and female flowers. Thus the amount of neutral HCA's was always greater in the male than in the female flowers and the female flowers generally contained more basic HCA's. In the inflorescences of some Araceae species in the Monsteroideae and Philodendroideae (genera *Monstera*, *Raphidophora* and *Philodendron*), the aromatic amines tyramine and dopamine were very abundant, with concentrations ranging from 1 to 4 mg of each amine per g fr. wt.

INTRODUCTION

Hydroxycinnamoyl acid amides (HCA's) contain hydroxycinnamic acids (HC's) and amines (AM's) linked by an amide bond. There are two types: basic HCA's (water soluble) having a primary amine function and neutral HCA's (water insoluble) showing no strongly ionizable functions. The amines of basic HCA's include aliphatic di- and polyamines such as putrescine, cadaverine, spermidine and spermine whereas those of neutral HCA's include both aliphatic and aromatic amines (tyramine, dopamine, octopamine and tryptamine).

HCA's have been found in flowers from a wide range of plants [1]; in tobacco they occur in the leaves and apices at the beginning of flowering and accumulate in sex organs and seeds [2–4]. In *Zea mays* HCA's have been observed to be the major components of male and female flowers and of the embryo after fertilization [5]. In *Z. mays* and *Nicotiana tabacum* flowers, male and female sex organs could be separated by their different HCA content. HCA's have been identified as newly synthesized compounds in leaves of *N. tabacum* infected with tobacco mosaic virus (TMV); HCA's were formed during the hypersensitivity reaction of tobacco to TMV and exhibited *in vitro* antiviral properties [6, 7]. HCA's have also been found in *Salsola subaphylla* var. *arenaria* [8], *Citrus* leaves [9], in young barley shoots [10], *Evodia belahe* bark [11], rust-infected resistant wheat leaves [12], *Pentaclethra macrophylla* seeds [13, 14], cultured callus tissue of *N. tabacum* [15], in apices and flowers of tobacco [16], kernels of *Z. mays* [17], in *N. tabacum* leaves showing mineral deficiencies [18], eggplant roots [19] and *N. tabacum* cell cultures [20]. At least some HCA derivatives (i.e. glycocinnamoylspermidines) have been considered as a new class of antibiotics [21]. Thus, HCA's appear to be very common constituents of higher plants, present especially during sexual reproduction (flowering, fertilization).

RESULTS AND DISCUSSION

The results of a survey of some Araceae species for HCA's and AM's are given in Table 1. Four unknown fluorescent compounds releasing AM's (**1**–**4**) were found and until these are identified, we shall consider them as HCA's. Indeed all four had the characteristic UV spectra, fluorescence, AM release, and R_f values in all chromatographic systems (i.e. PC, liquid chromatography and TLC) of HCA's. HCA's were found as components of the inflorescences of Araceae species and were most frequently derivatives of putrescine, spermidine and tyramine with ferulic acid.

The fertility of tropical Araceae was not tested in the glasshouse since Araceae species generally need to be cross-fertilized with pollen from different inflorescences of the same plant or preferably of different plants and we did not have two or more plants of each species. In nature, cross fertilization is frequently secured by insects, e.g. flies, beetles and bees. It was, therefore, difficult in this study to associate HCA accumulation in reproductive tissues with fertility, except for European Araceae, e.g. *Arum*. Moreover in the fertile flowers of several species we always found HCA's while other species never ac-

Table 1. The distribution of hydroxycinnamyl acid amides and aromatic amines in unopened inflorescences of some Araceae species

Species	Tissue*	Hydroxycinnamyl acid amides†														Aromatic amines‡	
		Basic							Neutral								
		PP	CP	FP	PS	CS	FS	4	dPP	dFP	dPS	dFS	FT	1	2	3	T	D
Philodendron andreanum (Devans)	S	++	+++	+	—	—	—	—	—	+	—	—	++	+	+	+	+++	++
	M	—	(+)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	F	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	(+)	—
P. scandens Koch et Sello	S	—	—	+	—	—	—	—	—	+	—	—	—	—	—	++	+++	+
	M	—	—	+	—	—	—	—	—	—	—	++	—	—	—	+++	+++	(+)
	F	—	—	+	—	—	—	—	—	—	—	+++	—	—	—	+	+++	(+)
P. tripartitum (Jacq.) Schott	S	—	—	—	++	+++	++	—	—	++	—	—	—	—	—	+	++	+++
	M	—	—	—	—	—	—	—	—	—	—	—	—	—	—	++	+++	+
	F	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
P. selloum C. Koch	I	—	—	—	—	—	—	—	—	—	—	+	—	+	+	—	+	—
P. martianum Engl.	I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+++
P. glaziowi Hook. f.	I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
P. erubescens Koch et Aug.	I	—	—	—	—	—	—	+	—	—	—	—	—	—	—	++	++	+
Zantedeschia aethiopica (L.) Spreng	S	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	—	(+)
	M	—	—	—	—	—	—	++	—	—	—	—	+	—	—	—	—	(+)
	F	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	—	(+)
Remusatia vivipara Schott	I	—	—	++	—	—	—	—	—	+	—	—	—	+++	+	—	++	—
Monstera deliciosa Liebm.	S	—	—	++	—	—	—	—	—	—	—	—	(+)	+	+	—	+	+++
	M	—	—	—	—	—	—	—	—	—	—	—	++	+	+	—	(+)	+
	F	—	—	++	—	—	—	—	—	—	—	—	—	—	—	—	(+)	+++
Raphidophora decursiva Schott	S	—	—	++	—	—	—	—	—	—	—	—	(+)	+	+	—	—	+++
	M	—	—	—	—	—	—	—	—	—	—	—	++	+	+	—	—	+
	F	—	—	++	—	—	—	—	—	—	—	—	—	—	—	—	—	+++
Colocasia esculenta Schott	I	+	—	+	+	—	—	—	—	—	—	—	—	++	++	—	—	
Arum maculatum L.	M	—	—	—	—	—	—	—	+	—	+++	—	—	—	—	—	—	—
	F	—	—	—	—	—	—	—	+	—	++	—	—	—	—	—	—	—
A. italicum Mill.	M	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—
	F	—	—	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	—
Arisarum vulgare Targ.	I	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—
Dracunculus vulgaris Schott	M	—	—	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	—

*S, Spathe; M, male; F, female; I, inflorescence (spathe + spadix).

†PP, *para*-Coumarylputrescine; CP, caffeylputrescine; FP, ferulylputrescine; PS, *para*-coumarylspermidine; CS, caffeylspermidine; FS, ferulylspermidine; dPP, di-*para*-coumarylputrescine; dFP, diferulylputrescine; dPS, di-*para*-coumarylspermidine; dFS, diferulylspermidine; FT, ferulyltyramine. Unknowns: **1**, violet fluorescent (366 nm) neutral compound releasing tyramine (R_f BEW 0.85; H_2O 0.45–0.65); **2**, blue-green fluorescent (366 nm) neutral compound releasing tyramine (R_f BEW 0.85; H_2O 0.3–0.55); **3**, violet fluorescent (254 nm) neutral compound releasing tyramine (R_f BEW 0.95; H_2O 0); **4**, violet fluorescent (366 nm) basic compound releasing spermine (R_f BEW 0.21; H_2O 0.25).

‡T, Tyramine; D, dopamine.

Abundance (approx. by PC) —, undetectable; (+), trace; +, present; ++, abundant; +++; very abundant.

cumulated them. So we have presented all the results (Table 1) as a survey, in order to stimulate further physiological investigations.

Despite the difficulties of interpretation, the present work has furnished some useful information on the characteristics of Araceae inflorescences. Thus, the spathe seems to be a very important organ with respect to HCA content. This foliar organ often accumulates large amounts of basic HCA's and occasionally neutral HCA's. The spathe may be an important site for events leading to the organogenesis of reproductive cells. This 'floral leaf', developing before the sex organs, is thus of importance in the flowering of Monocotyledoneae; this has been demonstrated for *Pennisetum typhoides* Stapf et Hubard [22], both for reproductive physiology and for HCA content. In florets we often noted accumulation of HCA's. In male florets (especially in pollen) neutral HCA's were more abundant than basic, while basic and neutral HCA's accumulated in female flowers. However male and female florets can be separated on the basis of the HCA's they contain, or by quantitative differences in the same HCA. For example, in *Arum italicum* di-*para*-coumarylputrescine and di-*para*-coumarylspermidine are found in both the male and female florets, but in the anthers di-*para*-coumarylspermidine accumulates in the largest amounts. The sterile organs can be distinguished from adjacent fertile flowers by their total lack of HCA. These sterile organs accumulate large amounts of glucose esters, especially feruloyl- and *para*-coumarylglucose (these compounds were characterized by R_f values on PC, fluorescence in UV light and acid hydrolysis). In addition, the sterile appendices extending the spadix of Aroideae (genera *Arum*, *Dracunculus* and *Arisarum*) never accumulate HCA's.

In some Araceae species tyramine and dopamine were the main phenolic constituents of the flowers (Table 1). In fact these two AM's were present in all the Araceae inflorescences we studied but they accumulate in large amounts (between 0.5 and 4 mg/g fr. wt for one amine) only in tropical species of the Philodendroideae and Monsteroideae (*Monstera*, *Raphidophora* and *Philodendron*). A rapid analysis shows that these tropical creepers contain high concentrations of aromatic AM's, not only in the inflorescences but also in the leaves, stems, adventitious roots and vegetative buds, with frequent qualitative differences between these organs. For example in *Raphidophora decursiva* Schott we note a tyramine–dopamine ratio of greater than 1 for vegetative buds and a tyramine–dopamine ratio of less than 1 for the whole inflorescence. In addition, quantitative assay of AM's was achieved in the case of *Monstera deliciosa* Liebm. (Table 2), showing that dopamine was the predominant AM found in some parts of the inflorescence but that it was completely absent from the reproductive organs, i.e. the ovules and the anthers (the small amount found is due to the imperfect separation of the ovary from the ovules and the stamens from the anthers during the dissections). In addition we studied the AM content of unfertilized fruits: dopamine increased in the fruit tissues and accumulated in unfertilized ovules (which grow bigger) during the first month after spathe-fall, and then decreased (Table 2). In *M. deliciosa* the development of the fruit does not require fertilization, but at the end of its growth the fruit degenerates and no seed is formed. Fruit development is thus probably parthenocarpic. The study of aromatic AM's in these particular Araceae would be of interest in order to elucidate their function.

The phenethylamines are widely distributed in the plant kingdom [23, 24], but their occurrence in concentrations of more than 1 mg/g fr. wt in certain species is of interest if we consider their physiological activity in higher animals. Such activity is still unknown in plants. Moreover dopamine has been previously observed, in large amounts, in tropical Monocotyledoneae such as banana [23] and pineapple fruit [Martin-Tanguy, J., unpublished result]. Dopamine also occurs in high concentrations in the ovules of *Ceratozamia mexicana*, Cycadaceae [Ponchet, M., unpublished result]. Aromatic AM's like dopamine appear to be characteristic of some tropical

Table 2. Free aromatic amines found in the different parts of *Monstera deliciosa* Liebm. inflorescences

Organ		Ratio of aliphatic amines to tyramine and dopamine*			Dopamine (mg/g fr. wt)
		Aliphatic amines†	Tyramine	Dopamine	
Male flower	Stamens	1	1	28	3.7
	Filament	1	2.1	94	
	Anthers	1	0.2	0.5	
Female flower	Ovaries	1	2.2	186	3.2
	Ovules	1	0	0.4	
Spathes		1	0.4	89	2.3
Fruit (1 month old)	Ovaries	1	1.9	112	3.9
	Ovules	1	1.2	27	
Fruit (>3 months old)	Ovaries	1	0.9	103	2.3

*Ratio was obtained from amine assay in tissues (mol).
†Putrescine + spermidine + spermine.

plants, especially monocots and some more primitive families, like the Cycadaceae.

Other authors [25–27] have previously reported that the odoriferous compounds (volatile aliphatic amines), indole and skatole were produced by the inflorescences of different arum lily species during flowering. As the biogenesis of these compounds is very close to that of HCA's, i.e. aliphatic amino acid decarboxylation on the one hand, shikimic acid pathway on the other, both classes could be studied together in order to appreciate their inter-relationship and importance in reproduction.

EXPERIMENTAL

The tropical Araceae species were grown in a glasshouse with the exception of *Monstera deliciosa* Liebm. and *Raphidophora decursiva* Schott which were obtained from gardens. The European Araceae species were collected in the vicinity of Dijon and Antibes.

The different parts of the inflorescences before opening: spathe, male flowers, female flowers and sterile flowers, were examined separately. Plant tissues (10 g fr. wt) were homogenized in MeOH (100 ml) and the suspension filtered and washed twice with MeOH (2 × 100 ml). The MeOH extract was concd under vacuum to 5 ml, diluted with H_2O (100 ml) and extracted with EtOAc (3 × 100 ml). The aq. and EtOAc fractions were evaporated to dryness, dissolved in MeOH–H_2O (1:1) and MeOH, respectively (2.5 ml/g fr. wt for each). The aq. extract, containing basic HCA's was passed through a 10 × 2.5 cm i.d. column of Amberlite resin Serva AG CG 50 I (H^+ form). The resin was washed with H_2O (400 ml) then EtOH–H_2O (2:3) (400 ml). Basic HCA's were eluted with 3 N HOAc (800 ml). The eluate was taken to dryness and dissolved in MeOH (0.5 ml/g fr. wt). This extract was purified on a 40 × 2 cm i.d. Whatman CF 11 cellulose column, eluted with H_2O (150 ml), 150 ml MeOH–H_2O (1:9) containing 1% HOAc and 150 ml MeOH–H_2O (1:4) containing 1% HOAc. The successive fractions collected (50 ml) were analysed by 2D-PC (Whatman No. 2) using *n*-BuOH–EtOH–H_2O (4:1:2) and H_2O (pH 5.5) [6]. The fractions were combined on the basis of their chromatographic similarities, then rechromatographed in the same system. Basic HCA's were eluted in the following order: putrescine derivatives (MeOH–H_2O, 1:9) then spermidine derivatives (MeOH–H_2O, 1:4). The EtOAc fraction, containing neutral HCA's, was dried on Avicel microcrystalline cellulose powder (Merck) and chromatographed on a (20 × 2 cm i.d.) column packed with the same cellulose. Elution was achieved with MeOH–H_2O (3:7) 1% HOAc (100 ml), MeOH–H_2O (1:1) 1% HOAc (100 ml), MeOH. The fractions collected (50 ml) were evaporated to dryness and dissolved in MeOH (0.5 ml/g fr. wt). The MeOH–H_2O 1% HOAc fractions contained neutral HCA's and flavonoids, whereas MeOH fractions contained removable neutral substances (chlorophylls, lipids, etc.). Further neutral HCA purifications were carried out on Polyclar AT (Serva) (20 × 2 cm i.d.) or Sephadex LH-20 (Pharmacia) (30 × 2 cm i.d.) columns with the aid of MeOH as developing solvent [1, 4]. All the fractions were analysed by 2D-PC (Whatman No. 2) using *n*-BuOH–EtOH–H_2O (4:1:2) and H_2O (pH 5.5).

Basic and neutral HCA's were identified through their fluorescence in UV light (254 and 366 nm alone and with Na_2CO_3), R_f values and colour reaction with ninhydrin. Further investigations used UV spectra, hydrolysis of eluted chromatographic spots [1, 4, 6]. Acid hydrolysis (2 N HCl, 100° for 6 hr) led to the release of AM's which were analysed by ion-exchange liquid chromatography coupled with fluorimetric detection [28]. The HC moiety was studied after basic hydrolysis (2 N NaOH, 100° for 4 hr), extraction into Et_2O and identified by previously described techniques [29]. Purified HCA's were compared with synthetic HCA's as in ref. [6].

Free aromatic AM analysis was either by the method above for basic HCA's or by extraction with 0.1 N aq. HCl. The 3 N HOAc fractions eluted from Amberlite resin contained aromatic AM's which were identified on the 2D-PC's by UV (A_{254}), colour reaction with ninhydrin and R_f values. This did not allow quantification of AM's, but permitted rapid qualitative evaluation of aromatic AM's in plant tissues. On the other hand, AM extraction with 0.1 N aq. HCl [30] allowed quantification of AM's from plant tissues (with only a slight loss of substance), when a fluorimetric assay [28] was used.

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